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A study on the bacteria of dog bite wounds in dogs and their susceptibility to antimicrobials

by

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This dissertation is dedicated to my parents Anthony and Jennifer, for their constant support throughout my career and who have paved my way with love and encouragement.

Contents

Summary

To investigate the bacterial composition of infected and non-infected dog bite wounds (DBW), a prospective study was performed on dogs with various grades of bite wounds presenting at the Onderstepoort Veterinary Academic Hospital, University of Pretoria, and a nearby animal shelter. Fifty dogs with bite wounds inflicted within the previous 72 hours were selected. This represented 104 wounds. Wounds were clinically graded according to severity. Swabs were collected from all wounds for bacterial culture and cytology. Infection was diagnosed if 2 of the following 3 criteria were met: macroscopic purulence, microscopic presence of phagocytosed bacteria, or pyrexia. Non-infected wounds were either classed as sterile (established by culture) or contaminated (culture positive but bacteria not phagocytosed on cytology). To determine the origin of the bacteria, swabs were collected from the skin near the wounds and gingiva of 15 bite victims. All swabs were cultured aerobically and anaerobically and all aerobic cultures were evaluated for antimicrobial susceptibility using the Kirby Bauer disk diffusion test.

The victims were predominately male, uncastrated, small-breed dogs. Of the 104 wounds studied, 21 were judged to be infected and 83 non-infected. Infected wounds were significantly more likely to culture positive (Fisher's exact test: $p = 0.02$). Sixteen per cent of wounds did not culture bacteria, 67% grew aerobes only, 1% anaerobes only and 67% a mixture of aerobes and anaerobes. A total of 213 isolates were cultured representing a mean of 2 isolates per wound. Of the aerobe species cultured, 22%, 19% and 17% belonged to the genera of *Pasteurella*, *Streptococcus* and *Staphylococcus* respectively. The species of *Pasteurella multocida* (66%) and *Staphylococcus intermedius* (70%) were predominant. *Pasteurella canis* and pyogenic streptococci were common in infected wounds, whereas *Bacillus* spp., *Actinomyces* spp. and oral streptococci were usually found in contaminated wounds. Three anaerobic genera were cultured, namely, *Prevotella*, *Clostridium* and *Peptostreptococcus*, and were usually associated with wounds with dead space. This study also describes the first documented case of *Capnocytophaga canimorsus* in an infected dog bite wound.

Notably clinical and cytological assessment was capable of establishing whether antimicrobials were required or not. Although no single antimicrobials was considered to be effective against all the bacteria, amoxycillin plus clavulanic acid, 1st and 3rd generation cephalosporins, ampicillin or amoxycillin and potentiated sulphonamides gave the best *in vitro* sensitivity results.

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List of abbreviations

AGB: Anaerobic Glove Box

CBA: Citrated Horse Blood

CLSI: Clinical and Laboratory Standards Institute

º C: Degrees Celsius

DBW: Dog Bite Wounds

DVTD: Department of Veterinary Tropical Diseases

e.g. For example

MAC: MacConkey Agar Culture

n: Normal

OVAH: Onderstepoort Veterinary Academic Hospital

%: Percent

PBAB: Black-pigmented anaerobic bacilli

RSA: Republic of South Africa

SD: Standard Deviation

SEM: Standard Error of Mean

SOP: Standard Operating Procedures

SPCA: Society for the Prevention of Cruelty to Animals

sp (p): Species (plural)

subsp: Subspecies

USA: United States of America

Chapter 1. Literature review

Dog Bite Wounds are a common problem in human and veterinary medicine and may account for 1% of human emergency visits[4] and 10% of canine emergency admissions.[2] It is estimated that 1 to 2 million humans sustain significant animal bite wounds in the USA of which 80% to 90% are inflicted by dogs.[3,4] Based on the available veterinary literature, the bacteriology of DBW inflicted by other dogs is limited, with the majority of studies having focused on management alone.[2,3] In contrast, the complex polymicrobial environment of DBW in humans has been well documented, with the majority of reports having investigated infected wounds. Due to the paucity of animal studies, much of the data concerning their bacterial composition cited in the veterinary textbooks originates from the human literature.[11,12]

1.1 Dog bites inflicted on dogs:

1.1.1. Characteristics of DBW:

Dog bite wounds have several characteristics, which make them unique. The range of injury is almost limitless, from simple puncture wounds and lacerations to various combinations of crush and tear injuries. The large forces generated by the jaws and associated dentition results in deformation of tissues in the form of compression, stretching or shearing.[2] Because bite wounds penetrate through the elastic skin into the less elastic deeper tissues, relatively innocuous wounds often mask extensive, more serious injuries to underlying tissues: this is often referred to as the ñtip of the icebergò phenomenon.[17,18] Bite wounds are typically contaminated with the victimôs endogenous skin/hair bacteria, the attackerôs oral bacteria and soil organisms. The combination of devitalised tissue, ischaemia, serum accumulation and dead space provide the ideal climate in which inoculated bacteria can grow.[19,20]

In a number of studies, bite wounds were found to involve similar anatomical regions. The most common areas of injury, in order of occurrence, were the limbs, head and neck, followed by the thoracic, abdominal and perineal regions. However, a large proportion of cases had multiple wound sites.[11,18,21] In a canine study, Cowell et al investigated the factors related to the incidence of complications in the victims of dog bite wounds.[18] In contrast to a human study[4], treatment delay and wound age resulted in no statistically significant increase in complications. It should however be noted

that dogs sustaining severe injuries were generally presented immediately after trauma whereas those receiving less severe wounds often had treatment delays of more than 12 hours. Location of injury was a factor that was recognised to be related to complication rate, where wounds involving the head were less likely to develop complications than wounds to other regions.

1.1.2. The bacterial composition of DBW:

As little has been published on the bacterial composition of DBW in dogs, two common assumptions are made in the veterinary literature:

- The first is that the organisms cultured from dog bite wounds accurately reflect the oral microbes.
- The second is that *Pasteurella* spp. play a pivotal role in the contamination of these wounds. [23]

However, this is in contrast to two veterinary studies primarily associated with the bacteriology of canine bite wounds.

In a retrospective study, Kelly et al.[12] examined the culture results from swabs previously collected from bite wounds, adjacent normal skin and gingival mucosa in 37 untreated dog bite victims. The swabs were cultured for aerobic growth and antimicrobial susceptibility. In this study, 68% of cultures yielded bacterial growth. The most common bacteria isolated from wounds were *Staphylococcus intermedius* (23%), *Escherichia coli* (18%), nonï lactose fermenting coliforms (14%) and *Pseudomonas* spp. (14%). Other staphylococci, including *Staphylococcus aureus,* were uncommon in these bite wounds. An analysis of the organisms according to the site of the wound showed that wounds on the abdomen, pelvic limbs and tail were more likely to yield pathogens than wounds on the head, neck, thorax and thoracic limbs and this trend was reflected in the isolation of *S. intermedius*. The time from wounding to presentation varied greatly and due to the lack of facilities, anaerobic cultures were not performed.[12]

In a more recent study, Griffin et al. documented bite wounds in 37 dogs, which were prospectively evaluated.[11] The study was carried out at the University of Pennsylvaniaôs Veterinary Hospital. Information recorded for each animal victim included: breed, age, sex, weight, time between injury and presentation, location and number of wounds, wound classification, evidence of wound infection

and antibiotic therapy that was administered. The wound classification system was introduced in order to correlate wound severity with risk of infection and outcome. An infected wound was defined as any wound that showed a purulent discharge or abscess formation around the site of injury. Aerobic and anaerobic cultures were taken from each wound within an hour of presentation and again during surgery, after which antimicrobial susceptibilities were determined for each sample. Perioperative antibiotics were given intravenously after cultures had been collected. Of the 37 dogs evaluated 65% had positive aerobic cultures, 15% had positive anaerobic cultures and 33% had negative cultures. The most common aerobic isolates were *S. intermedius*, *Enterococcus* spp, *Staphylococcus* spp. and *E. coli*. The most common ôanaerobicôisolates noted by them were *Bacillus* spp, *Clostridium* spp. and *Corynebacterium* spp. Ninety-five percent of the cases presented within 12 hours of wounding, the remaining two animals were seen between 12 and 24 hours post-bite and the other was seen 6 days after having been bitten. The infection rate in this study was relatively low (8%) and although not statistically significant, appeared to be well correlated with more highly contaminated Class-4 wounds. *Acinetobacter* spp*., Enterococcus* spp., *Enterobacter* spp*., S. intermedius* and *E. coli* were isolated from the infected wounds.

Normal cutaneous and oropharyngeal flora of dogs and their role in contamination of DBW:

It is generally believed that dog bites result in the contamination of these wounds by either oral or adjacent skin microflora.[40] The knowledge of the normal bacterial flora-host relationships has formed an important basis to the understanding of bacterial skin disease and dog bite wound bacteria in human beings and dogs.[24] In a study on the occurrence of *Staphyloccocus aureus,* White et al. distinguished three main groups of residents; Gram-positive cocci, aerobic diptheroids and anaerobic diptheroids.[24] The genus *Staphylococcus* is distinguished into coagulase-positive *(S. intermedius, S. aureus* and *S. hyicus*) and coagulase-negative organisms on the basis of biotyping and DNA homology.[25,26] Prior to 1969 when Hajek and Marsalak first described *Staphylococcus intermedius* [27], all coagulase-positive staphylococci from dogs were called S. *aureus.*[27] Most studies have emphasised the more pathogenic coagulase-positive staphylococci [28], rather than coagulase-negative species, which are considered to be less virulent and only occasionally found in clinical lesions in pure culture.[29] The two veterinary studies which documented the bacterial contamination of bite wounds both had similar findings. *S. intermedius* was the most common isolate, being 23% and 12% of the isolates in the study of Kelly [12] and Griffin [11] respectively.

Staphylococcus intermedius is considered a normal bacterial inhabitant of the skin in dogs where it is both transient and resident. Although *S. aureus* may be isolated from up to 10% of canine pyoderma cases [30],*S. intermedius* is considered the principal canine cutaneous pathogen[31,32] requiring an alteration in surface homeostasis to multiply and result in pyoderma.[33] A variety of studies have attempted to define the distribution of *S. intermedius* on the skin.[34] There are two genetically undistinguishable populations of *S. intermedius* on dogs. Firstly, there is a population within the pilosebaceous units particularly at the oral, nasal and anal sites, which may be resident.[28,35,36] Secondly, a transient population on the distal hair shaft, which is thought to act as a filter or bacterial ñtrapò[36] Interestingly the large population of *S. intermedius* found on the abdominal hair is thought to be associated with environmental contamination, or seeding from the mucous membranes of the nose and anus during grooming.[35]

Improved sampling and anaerobic culture techniques have increased the isolation of obligate anaerobes from clinical specimens.[8,37-38] Anaerobes can be classified as obligate or facultative based on their utilisation of oxygen. Obligate anaerobic genera such as *Bacteroides, Fusobacterium,* and *Peptostreptococcus* do not utilise oxygen for metabolism, whereas facultative anaerobes such as *E. coli* and *Pasteurella multocida* can grow either aerobically or anaerobically. Many members of the genera: *Clostridium, Lactobacillus* and *Actinomyces*, although considered as obligate anaerobes, are oxygen-tolerant. In an infectious process, it is not unusual to find two different anaerobic species admixed with aerobic bacteria. The synergistic interaction which develops between these two microorganisms has been well described.[40] The organisms most often isolated in association with obligate anaerobes are the enteric bacteria (particularly *E. coli*), members of the genus *Pasteurella,* and coagulase-positive staphylococci.

The oropharyngeal microflora is the major source of obligate anaerobic bacteria in bite wounds.[37] Another potential source of these bacteria in bite wounds adjacent to the anus is possibly anaerobes originating from the intestinal tract. Anaerobes, which constitute up to 90% of the colonic flora, include: C*lostridium, Lactobacillus, Bifidobacterium,* and *Bacteroides* spp.[40] Healthy tissues are normally resistant to anaerobic infection through high redox potential and oxygen tension. In fact anaerobes are responsible for a significant portion of innate immunity, with metabolic by-products produced by this group of organisms playing an important role in the regulation of the number of aerobic species (facultative and obligate).[40] In bite wounds the oxygen supply to tissues is compromised by a number

of factors including: impaired blood supply; tissue necrosis and prior infection with oxygen utilising bacteria.[37] The formation of a pyonecrotic focus associated with a malodorous exudate, often described as being ñfruityò, may be strong indicators for an infectious process involving anaerobes.[38,40]

1.1.3. Contamination versus Infection in dog bite wounds:

Bite wounds are usually considered to be contaminated.[17] The most common definition of contamination versus infection in wounds is based on the replication status of existing bacteria. Wounds are considered contaminated if there has been inoculation with microorganisms without subsequent replication, whereas an infected wound is one in which the time elapsed since injury is sufficient for bacterial growth to occur.[19,62] Although a 6-12 hour period was seen as the necessary time for bacterial multiplication to occur, the other factors which may play a role in progression of contamination to infection include: blood supply to the tissue, amount and type of traumatized tissue, number and pathogen species of bacteria inoculated into the wound, amount and type of foreign material in the wound and patientôs age and immunocompetence.[21]

Based on organism quantization alone, the definition of infection in the available literature can be somewhat confusing. In one study[9], in which bacterial quantization was evaluated as a determinant in primary closure of wounds, wound infection was defined as a minimum of 10^5 organisms per gram of tissue.[59] The problem of separating contamination from infection is compounded by the lack of distinction between microscopic and clinical infection used in the available literature. To the best of the authorôs knowledge, bite wound studies in dogs have been primarily concerned with clinical wound infection as opposed to wound contamination. In two bite wound studies, wounds were considered infected if there was associated purulence in combination with positive cultures for bacteria.[11,18] In contrast to what has been reported in canine studies, one human study used strictly defined and prospectively applied criteria for wound infection in order to reduce selection bias. To be eligible for enrollment in this study, human patients had to meet one of the following three major criteria for infection of a bite wound: fever, abscess, or lymphangitis. Alternatively, four of the following five minor criteria must be met: wound-associated erythema, tenderness at the wound site, swelling at the site, purulent drainage, and leukocytosis.[8]

Cytological evidence of infection:

Microscopically, inflammatory conditions are classified according to the cell type that predominates. Lesions are called purulent or suppurative when more than 85% of leukocytes present are neutrophils.[64] These lesions can be further subdivided by classifying the neutrophils as non-degenerate or degenerate. Non-degenerate neutrophils are morphologically normal and predominate in relatively non-toxic and sterile environments.[64] The nuclei of non-degenerate neutrophils are characterised by intact membranes and densely aggregated chromatin.[63] Degenerate neutrophils however, predominate in bacterial infections, particularly Gram-negative types.[64] Cytological changes of degenerate neutrophils are nuclear swelling, loss of nuclear membrane and decreased staining intensity of the nucleus. These changes are termed ñkaryolysisò and are an indication of rapid cell death in a toxic environment.[63. 64] An end stage of cell death may be seen cytologically as the result of pyknosis of hypersegmented nuclei. This is termed karyorrhexis and may appear, cytologically, as dark, dense, round nuclear segments.[63,64] The cytoplasm of the neutrophils may also be vacuolated.[63] Infectious agents usually cause lesions characterized by the presence of inflammatory cells. Bacterial agents usually produce lesions that are composed of more than 85% neutrophils, many of which may be degenerate, and a few macrophages, lymphocytes, and plasma cells.[67] In the event of bacteria being found on a slide without an associated inflammatory response, the bacteria are contaminants. This is true if the bacteria are adherent to surrounding squamous epithelial cells, suggesting that they are commensals.[67] On the other hand, pathogenic bacteria are usually found phagocytosed within neutrophils, however they may also be present extracellularly.[67] The absence of bacteria microscopically does not necessarily imply that the lesion is not infected, especially when degenerate neutrophils are present. These lesions should be cultured to identify a covert infection.[67]

1.1.4. Antimicrobial susceptibility of bite wound contaminants:

A consistent recommendation for the selection of appropriate antimicrobial therapy varies greatly in the veterinary literature. In a veterinary study of 93 DBW, wound complications occurred in 20% (16 of 78) of the patients receiving antimicrobials and in 0% (0 of 7) of patients not receiving antimicrobials, which led the authors to conclude that wound complications may not necessarily be decreased by giving antimicrobials.[18] However, the study failed to mention the incidence of infection amongst complications. The findings of another study showed that for antimicrobials to be effective, they must

be given within three hours of contamination so as to reach therapeutic levels at the site of injury.[57] These factors, together with the general consensus that no one antimicrobial is effective against all bacteria in contaminated dog bite wounds[6,11,12,58], make the correct empirical selection of a single antimicrobial agent very difficult. In a dog bite wound study cited by Griffin and Holt, over 50% of bacteria cultured had similar susceptibilities.[60] Their recommendations for dogs requiring parenteral medication included, either penicillin combined with an aminoglycoside or a second-generation cephalosporin combined with a fluoroquinolone. However, the latter combination was thought to be ineffective against *Enterococcus* spp., which are generally sensitive to ampicillin, clavulanic acid and amoxycillin, ticarcillin and clavulanate or vancomycin. Aminoglycosides, erythromycin, clindamycin and the first generation cephalosporins (cephalexin, cephalothin) are generally seen as an inappropriate choice for empiric therapy due to their poor coverage of *P. multocida* .[6,59]

Anaerobes are potentially important pathogens of bite wounds, and infectious complication rates in dogs and cats were shown to be higher when initial treatment did not include an antimicrobial effective against them. [37] Despite the importance of anaerobes, antimicrobial therapy for bite wounds should also be directed at aerobes to prevent an imbalance from their persistence or proliferation.[40,61] In one report five types of antimicrobials were considered routinely effective against obligate anaerobes: penicillins, chloramphenicol, clindamycin, metronidazole, and some cephalosporins.[37] In contrast, Jang et al.[61] found that all anaerobic bacterial isolates were susceptible to amoxycillin-clavulanic acid, chloramphenicol and metronidazole. Only 71% of the *Bacteroides* spp. isolates were susceptible to ampicillin, and only 83% were susceptible to clindamycin. Isolation of *Bacteroides*, particularly *B. fragilis* is considered significant in view of the apparent increasing prevalence of its resistance to penicillins and first generation cephalosporins[61]. There are certain antimicrobials, such as the aminoglycosides and quinolones, to which obligate anaerobes are inherently resistant. Other antimicrobials such as trimethoprim-sulfonamide combinations and tetracyclines have been found to have unpredictable efficacy *in vivo*.[61] However, metronidazole, a relatively inexpensive antimicrobial has been found to have consistent antibacterial activity against most clinically important anaerobes, including B. *fragilis*. First generation and most second-generation cephalosporins have been shown to have poor efficacy against *Bacteroides*. An exception is cefoxitin, a second-generation cephalosporin, which is effective against most obligate anaerobes and many of the facultatively anaerobic Enterobacteriaceae family.[37] Although Kelly et al.[12] showed that chloramphenicol was effective against most isolates, its widespread use is discouraged because of the danger of the emergence and spread

of resistance plasmids to serious human pathogens. Another concern is the ability for chloramphenicol to produce idiosyncratic aplastic anaemia in people who inadvertently take in the drug when, for example, they administer it to their pets.

1.2 Dog bites inflicted on humans:

1.2.1. Characteristics of DBW:

The characteristics of dog bite related injuries have been well documented. Approximately 75% of people suffering from dog bites are less than 21 years of age, with many being less than 10 years old. Most bite wounds are to the arms and hands, however in children less than 10 years of age, 65% involve the face.[40,41] Most people are bitten by dogs within the household or by dogs belonging to a neighbour.[42] Reproductively intact, male, medium-sized or large dogs are responsible for most bites requiring medical therapy.[40] Most bite wounds are minor, although, at least 10% require suturing and between 1% and 5% of patients are hospitalized.[43]

1.2.2. Bacterial composition of DBW:

Numerous studies have examined the bacterial cultures of animal bite wounds affecting people, [15,8,9] with over 50 species of bacteria having been isolated.[16] Bite wounds are contaminated with aerobic and anaerobic bacteria, both of which are capable of causing infection and can also act synergistically.[6,43,45] Early studies on the bacteriology of animal bite wounds made little distinction between the bacteriology of non-infected and infected bite wounds. In one study where 33 of 39 patients cultured positive for aerobic bacteria, the most frequent isolates were alpha-haemolytic (oral) streptococci. In this study *Staphylococcus aureus* was isolated from 18 wounds.[44] In a later prospective study 41% and 74% of bite wounds contained anaerobic and aerobic isolates respectively. The most common aerobic pathogens included alpha-haemolytic streptococci, *S. aureus* and *P. multocida*. Anaerobes isolated included *Bacteroides* spp. and *Fusobacterium* spp.[41]

It has been shown that although 80% of DBW inflicted on people culture positive for bacteria, only 3% to 20% will become infected.[1,6] In a review of 10 bite wound studies, despite the broad diversity of bacteria isolated, only a few organisms accounted for those wounds which became infected.[7] These

infected wounds have been shown to have less bacterial diversity with greater numbers when compared with non-infected wounds.[6,8]

The role that *Pasteurella* species appears to play in infected human bite wounds has been highlighted. In a study by Talan et al.[8] data supports its reputation for pathogenicity and its association with a rapid onset of clinical signs.[8,46] *Pasteurella* species inhabits the nasal, gingival and tonsillar regions of between 12% to 92% of dogs.[47] Although it is commonly found in the saliva of dogs, the risk of infection in people is low in the absence of bite wounds.[48] The frequency of isolation in dog bite wounds is as follows: *P. canis*, 27%, *P. multocida* subsp. *multocida*, 13% and *P. multocida* subsp. *septica* 13%.[49] Most human infections with *P. multocida* result from direct inoculation of the organism into a bite wound. Bite wound infections associated *with P. multocida* may result in cellulitis, erythema, pain and swelling, which usually develops within 2 days of injury.[50] Systemic illnesses associated with *P. multocida* infections are normally found to affect people who have underlying disease processes or are immunocompromised.

Another organism which can cause potentially fatal septicaemias, particularly in immunocompromised individuals older than 40 years, is *Capnocytophaga canimorsus,* a filamentous, Gram-negative, facultative anaerobe that has been isolated from the oral cavity of 16% of clinically healthy dogs.[51]

The majority of bite wounds cultured in humans contain a mixture of aerobes and anaerobes which are thought to reflect the diverse oral flora of the biting animal and to a lesser extent the victimôs skin.[9,10] The oral cavity of dogs has been shown to contain more than 64 species of bacteria.[4,45] The bacterial oral environment is varied, being both aerobic and anaerobic. Some of these bacteria may be difficult to culture and therefore identify. In one study on the dental plaque flora of the dog, 47% of canine isolates could not be fully identified to species level. This is a reflection of the complexity of oral flora and subtle differences between bacterial species. The same study showed that most subgingival bacteria were aerobic, Gram-positive or anaerobic, Gram-negative bacteria.[16] Although most of the common isolates from animal bites contain aerobic organisms, approximately one third will contain anaerobes[53,54] and are often associated with abscess formation or potentially serious infection.[16] More recent studies have isolated anaerobic bacterial populations, which often included mixed cultures of *Bacteroides* spp, *Prevotella* spp. and other anaerobic Gram-negative bacilli.[55] Allaker and colleagues isolated black-pigmented anaerobic bacilli (BPABôs) that consisted of *Porphyromonas* and *Prevotella*

spp. from dental plaque flora of 91% of dogs. The authors suggested that BPABs as well as *Eikenella corrodens* might constitute a significant risk with respect to bite wound infections, both having been underestimated in previous reports.[16]

1.2.3. Antimicrobial susceptibility of bite wound contaminants:

Whilst some studies on humans promote the administration of antimicrobials for all penetrating bite wound injuries[40], others question their use in the majority of cases, rather advocating liberal irrigation and surgical debridement.[56] The findings of one study on humans suggested treatment according to the risk weighting of individual wounds.[50] Risk factors have yet to be identified for veterinary patients, however in bitten humans, factors include the presence of punctures, especially fang wounds; wounds associated with the hand or foot; cat bites; delay in treatment longer than 12 hours and immunocompromised patients.

Historically, people with infected bite wounds received penicillin as initial parenteral treatment. The limitations of penicillin against *β*-lactamase producing *Staphylococcus* or Gram-negative enteric bacteria has been recognized, with the suggestions for the combined use of clavulanic acid and amoxicillin.[6,45,59] In a human study, the majority of dog bite wound isolates were susceptible to ß-lactam antibiotics and a ß-lactamase inhibitor.[56,59] A recent bite wound study on humans recommended that empirical therapy include a combination of a ß-lactam antibiotic and a ß-lactamase inhibitor, a second-generation cephalosporin with anaerobic activity, or combination therapy with either penicillin and a first-generation cephalosporin or clindamycin and a fluoroquinolone.[8]

Chapter 2. Objectives

2.1 Background and motivation

Despite the common occurrence of DBW seen in veterinary practices all over the world, there remains little available information on the subject. This is in contrast to DBW affecting people in which their bacteriology has been well described. In an attempt to contribute to the little that is known, this study sets out to form a platform of data. With the accumulative knowledge gained from further research in the field, our basic understanding of ñtypicalò bacteriological populations and antimicrobial susceptibility becomes possible. This may allow for the early recognition of ñatypicalò populations in cases that are refractory to treatment or where resistance is suspected. Furthermore, the early recognition of infected wounds by means of a practical tool may facilitate decision making, management and the judicious use of antimicrobials in DBW studies.

2.2 Problem statement

The lack of research into the bacterial composition and antimicrobial susceptibility of the bacteria within DBW has meant that this field is poorly understood. With the increased prevalence of resistance to bacteria in our patients and super-infections apparent in the hospital environment, the need for a greater understanding of these bacterial populations and the judicious use of antimicrobials has become a priority.

2.3 Research problems

- The bacterial composition of canine bite wounds presenting at the OVAH has never been investigated.
- The past veterinary studies have not clearly shown the prevalence of true obligate anaerobes in dog bite wounds (DBW).
- The relationship of anaerobes to bite wound location, time after injury and bite wound grade have yet to be elucidated.
- The source of bacteria (dermal, mucosal, and environmental) in DBW has not been fully investigated.

- The antimicrobial susceptibility of bacteria originating from DBW should be monitored on an ongoing basis to detect potential changes in the development of antimicrobial resistance.

2.4 Research questions

- What percentage of DBW has positive culture results at presentation?
- What is the relative proportion of contaminated and infected dog bite wounds that have positive culture results at presentation?
- What species of bacteria are found in DBW at presentation (aerobic and anaerobic)?
- What are the differences in species found in contaminated versus infected bite wounds at presentation (aerobic and anaerobic)?
- What is the association between bite wound location and these bacterial populations?
- Is there an association between the presence of anaerobes and the grade of severity of bite wounds?
- What are the antimicrobial susceptibility patterns of bacteria in DBW?

2.5 Hypothesis

- On presentation, 68% to 70% of DBW will have positive culture results.
- A greater proportion of infected bite wounds will have positive culture results compared with contaminated wounds at presentation.
- A significant proportion of bacteria found in DBW at presentation are aerobic in contrast to anaerobic isolates or negative culture results.
- A greater variety of bacterial species will be found in contaminated DBW compared to infected wounds at presentation.
- The average number of species isolated from the caudal area of the body (abdomen and pelvic limbs) will be greater than the average number of species isolated from the cranial area (head, neck, chest and thoracic limbs).
- Anaerobes will be more commonly found in Grade 2 and Grade 4 bite wounds (puncture wounds).
- The antimicrobial susceptibility pattern will be that normally associated with the bacterial species isolated from lesions, the skin and oral cavity of dogs admitted to the OVAH.

2.6 Benefits

Benefits of the present study are:

- To supplement the limited published veterinary data on the bacterial composition of DBW.
- To determine the prevalence of bacteria in infected and non-infected bite wounds at the OVAH in order to formulate a platform for examining existing and future bite wound therapies?
- To determine the antimicrobial susceptibility of the bacteria in DBW in order to assess present antimicrobial protocols.
- To provide a practical means of determining if DBW are infected.
- To supplement current knowledge on the site of origin of bacteria in bite wounds.

2.7 Objectives

This study aimed to document the complex microbiological population, their origins and antimicrobial susceptibilities as found in non-infected and infected DBW in which specific criteria were used to define infection.

Chapter 3. Materials and Methods

3.1. Model system

This study was approved by the University of Pretoriaôs Animal Use and Care Committee and the Research Committee (V046/05). This project was a prospective, cross sectional, descriptive study involving clinical cases. Owner consent was obtained prior to inclusion in the trial, after which every dog was treated within guidelines of standard operating procedures (SOP) for DBW.

3.2. Experimental design

3.2.1. Patient Selection

Forty-seven dogs admitted to the Outpatients clinic of the Onderstepoort Veterinary Academic Hospital and three dogs presented to the Society for the Prevention of Cruelty to Animals (SPCA) between August 2005 and May 2006 for the treatment of DBW were prospectively included in the study. A maximum of three samples were taken from separate bite wound locations on each dog. The following selection criteria applied:

Inclusion criteria:

- Dogs of any age, weight, breed or sex.
- Dogs with one or more cutaneous wounds caused by a dog bite.
- Wound types include full thickness puncture wounds, lacerations or both.
- Dogs that have sustained bite wound injuries within 72 hours of admission.

Exclusion criteria:

- Treatment with antibacterial agents or glucocorticoids within 72 hours prior to presentation.
- Cases with severe life-threatening bite wounds.
- Dogs with pre-existing skin or bone infections.
- Dogs with bite wounds sustained longer than 72 hours prior to admission.
- Samples that cultured positive for *Proteus* spp.

The rationale behind incorporating inclusion and exclusion criteria in research is to limit variability and bias from the study. The exclusion of glucocorticoids and antimicrobials ensure that the patientsô

immune systems and the natural ability to counteract infection are not externally influenced. Through the inclusion of healthy animals, free from any local or systemic infections such as pre-existing open skin or musculoskeletal infections, culture results should provide a better reflection of the true source of bacteria. A 72-hour inclusion/exclusion time frame is estimated to be a reasonable time to allow for wounds to be become infected. It also provides enough time to allow for wounds to become contaminated with commensal and environmental flora, thus mimicking the factors which normally play a role in bite wound events. A limit of 72 hours was set to ensure that wounds have not had the opportunity to ñself-cleanò through the formation of granulation tissue. It also creates a relatively narrow sample group. Due to its ability to overgrow other bacteria, any samples which cultured swarming *Proteus* spp, would be excluded.

3.2.2. Observations

Patient bite wound parameters were recorded for each animal, and are described in the Appendices (Figures A-1, A-2).

3.3. Experimental procedures

The study was divided into three parts: A component where dogs were clinically assessed and wounds characterised according to their nature, severity and location; a wound cytology component; and a wound culture and antimicrobial susceptibility component. A sub-component of this part of the study included the sampling of healthy skin and oral microflora in the last 15 bite wound cases.

3.3.1. Wound characterisation study

Wounds were classified according to grade of severity. Grade 1 and 2 wounds included full thickness skin lacerations and puncture wounds respectively. Grade 3 wounds were those with full skin-thickness lacerations and dead space present and Grade 4 wounds were puncture wounds with dead space present. Lacerations were defined as irregular edged wounds in which the length was greater than 10mm. Puncture wounds were wounds less than 10mm in length. This wound classification system is an adaptation of that used by Griffin and Holt[11], where, in the current study partial thickness

lacerations of the skin were omitted since the majority of cases requiring veterinary intervention as seen at the OVAH are those of a more severe nature.

3.3.2. Wound infection study

Each dog was evaluated clinically with special attention paid to its rectal temperature, pulse, respiratory rates, capillary refill time and wound discharge characteristics (see **Observations** in **Material and Methods**). All wounds were then individually assessed and accorded an infection status and wound grade.

ñInfectedòwounds were considered to have met **two of three** criteria:

1) Patients were pyrexic (rectal temperature of more than 39.7°C).

2) Wounds had a purulent discharge.

3) Cytological indicators of wound infection were present. [64]

For the purposes of this study, the cytological indicators of wound **infection** included either degenerate neutrophils alone or neutrophils with phagocytosed bacteria, often in addition to extracellular bacteria.[64,67] If bacteria were found on a slide without an associated inflammatory response, the bacteria were considered as **contaminants**. This was particularly true if the bacteria were adherent to surrounding squamous epithelial cells.[67] Since contaminated wounds may also yield bacterial growth, positive culture results alone was not considered to be criterion for infection in this study.

3.3.2.1. Sampling, transport and handling of cytology samples

Specimens for cytological evaluation were then taken from the same wounds used for culture using sterile cotton-tipped applicators and then gently rolled onto glass slides. Once air-dried, each slide was secured between two layers of cardboard and submitted to the Clinical Pathology Laboratory of the Faculty of Veterinary Science, University of Pretoria.

3.3.2.2. Microscopic assessment (wound cytology)

The glass slides were stained according to standard protocol with a Cams quick stain (Kyro-Quick stain, Kyron Laboratories). Each glass slide was examined under low (10x) and high (50 ï 100x) power magnification for evidence of contamination or infection. On the basis of cytology, wounds were considered infected if more than 85% of leukocytes present were degenerate neutrophils alone or degenerate neutrophils in the presence of bacteria.

3.3.3. Culture and antimicrobial susceptibility of wounds and selected healthy tissues

3.3.3.1. Specimen sampling**:**

Culture specimens were collected within 1 hour of presentation. The area of skin around each wound was clipped of hair using a no. 40 clipper blade (OsterÊ) and carefully cleansed using 70% ethanol swabs. Although it is acknowledged that 70% ethanol is not sporicidal, it does have wide bacteriocidal and fungicidal activity and has the advantage that it is highly volatile with no residual effect allowing sampling to be done without the concern that the disinfectant would contaminate the sample. Small cotton-tipped swabs (LabChem, South Africa) were used for sampling deep within puncture wounds and from deep pockets within lacerations. In order to prevent contamination from the wound edges, swabs were taken from between tissue planes spread open by the jaws of sterile curved mosquito forceps. They were then placed in 10 ml glass sample bottles containing a deep column of brain-heart infusion broth (Difco Laboratories, USA) supplemented with 0,2% cysteine and 1% agar (anaerobic transport medium) to make a semi-solid agar.

Since an objective of the study was to discover whether the bacteria in wounds originated from the skin or oral cavity, it was decided to swab these areas from the bitten dogs in the last 15 bite-wound cases. Areas included any unaffected skin close to a wound, but not disinfected, and the gingival margin adjacent to the upper premolar teeth.

3.3.3.2. Transport and handling of microbiological samples

All the specimens were labelled, packaged and either submitted directly to the Tropical Diseases Bacteriology Laboratory of the Faculty of Veterinary Science, or immediately stored in a refrigerator. The maximum time from sampling to culture was 12 hours.

3.3.3.3. Bacterial isolation and identification

A maximum of 3 swabs were taken from the bite wounds of 50 dogs resulting in 104 separate samples. Bacteriology was performed according to the standard operating procedures of the laboratory.[65] On receipt each specimen was immediately registered and processed as follows: One 90mm diameter, Columbia agar plate (Oxoid Products, UK) containing 7% citrated horse blood (CBA) and one 90 mm diameter, MacConkey agar plate (MAC) (Oxoid Products, UK) are labelled. The specimen was placed in the anaerobic glove box (AGB) (Bactron Anaerobic, Sheldon manufacturing, Oregon, USA) and streaked onto one labelled pre-reduced CBA that was stored in the (AGB) for at least 24 hours. This plate was then incubated at 37°C for 48 to 96 hours in the incubator section of the AGB. The specimen was removed from the AGB and streaked onto the CBA and MAC and the CBA incubated in 5 ï 10% CO_2 in air and the MAC incubated in air at 37°C for 24 to 96 hours. The specimen was then replaced into the transport medium, which acted as an enrichment culture medium for both aerobes and anaerobes, and incubated under the same conditions as the MAC plates for up to 7 days. The incubated specimens were only plated and incubated, as previously described, when no growth was obtained from the original plating after 72 hours of incubation and there was an increase in the opacity in the transport medium. This was done to ensure that all viable bacteria were detected.

The plates were checked daily for up to 4 days for the presence of bacterial colonies. A representative of each colony type was sub-cultured under the same conditions as its parent plate. Anaerobically-grown colonies that had been isolated from the CBA were also sub-cultured under aerobic conditions, to test whether they were true obligate anaerobes. Once isolated on subculture each organism was either identified to genus or species level. Potential pathogens were identified to species level and those not known to cause disease to genus level.

In order to determine the relevance of each isolate, semi-quantitative analysis was recorded by means of a scoring system (Figure 1).

Figure 1. Scoring System for semi-quantitative bacterial counts (SABS).

Number of colonies	Scoring
No growth	**0**
1-15	**1**
16-50	**2**
51-75	**3**
Too numerous to count	**4**

Phenotypic identification followed the standard operating procedures (SOP) of the laboratory.

In brief they were the following. [65]

- All isolates: Gram's stain, catalase, oxidase, glucose fermentation and spot indole tests and motility, aerobic preference and gelatinase production using thiogel (a mixture of thioglycollate broth and gelatine).
- Aerobic, Gram-positive, non-motile, catalase-positive cocci: DNase with mannitol, purple agar with maltose and polymixin B susceptibility. Extra biochemical tests were used for those bacteria that did not fully identify with these tests.
- Aerobic, Gram-positive, non-motile, catalase-negative cocci: Lancefield (cell wall antigens) grouping (Oxoid Ltd, UK) and if where necessary, additional sugars, 6.5% salt tolerance and aesculin positivity.
- Aerobic, Gram-negative rods that grew on MAC and were oxidase negative and catalase-positive: API10S (Merieux, France) and if where necessary additional sugars.
- Aerobic, Gram-negative rods, that were non-motile, oxidase-positive, nitrate reduction positive and glucose fermenters: *Pasteurella* biochemical test panel (in-house).
- Aerobic, motile, Gram-negative rods that were oxidase- and catalase-positive and glucose fermenters: *Aeromonas/Vibrio* test panel (in-house)
- Aerobic, non-glucose fermenting rods or cocci were placed on the *Pseudomonas* pane test panel (in-house). Additional tests were used as deemed necessary.

- Obligate anaerobic bacteria that stained Gram-negative were identified by the Maststring anaerobic antibiotic susceptibility test (Difco laboratories, USA) and, if necessary, extra tests were done.
- Obligate anaerobic, squat, Gram-positive rods that had the morphology of clostridia were placed on lactose-egg-yolk-milk agar (made up in-house) and further identified using sugar fermentation tests.
- Other obligate Gram-positive bacteria were identified using the API 20A (Merieux, France)
- These ñongò Gram-negative rods were fermentative, catalase and oxidase-positive, ONPG-positive and fermented glucose, lactose, galactose, maltose, mannose, sucrose and D-xylose.[74]
- *Capnocytophaga canimorsus* were identified as fine, non-haemolytic, yellow colonies that grew only on blood agar in an enriched carbon dioxide atmosphere.
- For the purposes of this study aerobes and facultative anaerobes were grouped together as aerobes.
- Only obligate anaerobes were considered to be true anaerobes

3.3.3.4. Susceptibility of isolates to antimicrobials**:**

Antimicrobial susceptibility tests were done on pure, 1-day-old cultures of all the aerobic bacteria, except *Bacillus* spp., using the Kirby-Bauer disk diffusion test and Clinical and Laboratory Standards Institute (CLSI formerly NCCLS) interpretate values.[66] The following antimicrobials were tested: ampicillin, amoxycillin + clavulanic acid, penicillin G, cloxacillin, cephalexin, enrofloxacin, orbifloxacin, doxycycline, a combination of sulphamethoxazole and trimethoprim, gentamicin, amikacin, kanamycin, lincomycin, lincospectin (lincomycin and spectinomycin) and tylosin.

3.3.4. Patient and wound management

The patients were treated according to the recommended guidelines used by the Section of Small Animal Surgery which included wound debridement, lavage using sterile Ringerôs lactate under pressure . The type of antimicrobial used was at the discretion of the attendant clinician, however most commonly included amoxycillin alone or in combination with clavulanic acid. Administration of antimicrobials only occurred after wounds had been cultured.

3.4. Analytical procedures

All data was entered onto a spreadsheet.[a] Results were depicted as proportions, median and range. Two by two tables were constructed. The Fisher's exact test was used to compare proportions between the following dichotomous outcome variables: ñinfectedò and ñnon-infectedò; wound age ñmore than 24 hoursò and ñ24 hours or lessò; ñculture positiveò and ñculture negativeò as well as ñmore severeò (grades 3 and 4) and ñless severeò wound grade (grade 1 and 2). A Chi squared test was used to determine a correlation between the numbers of isolates and species cultured from infected and non-infected wounds. Statistical analysis was performed using commercial software[b]. A p value of < 0.05 was considered statistically significant.

[a] Excel, Microsoft Corporation, Seattle, USA
[b] R (Development, 2005), R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0.URL http://www.R-project.org

Chapter 4. Results

4.1. Victim and wound characteristics

Fifty dogs were included in the study. Ages ranged from 6 months to 16 years (median 4 years). Weights ranged from 1 to 50 kg (median 9kg). Dog breeds varied from Jack Russell Terriers 16 (32%), Dachshunds 7 (14%), Bull Terriers 3 (6%), cross breeds 10 (20%) and other varied breeds 2 (28%). Of the 50 dogs, 20 (40%) were intact males, 10 (20%) castrated males, 4 (8%) intact females and 16 (32%) sterilized females. The median time from being bitten to sampling was 3.5 hours (range 40 minutes to 72 hours). Thirty-nine of 50 patients (78%) presented within 24 hours after injury and 11 patients presented (22%) between 24 to 72 hours after injury.

One hundred and four wounds were cultured from 50 patients (median number of wounds per patient, 2). Twelve dogs (24%) had one wound, 22 (44%) had two wounds and 16 (32%) had three or more wounds (range 1 - 6). Seventy-eight of 104 (75%) of the bite wounds cultured were on the cranial half of the body (head; neck; chest and thoracic limbs) and 26 (25%) were on the caudal half (abdomen; pelvic limbs and tail) of the body.

Using the previously described wound classification system to quantify severity or all the wounds, 15 (14%) were classified as Grade 1, 29 (28%) as Grade 2, 27 (26%) as Grade 3 and 33 (32%) as Grade 4.

4.2. Wound infection status

4.2.1. Criteria for infection

Results of non-infected and infected wounds were considered separately. The complete data set of results is in the annexure, Table A-1.

The combination of pyrexia and purulence was only recorded in 2 of 21 cases. The remaining cases were a combination of pyrexia and cytology; purulence and cytology or all 3 criteria; pyrexia, purulence and cytology. See the annexure, Table A-2.

4.2.2. Non-infected wounds:

Eighty three wounds were found to be non-infected, of which 66 had positive culture results. Median time between being bitten to sampling was 2 hours (range 40 minutes to 24 hours). Thirty-four of 39 patients (87%) presented within 24 hours after injury and 2 of 11 patients presented (18%) between 24 to 72 hours after injury. By wound classification, 13 (16%) were Grade 1, 26 (31%) as Grade 2, 16 (19%) as Grade 3 and 28 (34%) as Grade 4.

4.2.3. Infected wounds:

Using the criteria for infection in this study, 14 animals (28%) had one or more infected wounds (range 1-3) resulting in a total of 21 (20%) infected wounds. Positive cultures were obtained from all 21 of the wounds that were judged to be infected. Thus compared to non-infected wounds, infected wounds were significantly more likely to culture positive (Fisher's exact test: p = 0.021, Table 1). The median time from being bitten to sampling was 72 hours (range 12 hours to 72 hours). Most infected wounds presented close to the upper limit of 72 hours after having been bitten. Significantly more bites presenting greater than 24 hours after the event were infected, as compared to those presenting within 24 hours or less (9 of 11 versus 5 of 39; Fischerôs exact test p=0.004).

Table 1. Number of samples that cultured positive and negative from infected and non-infected wounds.

	Infected	Non-infected
Positive culture	21	66
Negative culture	0	17

(Fisher's exact test: p = 0.021).

A comparison was made between the severity of wound grade and the infected status of each wound. Of the 21 infected bite wounds, 16 (76%) were associated with Grade 3 and 4 severities, whilst 5 (24%) were associated with Grade 1 and 2 severities. Despite this higher proportion, more severe bite wounds (Grades 3 and 4), although showing a tendency toward significance, were statistically not more likely to be classed as infected than less severe (Grades 1 and 2) bite wounds (Fisher's exact test: p = 0.082, Table 2), nor were they more likely to culture positive (Fisher's exact test: p = 0.18, Table 3).

Table 2. Number of infected and non-infected wounds from wounds containing dead space (3 and 4) versus those not containing dead space.

	Grades 1 and 2	Grades 3 and 4
Infected	5	16
Not-infected	39	44

(Fisher's exact test: p = 0.082).

Table 3. Number of samples that cultured positive and negative from wounds containing dead space (3 and 4) versus those not containing dead space.

	Grades 1 and 2	Grades 3 and 4
Negative	10	7
Positive	34	53

(Fisher's exact test: p = 0.18).

Using a 1 to 4 scoring system, the numbers of each species in each wound was semi-quantified (Figure 1). Of the 66 contaminated wounds only 58 were scored and of the 21 infected wounds, only 19 were scored. For each wound an average score was then calculated. The average wound score for the contaminated wounds was 2.2 and for the infected wounds it was 3.2. The difference was considered to be statistically different (Student t-test, p=0.0003, Table 4).

Table 4. The numbers of wounds that were infected and non-infected and the number of isolates from these two types of wounds.

	Infected	Non-infected
Mean	3.237	2.298
SD	0.878	0.944
SEM	0.201	0.124
N	19	58

(Student t-test, p=0.0003).

Therefore there were significantly more bacterial colonies obtained from infected wounds than non-infected wounds. Similarly there were significantly more bacterial species isolated from infected wounds than non-infected wounds ($chi^2 = 5.218$, $p = 0.022$, Table 5).

Table 5. The numbers of wounds that were infected and non-infected and the number of species isolated from these two types of wounds.

	Infected	Non-infected
Wounds	21	83
Number of species	20	43
Number of isolates	55	156

($\chi^2 = 5.218604$, $p = 0.022$).

Of the 17 samples which cultured positive for anaerobes, 12 (71%) were from infected bite wounds of Grade 3 or 4 severities. The more severe grades of wound (3 and 4) were thus more likely to be infected by anaerobes than wounds of Grades 1 and 2 (Fisherôs exact test, $p = 0.017$ Table 6).

Table 6. Number of anaerobes from infected and non-infected wounds containing dead space (3 and 4) versus those not containing dead space.

	Grades 1 and 2	Grades 3 and 4
Infected	1	12
Not-infected	39	44

(Fisher's exact test: $p = 0.017$).

4.3. Wound culture and antimicrobial susceptibility study

Of the 104 bite wound samples used in the study, 87 (84%) wounds cultured positive giving a total yield of 213 isolates (mean of 2 isolates per bite wound). An isolate represented a distinct colony type or bacterial species per wound, not the total number of colonies, which were semi-quantified using the scoring system (Figure 1). A mixture of aerobes and anaerobes were cultured from 16% (17/104) of the wounds, aerobes alone from 67% (69/104) of the wounds, anaerobes alone from 1% (1/104); 16% (17/104) of cultures had no growth. The distribution of Gram-positive to Gram-negative bacteria was 112 (53%) to 101(47%) respectively. Statistically this proportion was not different (Fisher's exact test, p= 0.30) and thus wounds were as likely to culture Gram-positive as Gram-negative bacteria. A summary of all ungrouped aerobic and anaerobic culture results corresponding with the individual patient and their wounds is listed in the Appendix (Table A-1).

All aerobic bacteria isolated from the wounds are listed in Table 7. In order to highlight the importance of potentially pathogenic bacteria, and for the sake of clarity, some species were considered apart whilst others were grouped together. For example the Gram-positive rods, *Bacillus*, *Actinomyces* and *Corynebacterium* spp. were common genera that were isolated and thus given their own place in Table 7. Other Gram positive bacteria that were grouped together consisted of *Lactobacillus* spp., *Enterococcus faecalis* and *Micrococcus* spp. All anaerobic are listed in Table 8.

4.3.1. Non-infected culture results:

A mixture of aerobes and anaerobes was cultured from 6% (5/83) of the wounds, while aerobes alone were cultured from 72% (60/83) and anaerobes alone from 1% (1/83); while 21% (17/83) of cultures had no growth. The results are presented in Table 7. Coagulase-negative staphylococci were considered separately from the pyogenic *Staphylococcus intermedius* which is an important cause of infection in dogs. Similarly the predominantly oral streptococci were dealt with separately from *Streptococcus canis,* another pyogenic streptococcus associated with infections. Among the Gram negative bacteria the Enterobacteriaceae, including *Escherichia coli*, *Klebsiella* species, *Proteus* spp., *Yersinia enterocolitica* and *Citrobacter* spp. were grouped together. The salt-intolerant members of *Vibrio* species, *Aeromonas* spp. and *Plesiomonas shigelloides,* were included in the Vibrionaceae. Pasteurellaceae were recorded separately to *Pasteurella multocida* and *Pasteurella canis* which are common causes of wound infection.

Table 7. Aerobic bacterial isolates from 50 cases of dog bite wounds (n = 213)*.

	Non-infected		Infected		**Total isolates (%)**
	Isolates	Species	Isolates	Species	
Gram positive					
Coagulase negative *Staphylococcus* spp§.	8	2	3	2	11 (5%)
Staphylococcus intermedius	21	1	5	1	26 (12%)
Commensal *Streptococcus* spp. (mainly oral streptococci)	11	3	2	1	13 (6%)
Streptococcus canis	7	1	8	1	15 (7%)
Pyogenic streptococci (excluding *S. canis*)	4	4	9	3	13 (6%)
Bacillus spp.	13	3	0	0	13 (6%)
Actinomyces spp.	7	4	0	0	7 (3%)
Corynebacterium spp.	7	4	2	1	9 (4%)
Other Gram positive bacteria	3	2	2	1	5 (2%)
Total species		**24**		**10**	**112 (53%)**
Gram negative					
Pasteurellaceae (excluding *P. multocida* and *P. canis*)	4	3	1	1	5 (2%)
Pasteurella multocida	26	1	5	1	31 (15%)
Pasteurella canis	4	1	7	1	11(5%)
Enterobacteriaceae	11	5	4	2	15(7%)
Vibrionaceae	2	2	2	2	4 (2%)
Non-glucose fermenters	31	7	4	3	35 (16%)
Total species		**19**		**10**	**101 (47%)**
Total species		**43**		**20**	
Total isolates (%)					**213 (100%)**

* Each isolate represents a distinct colony type or bacterial species isolated per wound.

§ Since there was a large number of species cultured and only known pathogenic bacteria were identified to species level, it was decided to group them in such a way as to highlight the known pathogenic and common bacteria.

4.3.2. Infected culture results:

A mixture of aerobes and anaerobes was cultured from 57% (12/21) of the infected wounds, while aerobes alone were cultured from 43% (9/21). The aerobic bacteria isolated from infected wounds are listed in Table 7. The most common Gram positive bacteria were the pyogenic streptococci, *Streptococcus canis* and *Staphylococcus intermedius*. *Staphylococcus aureus* was not cultured. The most common Gram-negative bacteria were *Pasteuralla canis* and *Pasteurella multocida*, followed by the Enterobacteriaceae and the non-glucose fermenters. In this study the Gram-negative, non-glucose fermenters consisted mainly of oral microflora such as: *Acinetobacter* spp.; *Moraxella* spp.; *Burkholderia* spp.; *Flavobacterium* spp.; and *Pseudomonas* spp. and were thus grouped together. Included in this group was a single isolate of *Capnocytophaga carnimorsus*. Anaerobic isolates included *Prevotella melaninogenica* (59%) and *Clostridium* spp. (18%) (Table 8).

Table 8. Anaerobic bacteria cultured from 50 cases of bite wounds (n = 17).

	Non-infected wound Isolates	Infected wound Isolates	Total isolates (%)
Prevotella melaninogenica	0	10	10 (59%)
Clostridium spp. (Not *C. perfringens*)	2	2	4 (23%)
C. perfringens	1	1	2 (12%)
Peptostreptococcus spp.	1	0	1 (6%)
Total:	4	13	17 (100%)

4.3.3. Oral and normal skin culture results

Although a very small number (n = 15) of animals were tested, it is clear that *S. intermedius and Bacillus* spp. occurs predominantly on the skin and *Pasteurella multocida*, Pasteurellaceae, the non-glucose fermenters and *Actinomyces* spp. in the oral cavity. Of the anaerobes, *Clostridium perfringens* was cultured from the unaffected skin of 1 bite wound patient. *Prevotella melaninogenica* was the only anaerobe cultured from the mouth of 1 patient (Table 9).

Table 9. Oral and skin microflora from 15 bite wounds.

	Oral flora Isolates	Skin flora Isolates
Gram positive		
Staphylococus spp	1	4
Staphylococus intermedius	2	7
Commensal *Streptococcus* spp. (mainly oral streptococci)	2	2
Streptococcus canis	1	1
Pyogenic streptococci (excluding *S. canis*)	0	1
Bacillus spp.	2	7
Actinomyces spp.	5	1
Corynebacterium spp.	1	2
Other Gram positive bacteria	1	2
Clostridium perfringens	0	1
Gram negative		
Pasteurellaceae	5	0
Pasteurella multocida	10	3
Pseudomonas aeruginosa	0	1
Enterobacteriaceae	2	2
Non-glucose fermenters	10	5
Vibrionaceae	2	0
Prevotella melaninogenica	1	0
Total:	45	39

Table 10. Percentage antimicrobial susceptibility of the most common bacteria isolated in 50 cases of dog bite wounds.

	Pasteurella multocida n = 30	Pasteurellaceae n = 13	*Staphylococcus intermedius* n = 23	Pyogenic* streptococci n = 27	*Escherichia coli* n = 10
Ampicillin/amoxycillin	83%	100%	74%	74%	70%
Amoxycillin-clavulanate	87%	100%	91%	78%	80%
Cloxacillin	64%	83%	90%	70%	22%
Penicillin G	93%	92%	65%	81%	10%
Cephalothin	93%	92%	100%	86%	20%
Ceftiofur	93%	44%	65%	81%	44%
Enrofloxacin	93%	85%	91%	7%	50%
Orbifloxacin	64%	90%	74%	48%	50%
Doxycycline	93%	85%	57%	67%	10%
ï Sulphamethoxyzole + trimethoprim	90%	100%	74%	89%	60%
Gentamicin	43%[§]	92%	91%	19%	60%
Amikacin	65%	90%	100%	7%	89%
Kanamycin	83%	92%	95%	11%	50%
Lincomycin	17%	31%	40%	0%	10%
Lincospectin	33%	50%	33%	n/a	0%
Tylosin	76%	92%	95%	77%	10%

Pyogenic streptococci included: Group G (S. canis), Group A (S. pyogenes), Group B (S. agalactiae) and Group C (S. dysgalactiae subsp. dysgalactiae, S. dysgalactiae subsp. equisimilis).*

4.3.4. Antimicrobial susceptibility results

Antibiotic susceptibility of the most common pathogens are summarised in Table 10.

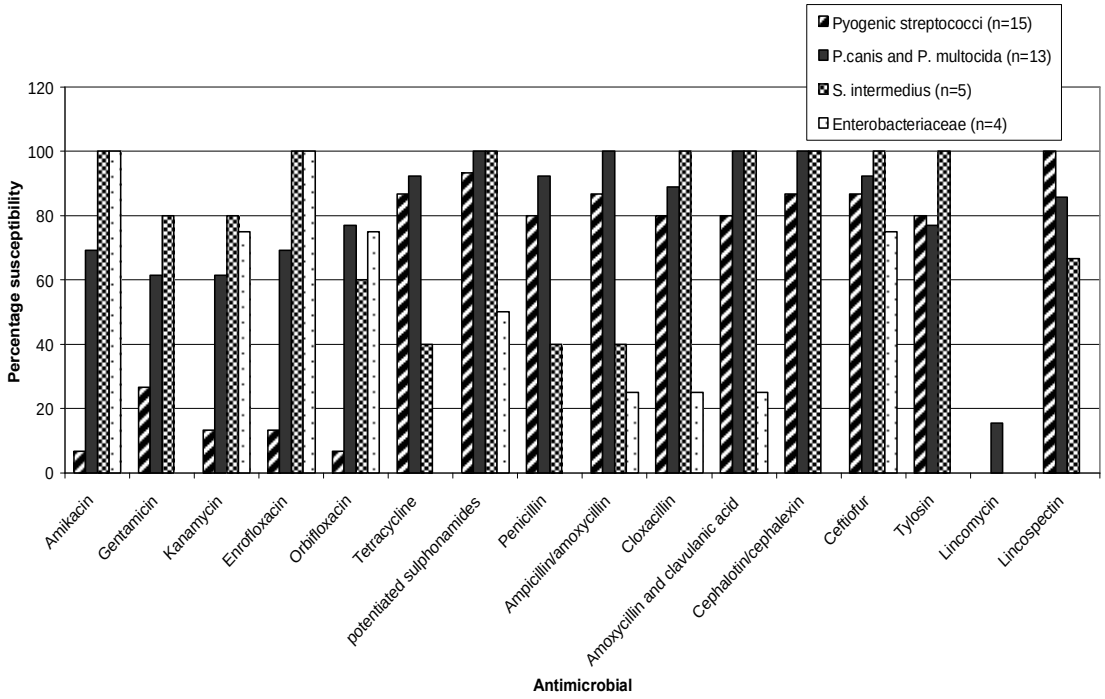
4.3.4.1. Non-infected wounds

Pasteurellaceae and *Pasteurella multocida* (with exception of gentamicin) and *Staphylococcus intermedius* were susceptible to the majority of selected antibiotics. However, *Escherichia coli* and in particular the pyogenic streptococci, showed greater levels of resistance. Due to cost restraints anaerobic antimicrobial susceptibility testing was not performed in this study.

4.3.4.2. Infected wounds:

Concerning the antimicrobial susceptibility of all the bacteria isolated from infected wounds, the most effective antimicrobials *in vitro* were potentiated sulphonamides (89% susceptibility), amoxycillin plus clavulanic acid (85.4%) and cephalexin/cephalothin (83%), the least effective were lincomycin (3.6%), gentamicin (43.6%) and kanamycin (49%). However this pattern changed slightly when only the common pathogens were taken into consideration. In this case potentiated sulphonamides, cephalexin and ceftiofur followed by amoxycillin and clavulanic acid and amoxycillin were the most effective *in vitro* as shown in Figure 2.

Figure 2. Percentage antimicrobial susceptibility of known bacterial pathogens isolated from infected DBW.



Chapter 5. Discussion

Animal bites inflicted on humans, have been referred to as an ñunrecognised epidemicò In fact it is estimated that 1 in 20 dogs will bite a human being during the dogôs lifetime. The high annual incidence of dog bites in the United States of America (USA) reflects a canine population that has grown four times faster than the human population.[68] As a consequence there has been extensive research into this field resulting in a large body of knowledge covering the epidemiology, risk factors for complications, evaluation components, bacterial composition, antimicrobial susceptibility patterns and recommended treatments. The relative paucity of information in the veterinary literature is somewhat surprising considering that the incidence of dog and cat bite wound trauma is cited as ranging between 10 to 15 percent of veterinary emergency visits.[2,20] Although accurate figures have not been calculated, the incidence of dog bite wounds presenting to the OVAH (Onderstepoort Veterinary Academic Hospital) amounts to 2-3 patients each week. Nevertheless, there is little objective information for veterinarians to make informed decisions, particularly with regard to status of wounds infection and antimicrobial use. Although studies of dog bite wounds involving humans may provide a platform for further veterinary research, this data has been shown to be biased towards human patients with more severe or infected wounds and should thus be interpreted cautiously.[68]

5.1. Wound characteristics study

The results of the authorsôstudy showed that of the patients presented to the Outpatients Clinic of the OVAH, the most common dogs requiring veterinary attention for bite wounds were juvenile, small breed, pure bred intact males. The findings of a previous study are similar in respect to age and sex. However large, cross-bred dogs were more prevalent in that study.[11] The differences between the two studies may be due to regional differences in the dog profiles. In separate Pretoria households, where dog owners are security conscious, there tends to be a predominant population of large, aggressive breeds housed together with noisy, small pure-breeds. Strict confinement and little contact with the outside world may result in referred aggression where overexcited larger dogs bite their smaller companions. The majority of cases presented within 48 hours after being bitten.

Unfortunately, since no record was made of wound position in patients with more than 3 wounds in this study, conclusions pertaining to anatomical location could not be precisely made. The overall trend

however did appear to favor the cranial half of the body (158 wounds) which included the head, neck thoracic limbs and thorax as a more common location for bite wounds than the caudal half of the body (55 wounds).

5.2. Wound infection study

The definition of what constitutes wound infection as used in the available veterinary literature is somewhat unclear. Historically the difference between contaminated and infected wounds has been based on factors such as the time taken for inoculated bacteria to start proliferating,[19,59] the quantification of bacteria in tissue,[19,20,59] and the presence of a purulent discharge or abscess formation.[11,18]

The combined use of the three criteria, pyrexia, purulence and cytology is unique to the present study. In a previous human study, two criteria pyrexia and purulence were also used as a determinant of infection, although in combination with additional criteria such as erythema, swelling, tenderness, lymphangitis and leukocytosis. However, many of these criteria are difficult to apply directly in a DBW study: erythema and swelling become subjective parameters, particularly with respect to the variety of sizes of dogs, skin colour and coat characteristics. Tenderness is based on subjective pain scoring which may be difficult to interpret in animals with severe wounds, or in more stoic animals. Lymphangitis may be more useful in people in which the majority of DBW occur to the distal extremities with accessible lymph nodes. Total leukocyte count however, may be a useful tool to consider using in future studies.

It is interesting to note that of the 21 wounds judged to be infected in the present study, all were found to show positive culture results, whilst 17 samples of the 83 non-infected wounds had no bacterial growth. The relatively low level of infection in the results reported herein (21 wounds or 20%) parallels the 3-20% incidence infected wounds cited in human clinical studies.[1,6,8] Although direct comparisons cannot be made with the Griffin study[11], which selected clinically uninfected cases at presentation, the acquired infection rate was comparable at 19%.

In comparing the number of isolates and variety of species found in infected and non-infected wounds, our findings equate to those of other studies.[8,68] As expected, the infected wounds yielded more

colonies but unexpectedly yielded relatively more species. Note, however, in real terms the number of species in the infected wounds were fewer, but were not statistically recognised as such due to the low proportion of infected wounds. Thus a study with a larger number of infected wounds may give a completely different result.

Although this study selected three criteria in order to asses the infection status of wounds, the author recognises limitations, such as: the difficulty in distinguishing between hyperthermia and pyrexia; despite its wide use in the available literature, the accuracy of wound purulence and the potential bias associated with the use of two highly subjective parameters (pyrexia and purulence).

5.3. The bacteriology of bite wounds

The high percentage (84%) of positive non-infected and infected cultures in the authorôs study is significantly higher than the 68% isolation rate found in the study by Griffin et al[11]. If however, two of the exclusion criteria in Griffinôs study are re-included, namely infected wounds at presentation and wounds older than 72 hours, a comparative level of 80% is evident. The 67% isolation rate in the study by Kelly et al.[12] should be viewed in light of the fact that aerobes alone were cultured and that two-thirds were from older wounds sampled after 72 hours of biting.

It appears from the culture results in the authorôs study that wound factors such as wound location and grade of severity were not predictive of whether a wound would culture positive or not. Although wounds of a greater severity (Grades 3 and 4) showed a tendency (p=0.082) to be infected, larger wound numbers would have to be evaluated to prove this conclusively. The proportion of Gram-positive to Gram-negative bacteria was not statistically different. This implies that when considering antimicrobial therapy for bites wounds both groups should be taken into consideration.

The three genera ï *Pasteurella, Streptococcus and Staphylococcus* were predominant in the present study. This finding is similar to many human dog bite wound investigations and supports popular dogma in many veterinary texts.[13,21,62,71] It is however different to previous veterinary bacteriology studies insofar as more members of the Pasteurellaceae and proportionally fewer enteric bacteria and *Pseudomonas* species were cultured by our group. In a retrospective study performed by Kelly et al.[12] of 87 untreated dog bite victims, wounds were cultured for aerobic growth and antimicrobial

susceptibility. The most common pathogen isolated from wounds was *S. intermedius* (23%), *E. coli* (18%), nonï lactose fermenting coliforms (14%) and *Pseudomonas* spp. (14%). Due to the lack of facilities, anaerobic cultures were not performed. In a more recent study, Griffin et al.[11] documented bite wounds in 37 dogs, which were prospectively evaluated. Aerobic and anaerobic cultures were taken from each wound within an hour of presentation and again during surgery, after which antimicrobial susceptibilities were determined for each isolate. The most common aerobic isolates were *S. intermedius* (20%), *Enterococcus* spp. (15%), coagulase-negative staphylococci (13%), *E. coli* (13%) and *Pseudomonas* spp.(5%). Sampling differences may be ascribed to the disparity evident between these prior studies and the authorsô study in which the proportions were *S. intermedius* (12%), *Enterococcus* spp (8%), coagulase-negative staphylococci (7%), *E. coli* (5%) and *Pseudomonas* spp.(1%).

In the Kelly et al.[12] study a high percentage of wounds were older than 72 hours, which would account for the higher number of Enterobacteriaceae and pseudomonads, which are usually secondary bacterial invaders. Furthermore, the higher incidence of *Pseudomonas* species may also be ascribed to the late presentation of many of these wounds and the affiliation of these environmental bacteria for large and open wounds.

The two bacterial species *Staphylococcus intermedius* and *Pasteurella multocida* were the most frequent isolates cultured in the current study. *Pasteurella canis* and the pyogenic streptococci including *Steptococcus canis*, were well represented in infected wounds. Non-pathogenic genera such as the oral streptococci, *Actinomyces* and *Bacillus* spp., that are common in the oral cavity and the environment, were common in the contaminated wounds, but were not associated with infected wounds (Table 7). The 66% (8/13) incidence of *Pasteurella canis* in our study is comparable to the study in people reported by Talan et al [8] in which only infected bite wounds were considered. In spite of the marked similarities in the bacterial spectrum isolated from dog bites (that occur in dogs compared with those in humans) there is still one significant difference. Namely there is a greater predominance of *Staphylococcus aureus* [8] (a bacterium that is closely related to the canid bacterium *Staphylococcus intermedius*).

A single isolate of *Capnocytophaga canimorsus* was diagnosed in an infected bite wound involving the head. This is the first documented case of *C. canimorsus* in an infected dog bite wound, even though it

was isolated with other organisms. One possible explanation for this apparent low prevalence includes the fastidious nature of the organism and its tendency for slow growth. Another reason, is that in culture media, *C. canimorsus* may be overgrown in the presence of a polymicrobial environment.[72] This Gram-negative facultative anaerobe that has been isolated from the oral cavity of 16% of clinically healthy dogs[51] is known to cause potentially fatal septicaemias in immunocompromised humans. Although *C. canimorsus* has zoonotic importance, its clinical relevance for domestic animals has not been established, with a single case reported in a dog-inflicted bite wound in a pet rabbit.[72]

In the current study, using anaerobic transport medium and culturing techniques, three obligate anaerobic genera were cultured; *Prevotella, Clostridium, and Peptostreptococcus*. Since only one isolate of *Clostridium perfringens* was cultured from the unaffected skin and one of *P. melaningenica* was cultured from the oral cavity, it was not possible to make any conclusions regarding the origin of the anaerobic microflora in the dog bites under investigation. However, it may be speculated that these anaerobes with the possible exception of *Clostridium* spp. are thought to originate from the oral cavity which has been shown to be rich in populations of *Bacteroides* spp. and the black pigmented anaerobic bacilli (BPABs), consisting of *Porphyromonas* and *Prevotella* spp.[16,55] It may be argued that the relatively high level of clostridia isolated in this study could have arisen from contamination of the surrounding skin or environment .This would seem unlikely considering that great care was taken to avoid the skin edges. This is supported by the fact that the ubiquitous, non-pathogenic, spore-forming *Bacillus* species were not cultured from the infected wounds. Of the 18 anaerobic isolates identified, only one was found by itself, whereas the majority was admixed with aerobes. Fifty six percent (10/18) of all anaerobes cultured were *Prevotella* spp.. These were cultured from Grade 3 and 4 infected bite wounds, of which 50% involved the head. Although low case numbers in this study do not allow for statistical evaluation, it would appear that bite wounds of a severe nature that involve the head may particularly prone to infection with *Prevotella* spp. This may be due to the presence of dead space associated with these wounds, an environment which may be more conducive to anaerobic replication. It is interesting that *Clostridium* spp. was the only true obligate anaerobe isolated in the Griffin et al.[11] study. The most frequently isolated bacteria in recent human studies include *Fusobacterium, Bacteroides, Peptostreptococcus* and *Prevotella,* spp. (particularly *P. melaninogenica* and *P. intermedia*).[8,46,68]

Oral and normal skin culture results

Culture findings of the skin and oral cavity showed that *Staphylococcus intermedius* and *Bacillus* spp. predominated on the skin, whereas *Pasteurella multocida,* the Pasteurellaceae including non-lactose fermenters and *Actinomyces* spp. predominated in the oral cavity. A variety of studies have attempted to define the distribution of *S. intermedius* on the skin.[34] in which it is proposed to have two populations. Firstly, a resident population within the pilosebaceous units particularly at the oral, nasal and anal sites.[28,36,35] Secondly a transient population on the distal hair shaft, a filter which is thought to act as a bacterial ñtrapò[36] Interestingly the large population of *S. intermedius* found on the abdominal hair is thought to be associated with environmental contamination, or seeding from the mucous membranes of the nose and anus during grooming.[35]

Although the oral cavity of the aggressor could not be cultured it, would seem plausible that *Staphylococcus intermedius* primarily originated from the normal skin of the victim whereas *Pasteurella multocida* originated mainly from the oral cavity. The notable presence of *Pasteurella multocida* from the gums of 10/15 (68%) dogs in this study is similar to other studies in which the organism has been shown to be a common inhabitant of the nasal, gingival and tonsillar regions of 12% to 92% of dogs.[47] Thus it would appear that both oral and skin microflora contribute to wound contamination and infection. However, due to the small sample size and inaccessibility of the aggressor dogs, I could not prove conclusively that this was the case.

Antimicrobial susceptibility

The indications for antimicrobial prophylaxis in surgical trauma have been well documented, where patient, wound and environmental factors contribute towards rational decision making. Although it is generally accepted that wound debridement and lavage are essential in encouraging wound healing, the use of parenteral antimicrobial prophylaxis is controversial in animals.[6,11,12,69] Whilst it is common practice for veterinarians to treat all penetrating wounds with antimicrobials[40], others have suggested prompt intravenous treatment in severely injured or compromised patients in combination with appropriate wound debridement and lavage.[73] In humans, antimicrobial therapy for bite wounds is considered therapeutic and not prophylactic.[52] In order to select an appropriate antimicrobial, it is recommended that aerobic and anaerobic cultures be performed on infected wounds as well as antimicrobial susceptibility testing. It is believed that cultures performed on wounds that are not

clinically infected are of little value in determining the potential infectious organism or selecting the correct antimicrobial.[9, 44, 71] Wounds may be more accurately classified by using wound cytology and the stricter definition of what constitutes an infected wound.

Unlike other members of the Pasteurellaceae, the resistance of *P. multocida* to some of the aminoglycosides has not only been well described in the literature. However, antimicrobial susceptibility patterns are commonly recorded in the DVTD Bacteriology Laboratory.[39] Findings show that kanamycin is most susceptible followed by an increased resistance to amikacin and gentamicin. A similar observation has been made with regards to erythromycin, clindamycin and the first-generation cephalosporins (cephalexin/cephalothin) which have been seen as inappropriate choices for empiric therapy for dog bites due to their poor coverage of *P. multocida.*[6,62] However, in the present study *P. multocida* was relatively susceptible to these antimicrobials. In addition, *Escherichia coli* isolates were generally resistant to the macrolides i.e. lincomycin, tylosin, the non-synthetic penicillins and first-generation cephalosporins, presumably because these antimicrobials are predominantly active against Gram-positive bacteria. Unusually there was a high level of resistance against the aminoglycosides, gentamicin (40%) and kanamycin (50%) third generation cephalosporins (56%) and the fluoroquinolones (50%). Although resistance to the tetracyclines, represented by doxycycline, is normally high in dogs, it was even higher in the isolates found herein (90%). The pyogenic streptococci including *Streptococcus canis* are known to have significant resistance to aminoglycosides and fluoroquinolones[39], an observation consistent with the current report. Overall the pleuromultalins (represented by lincomycin) fared poorly *in vitro* in the current study.

The recommendations for the use of penicillins and potentiated penicillins in DBW have been widely reported, including the authorõs study which confirmed a broad aerobic spectrum of activity for both.[6,11,62] Nevertheless, the increasing concern for ß-lactamase producing organisms has meant an increase in the use of amoxycillin and clavulanic acid.[8, 21, 68] *In vitro*, susceptibility of *E. coli* to the synthetic penicillins, especially the potentiated penicillins, is usually good. However this may not be the case *in vivo* and thus they should only be used if no alternative antimicrobials are available.

From the results of the current study, recommended empiric antimicrobial coverage for infected bite wounds of low severity should include ampicillin, amoxicillin or a potentiated sulphonamide. The widespread antimicrobial susceptibility to potentiated sulphonamides in this study may be as a

consequence of their infrequent use in veterinary practice. Trimethoprim has been shown to reach good levels in the skin and may be a good choice for canine pyoderma or other skin related infections when an inexpensive yet efficacious antibiotic is needed.[22] However, certain characteristics should be taken into account if it is to be considered for more than first line empiric use. There is a higher level of adverse drug reactions, particularly if used for long periods (> 21 days). Delayed hypersensitivity reactions to sulphonamides have been well described and should be avoided in Doberman Pinschers which may show familial idiosyncratic tendencies.[5] In addition sulphonamides tend to develop resistance rapidly, are bacteriostatic and are less effective in PABA-rich (purulent) sites of infection. For these reasons, sulphonamides are less desirable as first-choice therapy than ampicillin or amoxycillin.[22] Evidence suggested in the current study would indicate that for infected wounds of greater severity in which the potential risk to the patient is extreme, empiric coverage with amoxycillin-clavulanate should provide a broad spectrum of activity. The administration of ceftiofur, a third generation cephalosporin, is a second option.

The antimicrobial susceptibility of anaerobes has been extensively covered in the literature (see a summary in the Introduction). Irrespective of the therapeutic regimen selected, the synergistic interactions between anaerobes and aerobes are important to bear in mind as antimicrobial therapy should be directed at both. Metronidazole is an affordable and very effective choice, but it should be used in combination with other antimicrobials having aerobic activity. Although anaerobic susceptibility was not determined in the current study, reported data indicates that a third of *Prevotella spp.* are ß-lactam producers.[70] Thus amoxycillin-clavulanate or metronidazole and amoxicillin are the antimicrobials of choice when dealing with anaerobic infections. The use of most fluoroquinolones and the aminoglycosides such as gentamicin should be avoided prior to the culture results being available, as they are ineffective against anaerobic bacteria. The antimicrobials tested in the authorsô study were also found not to have poorer *in vivo* activity against these anaerobes.

Chapter 6. Conclusion and Recommendations

Epidemiological factors such as signalment, age, breed and wound characteristics have been described for the first time in those cases presented to the OVAH. In support of other bite wound investigations, the results of the present study are that the majority of DBW will have a positive culture result. The study has confirmed that the bacteriological populations regularly cited from DBW studies in dogs are in essence very similar to DBW studies in people where *Pasteurella* spp. and *Staphylococcus* spp. are important role-players. In addition, the results of this study show that anaerobes are more commonly found in bite wounds in dogs than previously thought and that with meticulous sampling, transport and culture techniques their true prevalence may be elucidated. Once again these findings mirror the numerous reports in human medicine. The association of *Prevotella* spp. with wounds of greater severity particularly to the head may alert the clinician to the potential for complications in these cases.

In this era when the prevalence of antimicrobial resistance in pathogens is increasing, there is a need to use antimicrobials in such a way that they assist in the recovery of animals from life-threatening diseases without markedly increasing bacteria resistance to critical antimicrobials. Thus the adage ñall bite wounds require antimicrobialsòis outdated and even hazardous. A simple cytological tool together with a clinical examination to identify infected wounds as was tested here will assist veterinarians in determining their treatment protocols. This may be of particular importance in more severe wounds that have associated dead space and are more likely to yield anaerobic growth and in wounds older than 12 hours which are more likely to be infected. Although 20% of the non-infected wounds were sterile, for practical purposes all wounds should be considered contaminated, making it essential that all wounds are debrided and lavaged. However, unless the patient is immunosuppressed, it is not recommended that these animals are treated with antimicrobials [17,70]. This is especially true as the grade of severity was not predictive of whether a wound would culture positive or not. Whilst awaiting culture and antimicrobial susceptibility results for those select cases, empiric antimicrobials should be tailored according to the nature of the wound, the risk to the patient and the expected bacterial populations, which in the authorsôstudy include pasteurellaceae, streptococci, staphylococci, coliforms and anaerobes.

6.1 Suggestions for further study

In a future study I would suggest repeating wound cultures and antimicrobial susceptibility testing 2 to 3 days after presentation in order to determine any changes in bacterial populations in patients that that had or had not received antimicrobials. In order to more accurately access antimicrobial susceptibility of anaerobes cultured, I would suggest the use of either liquid minimum inhibitory concentration tests or that an E-test be performed. In an attempt to test the accuracy of the combined criteria used to determine DBW infection in the current study, I would recommend that quantitative bacterial counts be performed on homogenised surgical biopsies.

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Appendices

Figure A-1. Patient record chart.

Student name:_____

Clinician:_____

Date:_____

Sticker OR:

Patient: é é é é é é é é é é é é é é é é é .

Patient number:é é é é é é é é é é é é é é é .

Species:é é é é é é é é é é é é é é é é é .

Breed:é é é é .. Sex:é é é .. Age:é é é é .

Owner:é é é é é é é é é é é é é é é é é é

Owner number:é é é é é é é é é é é é é é

Sticker

U.P number:é é é ..
(Unique Patient)

Clinical parameters	
Temperature	
Pulse	
Respiration	
CRT	

Bite wound characteristics	
Time factors	
Time of occurrence	
Time of presentation	
Time of culture / surgery	
Macroscopic wound assessment	
	Wound diagram completed (Over page)
Mark ñ**P"** for Punctures / ñ**L"** for Lacerations	Yes / No
Mark ñ**C"** for culture site	Yes / No
Exudate (Serous/Serosanguinous/Sanguinous / Purulent)	
Wound Classification (Class 1, 2 or 3)	
Head	
Neck	
Front Limbs	
Hind limbs	
Chest	
Abdomen /Rump	
Tail	
Wound treatment	
Lavage	Yes / No
Debridement	Yes / No

Figure A-2. Patient diagrams.

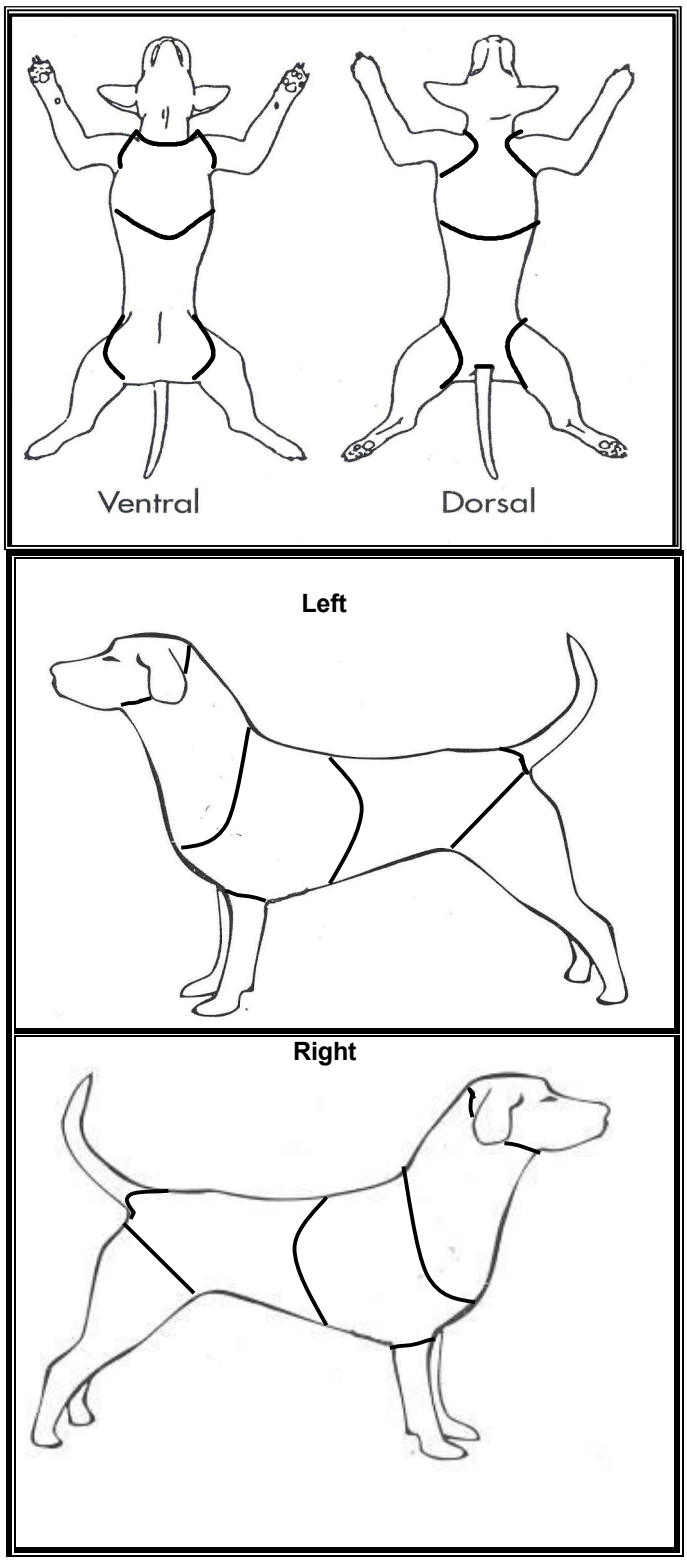

Table A-1. Ungrouped aerobic and anaerobic culture results.
(The infected bite wounds are shaded)

Patient number	Bite reference	Infection or non infected	Aerobes cultured	Anaerobes cultured	Bite wound grade	Site	Score	Aerobic culture	Anaerobic culture
1	1	Non infected	Yes	No	Gr. 2	Head		*Staphylococcus epidermidis*	
1	2	Non infected	Yes	No	Gr. 4	Fore Limbs		*Yersinia enterocolitica* *Moraxella* spp.	
1	3	Non infected	No	No	Gr. 2	Fore Limbs		*Negative*	
2	4	Infected	Yes	No	Gr. 2	Head		*Lactobacillus* spp. *CDC EF-4* spp. *Pasteurella multocida* *Pasteurella canis* *Capnocytophaga carnimorsus*	
3	5	Non infected	Yes	No	Gr. 3	Neck	2+ 2+ 2+	*Streptococcus* spp. *Pasteurella multocida* *Flavobacterium* spp.	
3	6	Non infected	Yes	No	Gr. 3	Neck	1+ 1+	*Flavobacterium* spp. *Pasteurella multocida*	
3	7	Non infected	Yes	No	Gr. 3	Neck	2+ 2+	*Corynebacterium bovis.* *Pasteurella multocida*	
4	8	Non infected	Yes	No	Gr. 4	Hind limbs	2+ 4+ 4+		
4	9	Non infected	Yes	No	Gr. 4	Neck	2+	*Staphylococcus* spp. *Streptococcus* spp. *Staphylococcus intermedius*	
4	10	Non infected	Yes	No	Gr. 1	Abdomen	2+ 4+ 2+	*Bacillus* spp. *Bacillus* spp. *Pasteurella canis* *Moraxella* spp.	
5	11	Non infected	Yes	No	Gr. 1	Fore Limbs	4+ 3+ 4+	*Streptococcus* spp *Streptococcus bovis* *Pasteurella multocida*	
5	12	Non infected	Yes	No	Gr. 4	Tail	4+	*Pasteurella multocida*	
5	13	Non infected	Yes	No	Gr. 2	Head	2+	*Pasteurella pneumotropica*	
6	14	Infected	Yes	Yes	Gr. 3	Chest	4+ 4+	*Streptococcus canis* *Escherichia coli*	*Provetella melaninogenica*
6	15	Infected	Yes	Yes	Gr. 3	Chest	4+ 4+	*Streptococcus canis* *Escherichia coli*	*Provetella melaninogenica* *Clostridium tyrobutyricum*
6	16	Infected	Yes	Yes	Gr. 3	Hind limbs	4+	*Streptococcus canis*	*Provetella melaninogenica*
7	17	Infected	Yes	Yes	Gr. 1	Hind limbs	4+ 4+	*Streptococcus canis* *Streptococcus pyogenes*	*Clostridium* spp.
7	18	Non infected	Yes	Yes	Gr. 3	Chest	2+ 3+ 2+	*Streptococcus canis* *Streptococcus pyogenes* *Escherichia coli*	*Clostridium* spp.
7	19	Non infected	Yes	Yes	Gr. 4	Tail	3+ 3+	Streptococcus canis Staphylococcus intermedius	*Clostridium* spp.
8	20	Non infected	Yes	N	Gr. 2	Hindlimbs	3+ 3+ 3+	*Moraxella* spp. *Streptococcus* spp. *Bacillus* spp.	.

8	21	Non infected	Yes	No	Gr. 2	Head	2+	Moraxella spp.	
9	22	Infected	Yes	No	Gr. 4	Head	4+ 4+	*Streptococcus Group C* *Pasteurella canis*	
9	23	Non infected	Yes	No	Gr. 1	Head	3+ 2+	*Moraxella* spp. *Streptococcus bovis*	
10	24	Non infected	Yes	No	Gr. 1	Fore Limbs	2+	*Manheimia haemolytica*	
11	25	Non infected	Yes	No	Gr. 3	Tail	2+ 3+ 2+ 2+	*Proteus mirabilis* *Streptobacillus monliforme* *Pasteurella multocida* *Escherichia coli*	
11	26	Non infected	Yes	No	Gr. 3	Hind limbs	1+	*Corynebacterium kutscheri*	
11	27	Non infected	No	No	Gr. 4	Chest		Negative	
12	28	Non infected	Yes	No	Gr. 4	Fore Limbs	1+	*Staphylococcus intermedius*	
13	29	Non infected	No	Yes	Gr. 2	Hind limbs		Negative	
13	30	Non infected	Yes	No	Gr. 2	Abdomen	2+	*Corynebacterium* spp.	
13	31	Non infected	Yes	Yes	Gr. 2	Tail	2+ 1+ 1+	*Enterococcus faecalis* *Micrococcus* spp. *Bacillus* spp.	*Clostridium perfringens*
14	32	Infected	Yes	Yes	Gr. 4	Head	4+ 4+ 4+	*Lactobacillus* spp. *Streptococcus canis* *Pasteurella canis*	*Clostridium perfringens*
15	33	Infected	Yes	Yes	Gr. 3	Head	3+ 4+	*Streptococcus agalactiae* *Staphylococcus epidermidis*	*Provetella melaninogenica*
15	34	Infected	Yes	Yes	Gr. 3	Head	 1+ 3+	*Staphylococcus intermedius* *Streptococcus pyogenes* *Vibrio parahaemolyticus*	*Provetella melaninogenica*
15	35	Infected	Yes	Yes	Gr. 3	Head	4+ 4+	*Streptococcus pyogenes* *Streptococcus agalactiae*	*Provetella melaninogenica*
16	36	Infected	Yes	No	Gr. 2	Head	3+ 4+	*Streptococcus agalactiae* *Escherichia coli*	
16	37	Non infected	Yes	No	Gr. 2	Fore Limbs	2+	*Streptococcus agalactiae*	
17	38	Non infected	Yes	No	Gr. 4	Hind limbs	3+ 3+ 2+ 3+ 1+	*Klebsiella ozaenae* *Streptococcus* spp. *Pasteurella multocida* *Streptococcus canis* *Staphylococcus epidermidis*	
18	39	Non infected	Yes	No	Gr. 1	Neck	2+ 3+	*Bacillus cereus* *Pasteurella multocida*	
19	40	Non infected	Yes	No	Gr. 4	Neck	3+	*Staphylococcus intermedius*	
19	41	Non infected	Yes	No	Gr. 4	Chest	3+	*Staphylococcus intermedius*	
19	42	Non infected	Yes	No	Gr. 1	Head	3+	*Staphylococcus intermedius* *Staphylococcus epidermidis*	
20	43	Non infected	No	No	Gr. 4	Head		Negative	
20	44	Non infected	No	No	Gr. 2	Neck		Negative	
21	45	Non infected	No	No	Gr. 1	Chest		Negative	
21	46	Non infected	No	No	Gr. 4	Chest		Negative	
21	47	Non infected	Yes	No	Gr. 4	Chest	2+ 2+	*Pasteurella multocida* *Moraxella* spp.	
22	48	Non infected	Yes	No	Gr. 4	Head	2+ 2+ 1+ 3+	*Streptococcus canis* *Pasteurella multocida* *Staphylococcus intermedius* *Pasteurella canis*	
23	49	Non infected	Yes	No	Gr. 1	Neck	3+ 2+	*Staphylococcus intermedius* *Pasteurella canis* *Streptococcus Group C*	

23	50	Non infected	Yes	No	Gr. 1	Neck	3+ 2+	*Streptococcus Group C* *Pasteurella canis*	
24	51	Infected	Yes	No	Gr. 3	Head	3+ 2+ 1+	*Streptococcus Group C* *Pasteurella canis* *Klebsiella pneumoniae*	
24	52	Infected	Yes	No	Gr. 3	Head	3+ 3+	*Streptococcus Group C* *Aeromonas hydrophilia*	
25	53	Non infected	Yes	No	Gr. 1	Neck		*Escherichia coli* *Streptococcus canis*	
25	54	Non infected	Yes	No	Gr. 2	Head		*Escherichia coli* *Streptococcus canis* *Pasteurella multocida* *Bacillus* spp. *Neisseria* spp.	
25	55	Non infected	Yes	No	Gr. 2	Hind Limbs		*Escherichia coli* *Pasteurella multocida* *Neisseria* spp.	
26	56	Non infected	Yes	No	Gr. 4	Head		*Pasteurella multocida* *Micrococcus* spp.	.
26	57	Non infected	No	No	Gr. 2	Head		*Negative*	
27	58	Non infected	No	No	Gr. 3	Fore Limbs		*Negative*	
27	59	Non infected	No	No	Gr. 2	Fore Limbs		*Negative*	
27	60	Non infected	No	No	Gr. 2	Fore Limbs		*Negative*	
28	61	Non infected	Yes	No	Gr. 2	Fore Limbs		*Bacillus* spp. *Pasteurella multocida*	
28	62	Non infected	Yes	No	Gr. 4	Hind limbs		*Pasteurella multocida* *Moraxella* spp.	.
28	63	Non infected	Yes	No	Gr. 3	Hind limbs		*Pasteurella multocida* *Moraxella* spp.. *Staphylococcus intermedius*	
29	64	Infected	Yes	No	Gr. 3	Head		*Moraxella* spp.. *Moraxella* spp.. *Streptococcus canis* *Staphylococcus intermedius*	
29	65	Non infected	Yes	No	Gr. 1	Hind limbs		*Staphylococcus intermedius*	
29	66	Non infected	No	N	Gr. 2	Neck		*Negative*	
30	67	Non infected	Yes	Yes	Gr. 3	Chest	3+ 3+	*Moraxella* spp. *Streptococcus*	*Peptostreptococcus* spp.
30	68	Non infected	Yes	No	Gr. 2	Chest	3+	*Moraxella* spp.	.
31	69	Infected	Yes	No	Gr. 3	Fore Limbs	4+	*Pasteurella multocida*	
32	70	Infected	Yes	Yes	Gr. 4	Head	3+ 2+ 3+ 2+	*Pasteurella multocida* *Streptococcus spp.* *Streptococcus canis* *Staphylococcus intermedius*	*Provetella melaninogenica*
32	71	Infected	Yes	Yes	Gr. 4	Head	2+ 3+ 2+ 1+	*Pasteurella multocida* *Streptococcus spp.* *Pasteurella canis* *Streptococcus canis*	*Provetella melaninogenica*
33	72	Non infected	Yes	No	Gr. 4	Head	3+ 2+ 3+ 2+ 2+ 3+	*Moraxella* spp. *Streptococcus canis* *Providencia stuarti* *Corynebacteriumpseodotuberculosis* *Staphylococcus intermedius* *Bacillus* spp.	
33	73	Non infected	Yes	No	Gr. 3	Neck	3+ 2+ 1+ 2+ 3+	*Moraxella* spp. *Staphylococcus intermedius* *Corynebacterium pseodotuberculosis* *Bacillus cereus*	

34	74	Non infected	Yes	No	Gr. 1	Head	3+ 4+ 3+	*Moraxella* spp. *Streptococcus Group D* *Staphylococcus intermedius*	
34	75	Non infected	Yes	No	Gr. 4	Head	3+ 4+ 3+ 3+	*Moraxella* spp. *Staphylococcus intermedius* *Streptococcus Group D* *Haemophilus aphrophilus*	
35	76	Non infected	Yes	No	Gr. 3	Hind limbs	4+	*Aeromonas hydrophilia* *Pseudomonas putida* *Escherichia coli*	
35	77	Non infected	Yes	No	Gr. 1	Hind limbs	4+ 3+ 2+	*Staphylococcus epidermidis*	
36	78	Non infected	Yes	No	Gr. 4	Hind limbs	3+ 3+	*Pasteurella multocida* *Staphylococcus* spp.	
36	79	Non infected	Yes	No	Gr. 3	Chest	3+ 3+	*Pasteurella multocida* *Staphylococcus* spp.	
37	80	Non infected	Yes	No	Gr. 2	Neck	3+	*Pasteurella multocida*	
38	81	Infected	Yes	Yes	Gr. 3	Neck	3+ 3+ 3+	*Staphylococcus* spp. *Pasteurella canis* *Corynebacterium bovis*	*Provetella melaninogenica*
38	82	Infected	Yes	Yes	Gr. 4	Neck	1+ 1+ 2+ 4+	*Pasteurella canis* *Staphylococcus intermedius* *Staphylococcus* spp. *Corynebacterium bovis*	*Provetella melaninogenica*
39	83	Non infected	Yes	No	Gr. 4	Hind limbs	4+ 3+ 1+	*Staphylococcus intermedius* *Pasteurella multocida* *Moraxella* spp.	
40	84	Non infected	Yes	N	Gr. 2	Fore Limbs	2+ 2+ 1+	*Staphylococcus intermedius* *Actinomyces neuii anitratus* *Bacillus* spp	
41	85	Non infected	Yes	No	Gr. 2	Hind limbs	1+ 1+ 1+	*Staphylococcus epidermidis* *Moraxella* spp. *Pasteurella multocida*	
41	86	Non infected	Yes	No	Gr. 4	Head	1+ 1+ 1+	*Staphylococcus epidermidis* *Moraxella* spp. *Pasteurella multocida*	
41	87	Non infected	Yes	No	Gr. 4	Neck	1+	*Streptobacillus milleri*	
42	88	Non infected	Yes	No	Gr. 3	Chest	1+	*Moraxella* spp.	
43	89	Infected	Yes	No	Gr. 2	Neck	1+	*Staphylococcus intermedius*	
43	90	Non infected	Yes	No	Gr. 4	Fore Limbs	1+	*Streptococcus* spp.	
44	91	Non infected	Yes	No	Gr. 3	Chest	2+ 1+ 2+ 1+	*Actinomyces odontolyticus* *Pasteurella multocida* *Actinomyces neuii neuii* *Bacillus* spp.	
44	92	Non infected	Yes	No	Gr. 4	Fore Limbs	3+ 3+ 1+ 3+	*Actinomyces odontolyticus* *Pseudomonas fluorescens* *Staphylococcus intermedius* *Pasteurella multocida*	
45	93	Non infected	Yes	No	Gr. 4	Neck	3+ 2+ 1+ 1+ 3+	*Actinomyces* spp. *Staphylococcus intermedius* *Bacillus cepacia* *Vibrio cholerae* *Streptobacillus moniliforme*	
46	94	Non infected	No	No	Gr. 2	Neck		Negative	
46	95	Non infected	No	No	Gr. 4	Hind limbs		Negative	
46	96	Non infected	No	No	Gr. 4	Tail		Negative	
47	97	Non infected	No	No	Gr. 2	Fore Limbs		Negative	
47	98	Non infected	No	No	Gr. 2	Fore Limbs		Negative	

48	99	Non infected	Yes	No	Gr. 4	Neck		*Bacillus* spp.	
48	100	Infected	Yes	No	Gr. 1	Chest	3+ 1+ 3+	*Pasteurella canis* *Pasteurella multocida* *Actinobacillus* spp.	
49	101	Non infected	Yes	No	Gr. 3	Neck	4+ 4+ 3+	*Actinomyces neuii anitratus* *Burkholderia cepacia* *Staphylococcus intermedius*	
49	102	Non infected	Yes	No	Gr. 2	Neck	4+ 4+ 4+ 3+	*Burkholderia cepacia* *Actinomyces neuii anitratus* *Corynebacterium* spp. *Staphylococcus intermedius*	
50	103	Non infected	Yes	No	Gr. 4	Abdomen	1+ 1+ 1+	*Escherichia coli* *Pasteurella multocida* *Flavobacterium* *Staphylococcus intermedius*	
50	104	Non infected	Yes	No	Gr. 2	Abdomen	1+ 1+	*Bacillus* *Pasteurella multocida*	

Table A-2. Infected bite wounds.

Patient number	Bite reference	Time	Purulence	Wound cytology	Pyrexia	Infected Non infected Negative
1	1	24h	No	No	No	Non infected
1	2		No	No	No	Non infected
1	3		No	No	No	Non infected (Negative)
2	4	8h	No	Yes	Yes (40.0°C)	Infected
3	5	2h	No	No	No	Non infected
3	6		No	No	No	Non infected
3	7		No	No	No	Non infected
4	8	7hr	No	No	No	Non infected
4	9		No	No	No	Non infected
4	10		No	No	No	Non infected
5	11	12hr	No	No	No	Non infected
5	12		No	No	No	Non infected
5	13		No	No	No	Non infected
6	14	72hr	Yes	Yes	Yes (40.0°C)	Infected
6	15		Yes	Yes	Yes (40.0°C)	Infected
6	16		Yes	No	Yes (40.0°C)	Infected
7	17	72hr	Yes	Yes	No (39.1°C)	Infected
7	18		No	No	No	Non infected
7	19		No	No	No	Non infected
8	20	2hr	N	No	N	Non infected
8	21		No	No	No	Non infected
9	22	32hr	No	Yes	Yes (39.7°C)	Infected
9	23		No	No	No	Non infected
10	24	0.4hr	No	No	No	Non infected
11	25	0.45hr	No	No	No	Non infected
11	26		No	No	No	Non infected
11	27		No	No	No	Non infected (Negative)
12	28	1hr	No	No	No	Non infected
13	29	12hr	No	No	No	Non infected
13	30		No	No	No	Non infected
13	31		No	No	No	Non infected
14	32	72hr	Yes	Yes	Yes (40.1°C)	Infected
15	33	72hr	Yes	Yes	No (38.9°C)	Infected
15	34		Yes	Yes	No (38.9°C)	Infected
15	35		Yes	Yes	No (38.9°C)	Infected
16	36	72hr	Yes	Yes	Yes (39.7°C)	Infected
16	37		No	No	No	Non infected
17	38	3hr	No	No	No	Non infected
18	39	0.75hr	No	No	No	Non infected
19	40	5hr	No	No	No	Non infected
19	41		No	No	No	Non infected
19	42		No	No	No	Non infected
20	43	3.5hr	No	No	No	Non infected (Negative)
20	44		No	No	No	Non infected (Negative)
21	45	1.5hr	No	No	No	Non infected (Negative)
21	46		No	No	No	Non infected (Negative)
21	47		No	No	No	Non infected
22	48	1.75hr	No	No	No	Non infected
23	49	17hr	No	No	No	Non infected
23	50		No	No	No	Non infected
24	51	72hr	Yes	Yes	No (38.6°C)	Infected
24	52		Yes	Yes	No (38.6°C)	Infected
25	53	1.5hr	No	No	No	Non infected
25	54		No	No	No	Non infected
25	55		No	No	No	Non infected
26	56	2hr	No	No	No	Non infected
26	57		No	No	No	Non infected (Negative)

Patient number	Bite reference	Time	Purulence	Wound cytology	Pyrexia	Infected Non infected Negative
27	58	1.5hr	No	No	No	Non infected (Negative)
27	59		No	No	No	Non infected (Negative)
27	60		No	No	No	Non infected (Negative)
28	61	1.5hr	No	No	No	Non infected
28	62		No	No	No	Non infected
28	63		No	No	No	Non infected
29	64	8hr	No	Yes	Yes (39.7^{o}C)	Infected
29	65		No	No	No	Non infected
29	66		No	Yes	No (39.5^{o}C)	Non infected (Negative)
30	67	2hr	No	No	No	Non infected
30	68		No	No	No	Non infected
31	69	72hr	Yes	Yes	No (39.0^{o}C)	Infected
32	70	48hr	Yes	Yes	No (39.3^{o}C)	Infected
32	71		Yes	Yes	No (39.3^{o}C)	Infected
33	72	4.5hr	No	No	No	Non infected
33	73		No	No	No	Non infected
34	74	1.5hr	No	No	No	Non infected
34	75		No	No	No	Non infected
35	76	5.5hr	No	No	No	Non infected
35	77		No	No	No	Non infected
36	78	1hr	No	No	No	Non infected
36	79		No	No	No	Non infected (Negative)
37	80	2.5hr	No	No	No	Non infected
38	81	72hr	Yes	Yes	Yes (39.9^{o}C)	Infected
38	82		Yes	Yes	Yes (39.9^{o}C)	Infected
39	83	24hr	No	No	No	Non infected
40	84	0.5hr	No	No	No	Non infected
41	85	1hr	No	No	No	Non infected
41	86		No	No	No	Non infected
41	87		No	No	No	Non infected
42	88	4.5hr	No	No	No	Non infected
43	89	8hr	Yes	No	Yes (40.3^{o}C)	Infected
43	90		No	No	No	Non infected
44	91	24hr	No	Yes	No	Non infected
44	92		No	Yes	No	Non infected
45	93	1.5hr	No	No	No	Non infected
46	94	3.5hr	No	No	No	Non infected (Negative)
46	95		No	No	No	Non infected (Negative)
46	96		No	No	No	Non infected (Negative)
47	97	1.5hr	No	No	No	Non infected (Negative)
47	98		No	No	No	Non infected (Negative)
48	99		No	No	Yes (40.8^{o}C)	Non infected
48	100	24hr	Yes	Yes	Yes (40.8^{o}C)	Infected
49	101		No	No	No	Non infected
49	102	6hr	N	No	No	Non infected
50	103	3hr	No	No	No	Non infected
50	104		No	No	No	Non infected

Table A-3. Skin and oral cavity culture results and infection status.

Patient Number	Infection Status	Skin Culture		Oral Cavity Culture	
36	Non infected	*S. canis* *Staphylococcus 1* *Staphylococcus 2* *Bacillus* sp	*P. multocida*	*S. canis* *Staphylococcus 1*	*P. multocida* *Flavobacterium*
37	Non infected		*Yersinia pseudotuberculosis*	*S. milleri*	*E. coli* *P. multocida*
38	Infection	*S. intermedius* *Staphylococcus* sp *Corynebacterium bovis*		*Corynebacterium bovis*	*P. canis*
39	Non infected	*S. intermedius*	*Moraxella 1*	*S. intermedius*	*Moraxella 2* *P. multocida*
40	Non infected	*P. aeruginosa*		*Moraxella*	
41	Non infected	*S. epidermidis* *Streptococcus* sp. *Actinomyces canis*	*Moraxella* *P. multocida*	*Actinomyces canis*	*Moraxella* *Prevotella melaninogenica*
42	Non infected		*Moraxella* sp.	*P. multocida* *Moraxella* sp.	
43	Infected	*S. intermedius* *Bacillus* sp.		*A. neuii anitratus*	*P. multocida* *P. canis*
44	Non infected	*S. intermedius* *Bacillus* sp.	*P. multocida*	*A. neuii neuii* *A. odontolyticus* *Bacillus* sp.	*P. multocida*
45	Non infected	*Bacillus* sp.		*S. intermedius* *Actinomyces* sp.	*V. cholerae*
46	Non infected	*S. intermedius* *E. durans*		*Dermatophilus congolensis*	*P. multocida* *Flavobacterium* *Plesiomonas shigelloides*
47	Non infected	*Bacillus* sp virudans *Streptococcus*	*A. lwoffii* *Pseudomonas*	.	*Pseudomonas* *P. canis* *P. multocida* *A. lwoffi* *(Candida albicans)*
48	Non infected	*Bacillus* sp *S. intermedius* *S. equisimilis*		Virudans *Streptococcus*	*Actinobacillus* *P. multocida* *P. canis*
49	Non infected	*Clostridium perfringens* *Bacillus* sp. *Enterococcus faecium*		*Bacillus* sp.	*Flavobacterium* sp.
50	Non infected	*S. intermedius* *Corynebacterium* sp.	*E. coli*		*E. coli* *P. multocida* *Flavobacterium* sp.