Bacterial profiles and antibiograms of the bacteria isolated of the exposed pulps of dog and cheetah canine teeth

A dissertation submitted to the
Faculty of Veterinary Science,
University of Pretoria.

In partial fulfillment of the
requirements for the degree
Master of Science (Veterinary Science)

Promoter: Dr. Gerhard Steenkamp
Co-promoter: Ms. Anna-Mari Bosman

Department of Companion Animal Clinical Studies
Faculty of Veterinary Science
University of Pretoria

Pretoria (January 2012)

J.C. Almansa Ruiz

Declaration

I declare that the dissertation that I hereby submit for the Masters of Science degree in Veterinary Science at the University of Pretoria has not previously been submitted by me for degree purposes at any other university.

J.C. Almansa Ruiz

Dedications

To one of the most amazing hunters of the African bush, the Cheetah, that has made me dream since I was a child, and the closest I had been to one, before starting this project was in National Geographic documentaries. I wish all of them a better future in which their habitat will be more respected.

To all conservationists, especially to Carla Conradie and Dave Houghton, for spending their lives saving these animals which are suffering from the consequences of the encroachment of human beings into their territory. Some, such as George Adamson, the lion conservationist, even lost their lives in this mission. To the conservationist, Lawrence Anthony, for risking his life in a suicide mission to save the animals in the Baghdad Zoo, when the conflict in Iraq exploded.

To my mother and father José Maria and Rosa. Thank you for always being with me, even when you are far away, and for letting me fulfill my dreams.

To my uncle and untie, Prof. Luis Ruiz and Prof. Maria Jesús Fernández, you were the ones which made me love this profession.

To my love Keri-lee.
Thank you for your support and understanding, and for always being there when I need you. Te quiere tu alma gemela.

Acknowledgements

To Dr. Peter Emily and his Foundation, thank you for partly sponsoring this research.

To the staff of the University of Pretoria for their time and financial assistance which has allowed me to perform this study.

To the Africat Foundation and to the Ann Van Dyk Cheetah Centre, for their support, and facilities which enabled me to undertake the project.

I would especially like to thank Dr. Gerhard Steenkamp, my promoter, for his support patience and active involvement in my training.

Miss Anna-Mari Bosman, Prof. Estelle Venter, Sr. Tammy Fisher and Sr. Michelle Cruywagen and all the personnel of the Bacteriology laboratory, without their help and guidance the study would not have been possible.

Also, I thank Dr. Macarena Sanz for always being there to lend me a hand in whatever I needed in dealing with all the bureaucracy, as well as for her critical opinion about any matter I showed her.

Summary

Objectives: The aims of this study was to investigate the RC microbiota in CCF canine teeth in the domestic dogs (*Canis familiaris*) and cheetahs (*Acinonyx jubatus*), identify the possible factors related to the presence of aerobic or anaerobic bacteria and evaluate and evaluate antibiotic susceptibility of bacteria isolated.

Animals: Thirty nine animals suffering from CCF of their canine teeth were included in this study, of which 20 were dogs and 19 were cheetahs.

Procedures: Evaluation of the oral cavity of animals while under general anaesthesia was performed and those without necrotic pulps or those that had received antibiotic therapy in the previous two weeks were excluded. Microbial samples were taken from 63 RC of which 27 were from dogs and 36 were from cheetahs. Strict anaerobic and aerobic techniques were used in parallel for plating, incubation and identification of the bacteria isolated in this manner. In an attempt to evaluate the sensitivity of the culture media and anaerobic technique used, additional samples were collected after the samples for bacterial isolation had been taken from the last eight pulps. These comprised those from six cheetahs and two dogs and were analysed using culture techniques and an initial screening with the 16S rRNA-specific PCR.

Results:

- **Dogs:** A total of 49 cultivable isolates were recovered belonging to 19 different bacterial species and 13 different genera. Individual RC yielded a maximum of four bacterial species. Of the bacterial isolates, 4.08 % were strict anaerobes, being represented by *Clostridium acetobulitycum* (2.04 %) and *Prevotella melalinogenica* (2.04 %). The incidence of aerobic bacteria and facultative anaerobic bacteria in this study were 18.36 % and 77.56 % respectively of all the bacterial isolates. Of these *Pasteurella multocida* (10.20 %), *Corynebacterium* spp. (10.20 %), *Moraxella* spp. (8.17 %), *Bacillus* spp. (6.12 %), *Aeromonas salmonicida* (6.12 %), *Escherichia coli* (6.12 %) and *Pseudomonas aeruginosa* (6.12 %) were the bacteria most frequently isolated. In summary, the RC microflora was found to be predominantly Gram negative facultative anaerobic microorganisms. The antibiotic agents that showed the highest efficacy *in vitro* against the different bacteria isolates were Enrofloxacin (85.21 %), Gentamicin (92.39 %), Chloramphenicol (89.13 %).

- **Cheetahs:** A total of 59 cultivable isolates, belonging to 19 different microbial species and 13 different genera were recovered from 36 RC sampled. Thirty-two (54.49 %) of the cultivable isolates were Gram positive while 27 (45.71 %) were Gram negative. Individual root canals each yielded a maximum of six species. Four RC had no cultivable bacteria. The bacterial microflora recovered from the RC of the animals showed a higher number of facultative anaerobes (62.72 % of all the bacterial isolates). Aerobic isolates were 28.81 %, and strict anaerobes 8.47 % of all the isolates. The latter species comprised *Clostridium sordelli* (5.08 %), and *Clostridium septicum* (3.38 %). The species with the highest isolation frequency were *Bacillus* spp. (22.13 %), *Pasteurella multocida* (10.16 %), *Corynebacterium* spp. (8.47 %), *Enterococcus* spp. (8.47 %), *Moraxella* spp. (8.47 %) and *Pseudomonas aeruginosa* (5.25 %). In summary, the bacteria isolated from the RC were Gram positive facultative anaerobic bacteria. The antibiotics, which showed the highest efficacy *in vitro* against the different bacteria isolates, were Enrofloxacin (91.96 %), Gentamicin (86.37 %) and Orbifloxacin (86.28 %).

- **Nucleic Acid-Base detection:** In dogs, Gram negative and Gram positive bacterial species were equally represented. Anaerobic bacterial species predominated at 83.3 % (5/6) of the species detected. On the other hand, in cheetahs, the bacterial species isolated by the PCR method showed a prevalence of anaerobic bacteria (60.8 %, 14/23), while facultative anaerobes were isolated in 30.2 % (7/23) of cases and aerobic bacteria in 8.6 % (2/23).

Conclusions and Clinical Relevance: This study has indicated that the microbial flora in any single infected RC is much more diverse than it has been shown using cultural techniques alone and can contain potentially uncultivable bacterial species. Some of these species may represent potentially new phylotypes, which may be involved in endodontic infections and ultimatelyin periradicular periodontitis, and should therefore be considered in any future studies involved in defining endodontic pathogens.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

AF	Africat Foundation
BLAST	Basic Local Alignement Search Tool
CBA	Citrated Horse Agar
CCF	Complicated Crown Fracture
CDC	Centers for Disease Control
CLSI	Clinical and Laboratory Standards Institute (CLSI)
MAC	MacConkey Agar
NCBI	National Centre of Biotechnology
OVAH	Onderstepoort Veterinary Academic Hospital
PCR	Polymerase Chain Reaction
RCT	Root Canal Treatment
TAVDCC	The Ann Van Dyk Cheetah Centre
UP	University of Pretoria
VAST	Veterinary Antimicrobial Susceptibility Testing
104	Right maxillary canine tooth
204	Left maxillary canine tooth
304	Left mandibular canine tooth
404	Right mandibular canine tooth

Chapter 1
Introduction

Endodontics is the branch of dentistry that addresses the diseases of the dental pulp. RCT is a routine procedure in humans and small animals, although it is performed sporadically in many other species of animals (1).

The goal of endodontic treatment, for example, RCT, is the attempt to retain periodontically sound strategic teeth that are affected with pulp injury. To achieve this goal, many new materials having different properties, and new techniques have been applied to improve the outcome of RCT. The role of microbes and their antimicrobial susceptibilities in both acute and chronic infections of the dental pulp in humans has been well studied (2-7). However, studies lack in veterinary dentistry. It is also important to note, that animals have different tooth anatomy and resident oral microflora compared to humans (8).

When teeth are subjected to RCT using aseptic techniques and according to accepted clinical principles, the success rate is generally high. Most follow-up studies in humans on endodontic therapy report overall success rates of 85 % to 90 % (9, 10); in veterinary dentistry the success rate is slightly higher, 95 % (11). Many cases which fail to respond to the treatment are the result of technical problems which arise during treatment, but some cases fail when apparently well treated. A number of factors have been associated with the failure of endodontic therapy including extraradicular infection, foreign body reactions and the presence of periradicular cysts (12-15). In veterinary dentistry the evidence of a preoperative periapical lucency, preoperative pulp necrosis, preoperative root resorption and the kind of tooth treated have been reported as factors that decrease the success rate when performing non surgical RCT (11). However, most treatment failures are caused by microorganisms persisting in the apical parts of RC of obturated teeth (16) and, for this reason, the application of appropriate antibiotics either locally or systemically should improve the success rate of this procedure (16).

In carnivorous animals, the canine teeth which are used for prey prehension and ripping, as well as for fighting, tend to be the most susceptible to traumatic injury. Therefore, domestic dogs (*Canis familiaris*) are the non-human species that most often require RCT. Cheetahs (*Acinonyx jubatus*) are endangered African carnivores that have been saved from extinction by successfully breeding them in captivity for later release in the wild. Any serious damage to their canine teeth

will prevent them from successfully hunting prey. These teeth are also used by cheetah in the mating ritual and hence reproduction. The Dental Clinic of the Veterinary Academic Hospital of the Faculty of Veterinary Science, University of Pretoria which is situated at Onderstepoort, Pretoria, South Africa is in a unique position in that the veterinary dentists are called upon to perform RCT on fractured teeth and worn teeth in domestic dogs and cheetahs as well as other animal species. The clinic assists in the animal dental health programmes of organisations such as TAVDCC in South Africa which operates a cheetah breeding programme, and a similar organisation, AF in Namibia. In captive cheetahs one of the main problems found is exposure of the pulp because of abrasion of the teeth (17). However, treatment of dental infections in dogs and cheetahs is limited as there is very little knowledge on the nature of the endodontic microflora as well as on their antimicrobial susceptibility.

For these reasons therefore the subject of the research work reported here is focussed on the isolation and identification of bacteria found in teeth with CCF in both the domestic dog and cheetah. In addition a variety of antibiotics were tested against the bacteria isolated in order to determine an appropriate antibiotic for use as a co-adjuvant antimicrobial agent when performing non-surgical root canal procedures in dogs and cheetahs with CCF of their canine teeth.

Chapter 2
Literature Review

Root canal therapy is commonly performed in veterinary dentistry in an attempt to retain periodontally sound strategic teeth that are affected by pulpal injury (18, 19). Bacteria present in the pulp canal have the potential of spreading to the surrounding alveolar bone through the apical delta. Once this has occurred periapical infection and inflammation will develop that may ultimately present as a periapical lesion (4, 20-34). Many studies in human dentistry have been done in which the bacteria from injured pulp were isolated and identified (4-7, 16, 21, 23, 27, 34-76); however, in veterinary dentistry just one study has been conducted according to the international literature (8).

The success of endodontic treatment is directly influenced by the control of the invading micro-organisms in infected RC (39, 77, 78). It is therefore important to consider the type of infection present when planning a treatment protocol (39, 77, 78). The primary RC infection is associated with endodontic microbiota generally composed of Gram negative anaerobic bacteria (39, 77, 78). Of the major dental diseases, infection of the root canal is unique since infection establishes where micro organisms have not previously been present. Other microbial diseases of the oral cavity, such as caries and periodontal disease, develop at sites where a microbial biofilm is already established and disease occurs with a change in the environmental conditions as well as the type and mix of microbial flora (79). Cleaning the RC is always difficult in endodontics in humans and dogs as dentin has a tubular histological structure (Figure 1) and complex anatomy (18).

Even though most necrotic pulp tissue is removed during chemomechanical procedures, remaining bacteria can also use necrotic tissue remnants as a nutrient source (80-82). Tissue remnants can be localized in isthmi, irregularities, dentinal tubules, and lateral canals, which very often remain unaffected by instruments and irrigants (80-82). There may even be part of the canal that remains untouched after thorough instrumentation (80-82). Most micro-organisms are located inside the main RC, yet they can also be observed in the dentinal tubules, RC branches and cement lacunae (83, 84). In vitro studies have shown that bacteria are able to penetrate dentinal tubules of the root up to 800 micrometers, when the cementum is removed from the root surface and the smear layer from the RC wall (85-87). In case of traumatic injuries

to the teeth resulting in pulpitis and damage to the cementum, it has been shown that bacteria can penetrate the dentinal tubules and contribute to external inflammatory resorption in monkeys (88, 89).

Microbes can reach the pulp by one of these five ways (2, 29, 67):

- Periodontal disease, exposing the accessory canals and apical foramina. Due to the perio-endolesions caused by periodontitis (90).
- Traumatic tooth fracture or pathological exposure due to tooth wear (91).
- Exposure during dental treatment.
- Extension from caries lesions into the dentine, and spread of bacteria to the pulp via the dentinal tubules. There is a low incidence of caries in dogs due to the pH of the saliva, the anatomy of the cusps, and the oral bacteria (92).
- Anachoresis, a process whereby bacteria can reach the pulp of teeth with clinically intact crowns through bacteraemia. In these cases a necrotic pulp is found in a tooth with a clinically intact crown.

Endodontic infection diffuses through the root canal system and is polymicrobial (26, 93). Any microorganism of the oral cavity, upper respiratory tract or gastrointestinal tract can gain access to the root canal system, but the species and the combination of the microbial flora developed are in response to a complex interaction between the local environment within the tooth and other microflora that may be present (94). In the first stages of pulpitis facultative anaerobic streptococci as well as staphylococci may be found. With time, pulpal necrosis and periapical lesions develop and many obligate anaerobes are then found. Zavistosky *et al.,* (1980) have studied the quantity of bacteria that are present in the RC of necrotic teeth in humans, showing that the amount is similar to the bacterial concentration in other anatomical areas in the presence of infections. The most frequently isolated bacteria inside the RC of infected teeth in humans are obligate anaerobes (*Fusobacterium nucleatum, Porphyromonas gingivalis, Prevotella melaninogenica,* and *Actynomyces odontolyticus),* facultative aerobes-anaerobes (*Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Aggregatibacter* (formerly *Actinobacillus*) *actinomycetemencomitans, Streptococcus mitis, Streptococcus mutans, Streptococcus salivarius,* and *Streptococcus sanguis* (64). Several studies investigated the possibility that certain bacteria may be responsible for specific clinical signs and symptoms (2, 95-98).

Endodontic microbiota (31, 99) and their products (60) are responsible for the accumulation of inflammatory cells in the periapical region (31), as well as biomechanical changes, such as changes in pH (25), the presence of inmunoglobulins (100), metabolites of arachidonic acid (31), enzymes (101) and pro-inflammatory cytokines (31). In addition to specific enzymes, actively metabolising and dying Gram negative bacteria release endotoxins from their outer cell walls (18). Endotoxins initiate the inflammatory response by triggering the release of pro-inflammatory cytokines from macrophages, neutrophils, lymphocytes (39, 78, 102, 103). Theobald Smith (1921) said that an infectious disease is the result of the interplay between microbial virulence, the number (load) of bacteria and the host response.

The opportunities for invading the root canal system are the same for all the bacteria within the oral cavity, but only a restricted group of species has been identified in infected RC (58, 62, 75). The reason for the disproportionate ratio between potential and actual number of species is that the RC is an unique environment where biological selection plays an important role driving the type and course of infection (38). An anaerobic milieu, interactions between microbial factors and the availability of nutrition are principal factors that define the composition of the microbial flora (38). The type and availability of nutrients is important in establishing microbial growth. Nutrients may be derived from the oral cavity, dentinal tubule contents, degenerating connective tissue, or a serum-like fluid from periapical tissue (38). Exogenous nutrients, such as fermentable carbohydrates, affect the microbial ecology of the coronal part of an exposed RC by promoting growth of species that primarily obtain energy by carbohydrate fermentation (38). Endogenous proteins and glycoproteins are the principal nutrients in the main body of the root canal system and this substrate encourages the growth of anaerobic bacteria capable of fermenting amino acids and peptides. After degradation of pulp tissue a sustainable source of proteins develops because bacteria induce periapical inflammation that leads to an influx of serum-like exudates into the canal (38). This fluid contains proteins and glycoproteins, and the bacteria that dominate this stage of the infection are likely to be those that either have a proteolytic capacity, or maintain a cooperative synergy with those that can utilize this substrate for bacterial metabolism (38). Oxygen and oxygen products play and important role as ecological determinants in the development of specific proportions of the RC microflora (88, 104).

Even when endodontic treatment does not succeed in completely eradicating the infection, the majority of bacteria are eliminated and the environment is markedly disturbed (6). To survive and therefore be detected in post-treatment samples, bacteria have to resist or escape intracanal

disinfection procedures and rapidly adapt to the drastically altered environment caused by treatment procedures (6). Bacteria detected in post-instrumentation samples in humans are remainders of the initial infection that resisted the effects of instruments and irrigants or were introduced in the RC as a result of a breach in the aseptic chain (6). Several strategies may help bacteria to resist treatment and persist in this environment. Possibly the most important survival tactic used by endodontic bacteria is to convert from the planktonic form to the sessile form by adhering to the RC walls, accumulating and producing a viscuous extracellular polymeric substance (EPS) which enables them to form highly organised multi-cellular, polymicrobial communities. These adherent communities are known as "biofilms" and communicate via signalling molecules, the so-called "quorum sensing" so that they can collaborate collectively to harvest nutrients and display some unusual -"survival tactics"- (40, 45, 46, 102). In the RC not only does the EPS act as a surface adherent but also forms a barrier to inflammatory cells, immunoglobulins, oxidising or charged biocides, metallic cations and some antibiotics (105). In fact, antibiotics have to be up to 1 000 times more concentrated than their minimum inhibitory concentration for planktonic bacteria to be effective (105). Furthermore a portion of the bacteria in biofilms are starved and in a stationary phase rendering them much more resistant to antibiotics as many antibiotics, especially the β-lactam antibiotics, require actively metabolizing bacteria to be effective. Stress regulon proteins produced by these bacteria can result in the up-regulation of efflux pumps which further enhances their resistance to antibiotics. These communities of bacteria live in close association and thus any transferrable antibacterial resistance coding genes can easily be transferred both within a species and to closely-related species (40). Duggan and Sedgley (2007) tested the hypothesis that the ability of *E. faecalis* to form biofilm is related to the source of the strains. Biofilm formation might be an important factor when considering the virulence phenotype of endodontic strains in general (106). These biofilms are found mainly on the walls of the RC, apical delta canals and the areas of apical cement resorption (103).

Chávez de Paz *et al.* (2007) evaluated the possible role of biofilm communities. Changes in the environment, such as calcium hydroxide-related pH increase or the effect of antimicrobials, are capable of triggering genetic cascades that modify the physiological characteristics of bacterial cells. An example of mechanism triggering in *E. faecalis*, is the activation of ion-transport systems to balance intracellular and extracellular pH levels, as a response to high pH proton-motive force (107, 108). Another factor that can result in the pre-resistance of bacteria in the RC is the development of antimicrobial resistance via selection pressure i.e. the concentration of antibiotic is such that only the highly susceptible members of a population will be killed. This

provides the more resistant members with a competitive advantage and thus they become the prevalent population (108). Furthermore, some bacteria in the presence of sub-lethal concentrations of antibiotics can be stimulated to mutate into a more resistant genotype. The classic example is a single base pair change in the *gyr*A gene will render a Gram negative bacterium resistant to the quinolones and a further base pair change in the same gene or in *gyr*B or *par*C will render this bacterium resistant to the fluoroquinolones. For these reasons bacterial resistance to antibiotics may take place very rapidly in evolutionary time (108).

Cultivation and other traditional identification methods have been demonstrated to have several limitations when it comes to microbial diagnosis (109), including the probable contribution of viable but uncultivable bacteria to disease and insufficient bacterial characterisation (110). Molecular genetic methods can sidestep many of these limitations associated with culture approaches (111). The past decade has brought many advances in microbial molecular diagnostics, the most prolific being in DNA-DNA hybridization as well as in polymerase chain reaction (PCR) technology and its derivates. Indeed, findings from cultivation-based methods with regard to the microbiota living in diverse ecosystems have been supplemented and significantly expanded with molecular biology techniques, and the impact of these methods on the knowledge about the oral microbiota in healthy and diseased conditions is astonishing (112). Bacteria detected from the oral cavity in humans fall into 11 phyla that comprise over 700 different species or phylotypes (113, 114). About 40-50 % of these bacteria are novel uncultivated species or phylotypes, which are known only by 16S rDNA sequences (113). This raises the interesting possibility that uncultivated and as-yet uncharacterised species that have remained invisible to studies using traditional identification methods may make up a large fraction of the living oral microbiota, and may participate in the aetiology of oral diseases (112). Table 1 represents a review of the bacteria isolated by several authors using traditional culture techniques and molecular methods; the diversity of species isolated using molecular methods can be noticed.

In fact, the introduction of molecular approaches in oral microbiology research has brought about a significant body of new knowledge with regard to the human oral microbiota in health and disease. The development of molecular bacterial identification methods has made it possible to study the role that uncultivable bacteria or even other micro-organisms such as Archaea (group of single-celled microorganisms without cell nucleus or any other membrane-bound organelles within their cells) play in oral diseases in humans (115-118). Consequently, a significant revolution in the knowledge of the human oral microbiota in health and disease has

taken place after the advent of molecular techniques for microbial identification. In this context, endodontic infections are far from being an exception (112).

Because of the physical constraints of the root canal system, obtaining a representative sample from this site is not often an easy task. As a consequence, the number of cells sampled can fall short of the detection rate of the identification method and the prevalence of a given species can be underestimated (69). As is commented on above, the development of effective strategies for root canal therapy is dependent upon understanding the composition of the pathogenic flora of the root canal system (35). Identification of the RC isolates from previous studies has traditionally been performed using standard microbiological and biomechanical techniques. However, correlation of the microbiological findings from these studies is affected by certain limitations of the culture techniques, leading to the underestimation of bacterial diversity within the root canal system (35, 119). Several molecular techniques have been used in humans to detect bacteria in endodontic infections using oligonucleotide probes (120) and chequerboard DNA-DNA hybridization analysis (121). However, the use of specific DNA probes limits the boundaries of the detection technique, as it assumes that these probes target the species of importance. The species selected are based on culture studies and do not account for any uncultivated bacteria or uncultivable biotypes of known species (35). Techniques utilizing the 16S rRNA gene sequence data have been developed for use in the field of microbial ecology to evaluate the members of diverse microbial communities included uncultivable microorganisms (122-124). These techniques have been adapted to study uncultivable microorganisms involved in disease (125); to study the bacterial diversity in dentoalveolar abscesses (126), subgingival plaque (127) and saliva (128); and to investigate the eubacterial and spirochete species involved in periodontitis (129, 130).

Polymerase chain reaction (PCR) – derived techniques show many limitations that can arise from variations in technology. The issues related with the ability of PCR to detect either a very low number of cells or dead cells are of special interest when one interprets the results of PCR identification procedures in endodontic microbiota research (112).

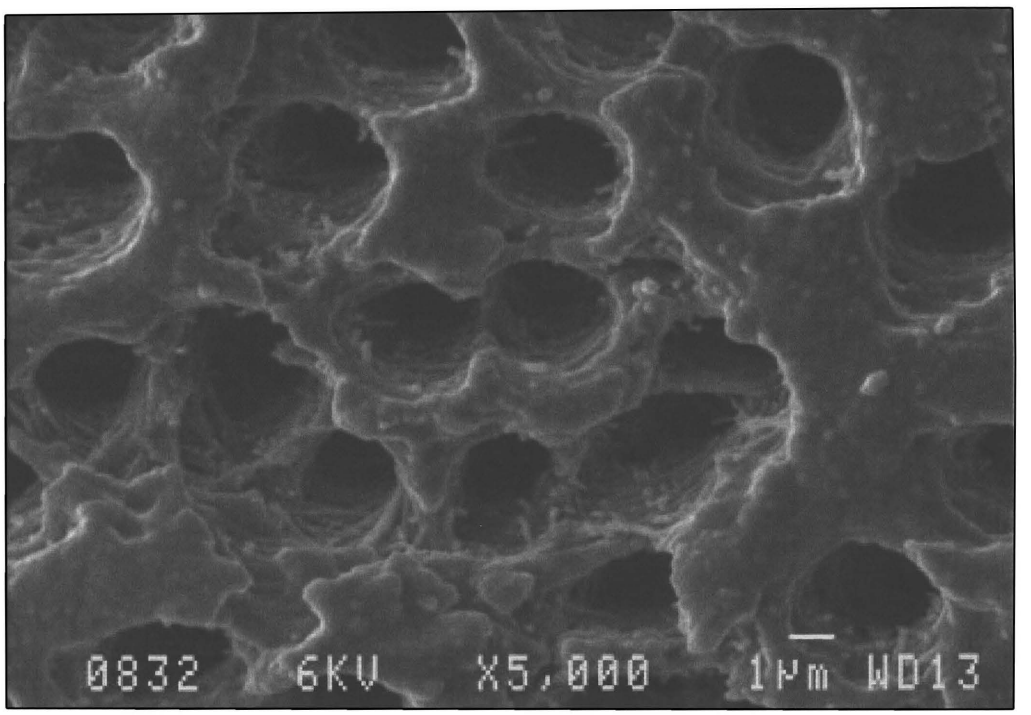

Figure 1 A scanning electron photograph of the dentin tubular structure, lining the pulp canal in the dog. (Courtesy Prof. Sonja Boy)

Table 1 Comparison review of the different bacteria isolated from infected human RC by several authors using traditional culture techniques and molecular methods (4, 5, 7, 33, 35, 63, 69, 131).

Bacterium	Culture	Molecular Methods
Enterococcus faecalis	(5, 7)	(35, 63, 69)
Streptococcus spp.		(63, 69)
Streptococcus anginosus	(4, 29)	(35, 131)
Streptococcus constellatus	(7, 33)	(131)
Streptococcus intermedius	(5, 7)	(35, 131)
Streptococcus mitis	(5, 7, 33)	(35)
Streptococcus mutans	(5)	
Streptococcus parasanguis	(7)	
Streptococcus oralis	(5, 33)	
Streptococcus sanguis	(4)	(35)
Streptococcus salivaris		(35)
Streptococcus gordonii		(35)
Peptostreptococcus spp.	(5, 33)	(35)
Peptostreptococcus asaccharolyticus	(5)	
Peptosreptococcus micros	(7, 33)	(69)
Peptostreptococcus prevotii	(5, 33)	
Peptostreptococcus anaerobius	(33)	
Actinomyces naeslundii	(5)	
Actinomyces meyeri	(5)	
Actinomyces odontolyticus	(4)	
Actinomyces radicidentis		(69)
Actinomyces viscosus	(4)	
Actinomyces israelii	(7)	(69)
Pseudoramibacter alactolyticus	(7)	(69)
Eubacterium spp.		(35)
Eubacterium timidum	(7)	
Eubacterium yurii		(35)
Eubacterium infirmum		(63, 131)
Eubacterium lentum	(5, 33)	
Lactobacillus spp.	(5)	(35, 63)
Lactobacillus salivarus	(5)	
Lactobacillus fermentum strain		(35)
Lactobacillus paracasei	(5)	(35)
Lactobacillus mucosae		(131)
Lactobacillus catenaforme	(7)	
Lactobacillus acidophilus	(5, 33)	
Propionibacterium spp.		(35)
Propionibacterium acnes	(7)	(35)

Table 1 continued

Bacterium	Culture	Molecular Methods
Propionibacterium propionicum		(63, 69)
Propionibacterium propionicus	(5, 7)	
Fusobacterium nucleatum	(5, 7, 33)	(35, 69)
Bacteroides fragilis	(4)	
Bacteroides gracilis	(7, 33)	
Gemella morbillorum	(5, 33)	
Gemella haemolysans	(33)	(35)
Prevotella spp.		(35)
Prevotella oris		(35)
Prevotella intermedia / Prevotella nigrescens	(5, 33)	(63, 69)
Prevotella denticola	(5)	
Prevotella corporis	(33)	
Prevotella baroniae		(63)
Prevotella loescheii	(5, 33)	
Prevotella tanerae		(63)
Prevotella oralis	(5)	
Prevotella nigrescens		(35)
Prevotella bucae	(5)	
Prevotella multisaccharivorax		(63)
Prevotella bucallis	(5)	
Veillonella spp.	(5, 33)	(35)
Veillonella dispar		(35, 131)
Veillonella parvula		(131)
Staphylococcus spp.	(5)	
Staphylococcus saccharolyticus	(33)	
Staphylococcus epidermides	(33)	
Cardiobacterium hominis	(33)	
Clostridium spp.	(5)	
Clostridium butyricum	(5)	
Clostridium clostriidiforme	(5)	
Clostridium bifermentans	(5)	
Clostridium subterminale	(5, 33)	
Tissierella praecuta	(33)	
Bifidobacterium adolescentis	(5)	
Bifidobacterium spp.	(5)	
Leuconostoc spp.	(5)	
Capnocytophaga sp.	(5)	
Capnocytophaga gingivalis		(35)
Porphyromonas gingivalis	(4)	(63, 69)

Table 1 continued

Bacterium	Culture	Molecular Methods
Porphyromonas endodontalis		(69)
Pantoea spp.		(35)
Selenomonas spp.		(35)
Selenomonas sputigena		(63, 131)
Selenomonas infelix		(35)
Cytophaga spp.		(35)
Dialister spp.		(35)
Dialister pneumosintes		(63, 69)
Dialister invisus		(63, 131)
Mogibacterium spp.		(35)
Mogibacterium neglectum / Mogibacterium punilum / Mogibacterium diversum / Mogibacterium vescum		(131)
Solobacterium moorei		(35, 63)
Atopobium rimae		(131)
Filifactor alocis		(63, 69)
Tanerella forsythia		(63)
Tanerella forsythensis		(69)
Campylobacter rectus		(69)
Campylobacter gracilis		(69, 131)
Treponema denticola		(63, 69, 131)
Treponema socranskii		(63)
Bulleidia extructa		(63)
Johnsonella ignava		(63)
Anaeroglobus geminatus		(63)
Olsenella genomosp. C1		(131)
Scardovia inopinata		(131)
Pseudomonas mephitica		(131)

Chapter 3
Materials and Methods

3.1 Patient selection

Only those animals showing necrotic pulps (Figure 2) were sampled, because we expected to find more anaerobic bacteria in them due to the environmental conditions in them. A tooth was deemed having a necrotic pulp when no bleeding was observed during the sampling procedure. No radiographic facilities were available in Namibia and for standardisation the same criteria was applied to the animals seen at the Dental clinic.

3.1.1 Dogs

All the dogs included in this study are dogs that live in South Africa, and include both working dogs and ordinary pets. These were presented to the Dental and Maxillofacial Surgery Clinic of the OVAH suffering from CCF, which required treatment (Figure 3).

For each dog the owner, sex, age, diet, weight, dental record, and the specific breed were recorded on a dental record sheet (Figure 4).

3.1.2 Cheetahs

The cheetahs used in this study, originated from the AF and TAVDCC. The Africat Foundation is situated in Namibia (Figure 5), south-west of Otjiwarongo on Okonjima farm (S 20° 51' 59" E 16° 38' 22"), where all cheetahs are kept in captivity in large enclosures having a minimum size of one hectare per animal. The Ann Van Dyk Cheetah Centre is a captive breeding facility close to Hartbeespoort Dam (Figure 6), north-west of Pretoria, South Africa (S 025° 40´ 421´´ E 027° 55° 423´´).

For each cheetah the sex, age, diet, weight and dental record were recorded on a dental record sheet (Figure 7).

All the cheetahs requiring therapy at AF were treated during the winter months when the Dental and Maxillofacial Surgery Clinic conducts its annual dental treatments there. Due to its proximity to the OVAH, all cheetahs from TAVDCC were presented at the Dental and Maxillofacial Surgery Clinic when a fractured tooth was noticed (Figure 8).

3.2 Sampling

Animals should not have received antibiotic therapy for at least two weeks before sample collection in order to be included in this study.

3.2.1 Dogs

All dogs presented underwent the standard evaluation and treatment in the Dental and Maxillofacial Surgery Clinic, which consisted of the following:

1. A full clinical examination was performed including a blood smear.
2. In animals older than eight years, a full blood count was performed and serum creatinine, and serum alkaline transferase levels were determined.
3. An intravenous catheter was placed into the cephalic vein and a crystalloid [Intramed Ringers-lactate solution, Bodene (Pty) Ltd] given at the rate of 10 ml/kg/h for the duration of the anaesthetic procedure.
4. Anaesthetic induction was performed with Propofol 1 % [Propofol 1 %, Fresenius Kabi South Africa (Pty) Ltd] at a dose of 6-8 mg/kg intravenously.
5. A cuffed endotracheal tube was inserted into the trachea and secured. Gaseous anaesthesia was maintained using 2 % Isofluorane [Isofor, Safe line pharmaceuticals (Pty) Ltd, Florida, South Africa].
6. Animals younger than eight years of age were premedicated with Medetomidine [Domitor®, Pfizer Laboratories (Pty) Ltd] at a dose rate of 5-10 µg/kg, but did not exceed a total of 0.1 ml irrespective of the size.
7. They were positioned in lateral recumbency, with the affected canine tooth uppermost.
8. An oral evaluation was performed and recorded on a dental record sheet (Figure 4) before sampling.

9. The RC was first opened using a sterile 25-40 sized Hedstrom- or Kerr-file. In the majority of RC this size file was adequate to open the RC in order to obtain a sample (Figure 11). In RC with necrotic pulp the material is often dry and therefore 2-3 drops of sterile Ringers-lactate [Intramed Ringers-lactate solution, Bodene (Pty) Ltd] was placed on the file while it was in the RC, and filing of the canal was then performed. This addition of Ringers lactate was done in order to place the bacteria present in a suspension.

10. Larger files, 45-70 in size were required in RC of very young dogs in order to also file the dentinal wall. This was necessary to obtain dentin that may have harboured bacteria.

11. A sterile paper point (size 25) was introduced into the root canal at the maximum depth possible using a dressing forceps, it's active part having been first sterilised in a bead steriliser (Dry Steriliser, Hot Glass Bead, Carlo de Giorgi Srl) for 30 seconds (Figure 12). The paper point was left in the canal for one minute (Figure 13).

12. After one minute the paper point was transferred to a 10 ml glass sample bottle containing a deep column of brain heart infusion broth (Difco Laboratories, USA) supplemented with 0.2 % cysteine and 1 % bacteriological grade agar (anaerobic transport medium) (Figure 14). The paper point was inserted into the medium, and the bottle was then placed directly in a refrigerator at 3-5 ºC.

13. Most samples were sent to the laboratory on the same day of sampling. If samples were collected after the time that the laboratory accepts new cases for the day, they were kept in the refrigerator at 3-5 ºC overnight and sent to the laboratory first thing in the morning.

14. All samples were accompanied by a transfer form (Figure 15) containing all the necessary details of the case, including the history.

3.2.2 Cheetahs

When dealing with wild animals it is often impossible to conduct a thorough clinical examination (as outlined for dogs) and therefore they were anaesthetised without the thorough work-up as presented for dogs. Once anaesthetised oral examination was performed in the same manner as described for dogs.

1. Animals were either darted with a pressurised darting system (Dan-Inject International S.A, Skukuza, South Africa) or hand injected in a crate. Some crated cheetahs were tame enough to allow the isolation of a hind leg and placement of an intravenous catheter into the saphenous vein.

2. Anaesthetic induction was performed with Propofol 1 % [Propofol 1 %, Fresenius Kabi South Africa (Pty) Ltd] at a dose of 6-8 ml/kg intravenously for those cheetahs where a catheter could be placed in the hind leg. If this was not possible they were darted (Figure 9) or hand injected with a Ketamine [Ketamine-Fresenius 100mg/1ml, Bodene (Pty) Ltd; 4 mg/kg] / Medetomidine [Domitor®, Pfizer Laboratories (Pty) Ltd; 40 µg/kg] combination of drugs.

3. Once the animal was anaesthetised, an intravenous catheter was placed in the saphenous vein and a crystalloid [Intramed Ringers-lactate solution, Bodene (Pty) Ltd] given at rate of 10 ml/kg/h for the duration of the anaesthetic procedure.

4. An endotracheal tube was then placed and secured and, gaseous anaesthesia was maintained using 2 % Isofluorane [Isofor, Safe line pharmaceuticals (Pty) Ltd, Florida, South Africa].

5. Animals were positioned in lateral recumbency, with the affected canine tooth uppermost (Figure 10).

6. An oral evaluation was performed and recorded on a dental record sheet (Figure 7) before sampling.

7. The RC was first opened using a sterile 25-40 Hedstrom - or Kerr-file (Figure 11). In the majority of RC these size files were adequate to open the RC in order to obtain a sample. In RC with necrotic pulps the material is often dry and therefore 2-3 drops of sterile ringers lactate [Intramed Ringers-lactate solution, Bodene (Pty) Ltd] were placed on the file, while in the RC, and filing of the RC was then performed. This addition of Ringers lactate was done in order to place the bacteria present in a suspension.

8. Larger files 45-70 were required in RC of very young animals in order to also file the dentinal wall to obtain dentin that may have harboured bacteria.

9. A sterile paper point (size 25) was introduced into the root canal at the maximum depth possible using a dressing forceps, it's active part having been first sterilised in a bead steriliser (Dry Steriliser, Hot Glass Bead, Carlo de Giorgi Srl) for 30 seconds (Figure 12). The paper point was left in the canal for one minute (Figure 13).

10. After one minute the paper point was transferred to a sterile container with an anaerobic culture medium (Figure 14). The paper point was inserted into the medium and placed directly into a refrigerator at 3-5 ºC.

11. Most samples were sent to the laboratory on the same day of sampling. If the sample was collected after the time that the laboratory accepts new cases for the day, it was kept in the refrigerator at 3-5 ºC overnight and sent to the laboratory the next morning. Samples collected in Namibia were kept refrigerated for between 4-7 days and submitted upon return to the laboratory.
12. All samples were accompanied by a transfer form (Figure 15).

3.3 Culturing

Once the samples were received by the Bacteriology Laboratory, of the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, the samples were processed according to a specific protocol:

The samples for the isolation of anaerobic bacteria were processed and cultured under anaerobic conditions in an anaerobic globe compartment (Figure 16). In the laboratory they were plated onto plates containing pre-reduced Columbia agar (Oxoid Products, UK) containing 7 % citrated horse blood (CBA), nonreduced CBA and MacConkey agar (MAC) (Oxoid Products, UK). The paper points were then reinserted in the transport medium. All plates and the specimens in transport medium were incubated at 37 ºC for up to 72 h with the pre-reduced CBA plates being incubated under anaerobic conditions, the unreduced CBA in 5 % CO_2 in air, and the MAC plates and specimens incubated in air. The bacteria in the samples were left to grow for a period of 72 h under anaerobic conditions and an environmental temperature of 37ºC.

When growth was noticed (Figure 17), the colonies were subcultured into several plates. Once pure colonies had been grown, they were identified using a specific algorithm (Figure 18).

3.4 Antibiogram

Once bacterial colonies had been established and typed, they were placed on a Mueller-Hinton agar plate containing a disk impregnated with specific antibiotics. The antibiogram analysis followed the currently recommended protocol by the Clinical and Laboratory Standards Institute (CLSI) subcommittee on Veterinary Antimicrobial Susceptibility Testing (VAST) for agar disk diffusion testing; the technique being known as Kirby-Bauer antibiotic testing or disk diffusion antibiotic sensitivity testing (Figure 19 and Figure 20). This is the most complete described

method for which interpretive standards have been developed and supported by clinical and laboratory data. The panel of antibiotics used is showed in Table 2 and Table 3.

3.5 Nucleic acid-based detection

In an attempt to evaluate the sensitivity of the culture media and anaerobic technique used, samples were collected (as described above) from the last eight dental pulps after the samples for bacteriology had been collected. Of these eight samples, six were from cheetahs and two from dogs. Paper points harbouring bacteria were stored in a sterile container with neither growth media nor any preservatives for a minimum of 82 days and a maximum of 220 days (time needed to collect these eight last samples).

The samples were prepared by the Molecular Biology Laboratory, Department of Tropical Diseases, Faculty of Veterinary Science, University of Pretoria and send to Inqababiotec, Pretoria, South Africa for molecular analysis. They based their analysis on a conserved primer pair that amplified 16S rRNA. These amplification products (PCR products) were cloned, sequenced and analysed by Inqababiotec, Pretoria, South Africa.

Sequences were further characterised using the BLAST (Basic Local Alignment Search Tool) function (http://0-blast.ncbi.nlm.nih.gov.innopac.up.ac.za/Blast.cgi). An excel spreadsheet was created with all the accession numbers (National Centre of Biotechnology Information (NCBI)) and the information of all the clones was sequenced.

3.6 Skull measurements

Twenty cheetah skulls that are kept in the OVAH were included in this part of the project. The majority of skulls were from animals that had died in captivity, and were sent for post-mortem analysis; details from some of them are known (i.e. those that died in captivity), but the details of those that died in the wild are unknown. All canine teeth of the animals had to be intact with minimal wear and had no fractures to be included in this study. The following tooth measurements were taken with a steel ruler, marked in millimetres:

- Length of the left (204) and right (104) maxillary canine teeth.
- Length of the left (304) and right (404) mandibular canine teeth.

The measurements were always performed on the buccal surface of the canine teeth, commencing the measurement from the cemento-enamel junction to the occlusal part of the canine teeth (tip of the clinical crown) (Figure 21).

- Distance between both canine teeth of the maxilla and the mandible.

The measurement was performed from the occlusal surface of the left maxillary canine tooth to the occlusal surface of the right maxillary canine tooth. The same measurement was performed between the canine teeth of the mandible (Figure 22).

3.7 Figures

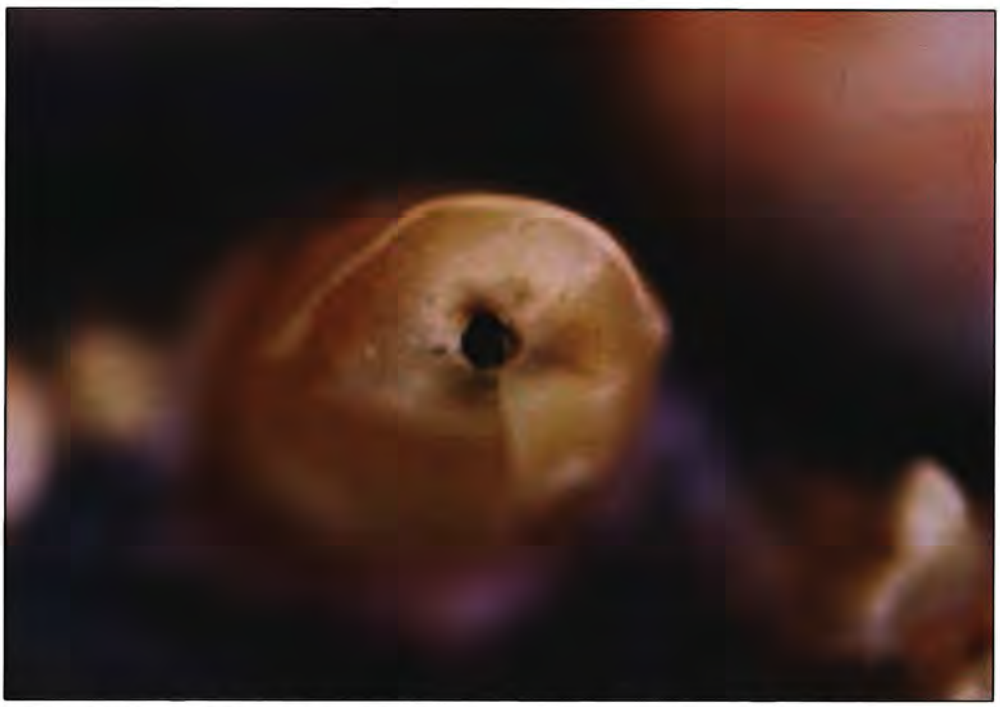

Figure 2 A long-standing CCF of a cheetah's maxillary canine tooth. Note the necrotic pulp.

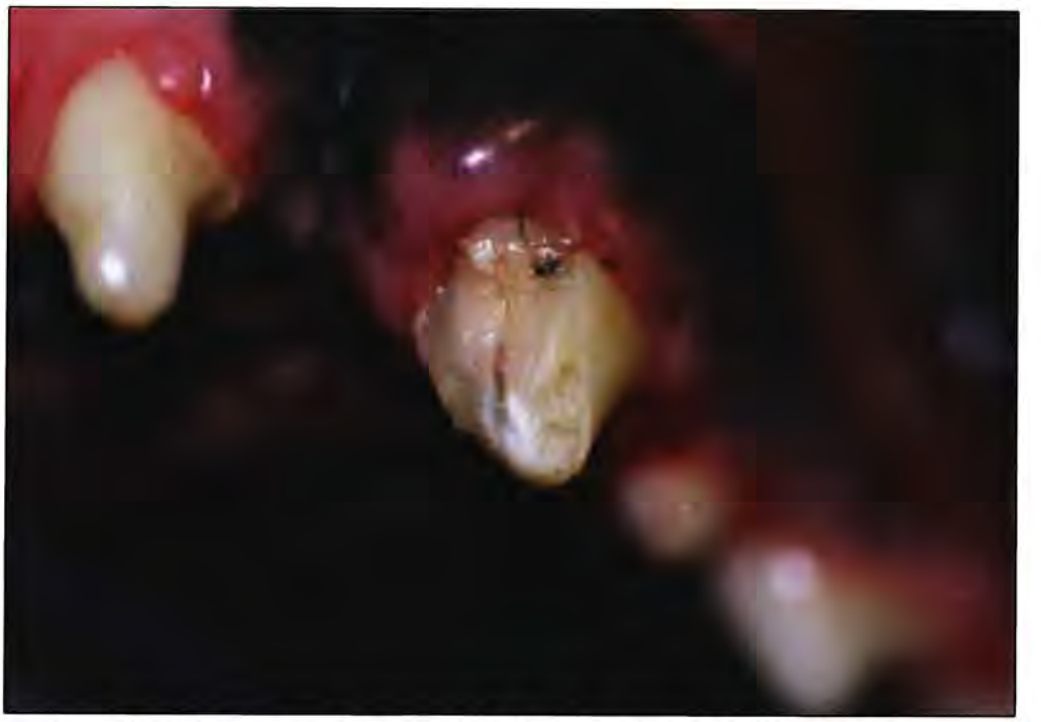

Figure 3 A complicated crown-root fracture in the left upper canine tooth (204) of a dog.

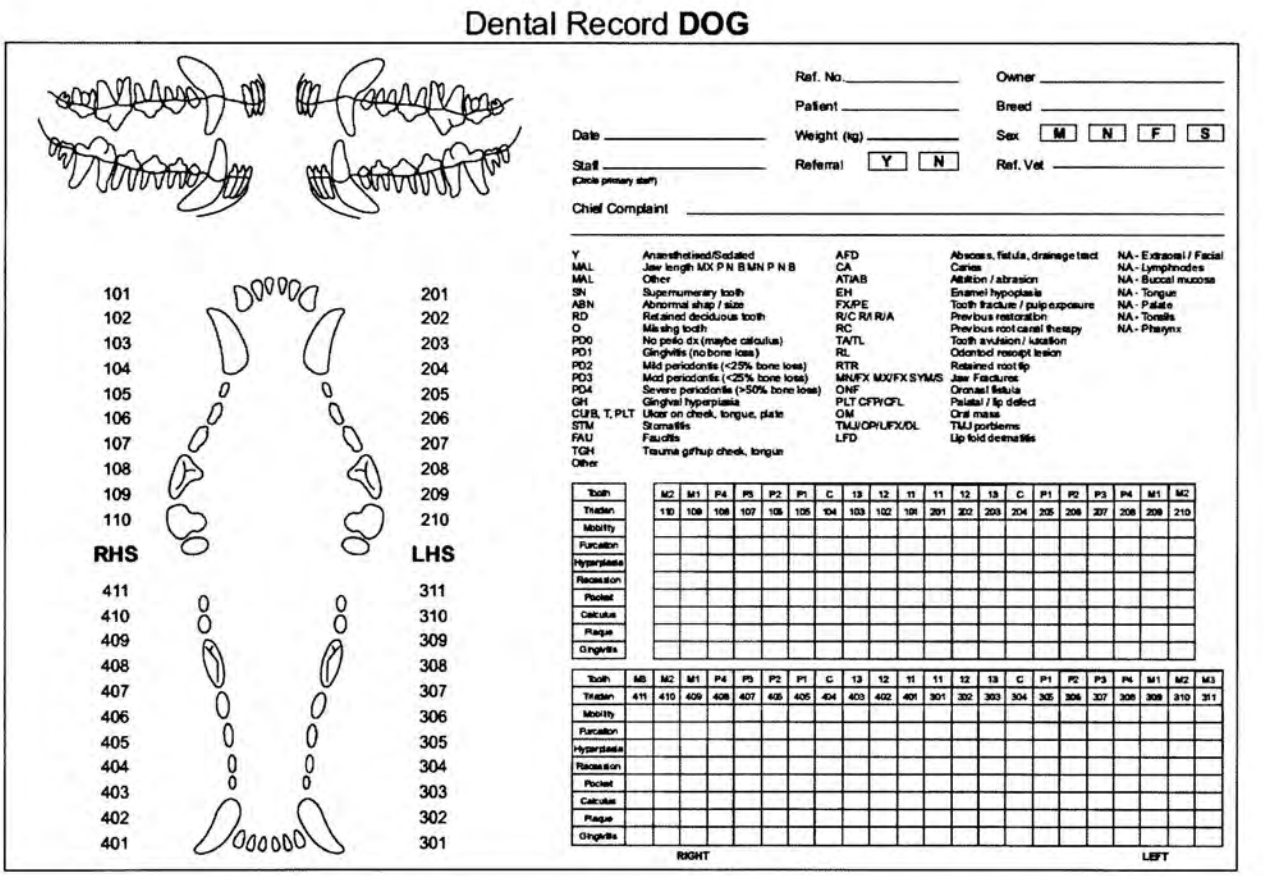

Figure 4 Document used to record the owner, sex, age, diet, weight, dental record and the breed of dogs.

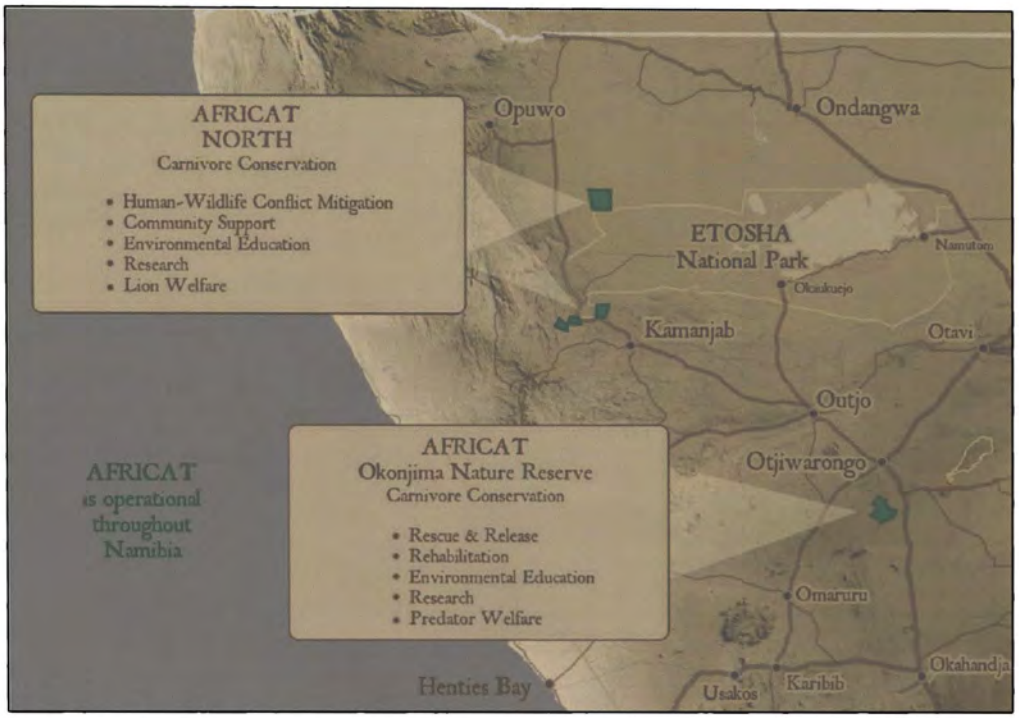

Figure 5 The Africat Foundation is situated on the Okonjima farm close to Otjiwarongo, Namibia. (Reproduced with permission from the Africat Foundation)

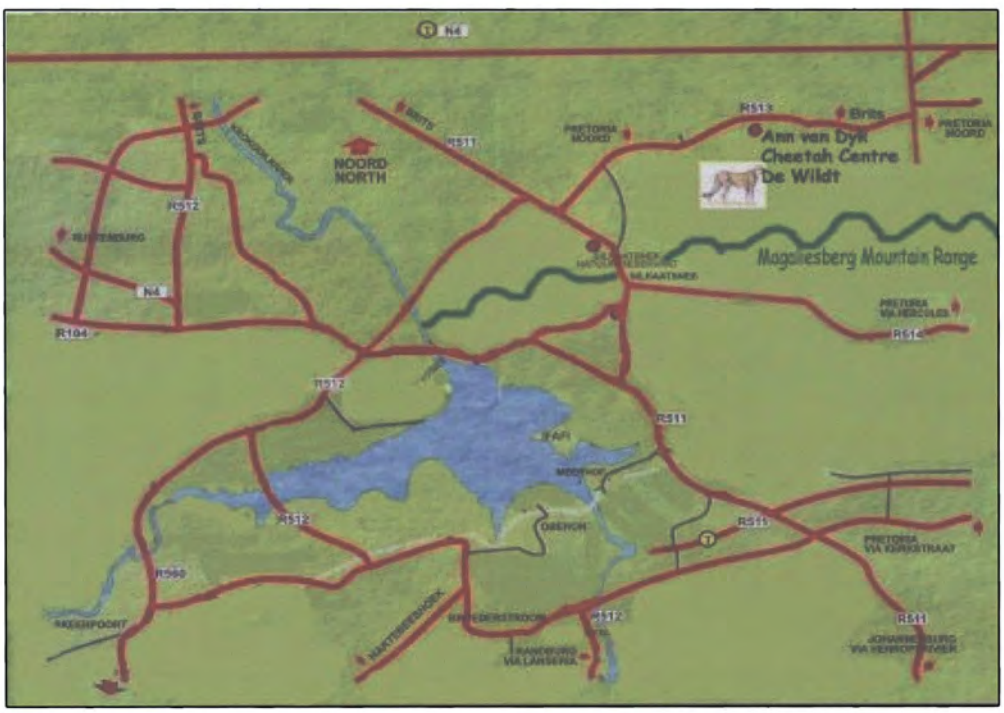

Figure 6 The Ann Van Dyk Cheetah Centre is located north-east of Pretoria, South Africa. (Reproduced with permission from The Ann Van Dyk Cheetah Centre)

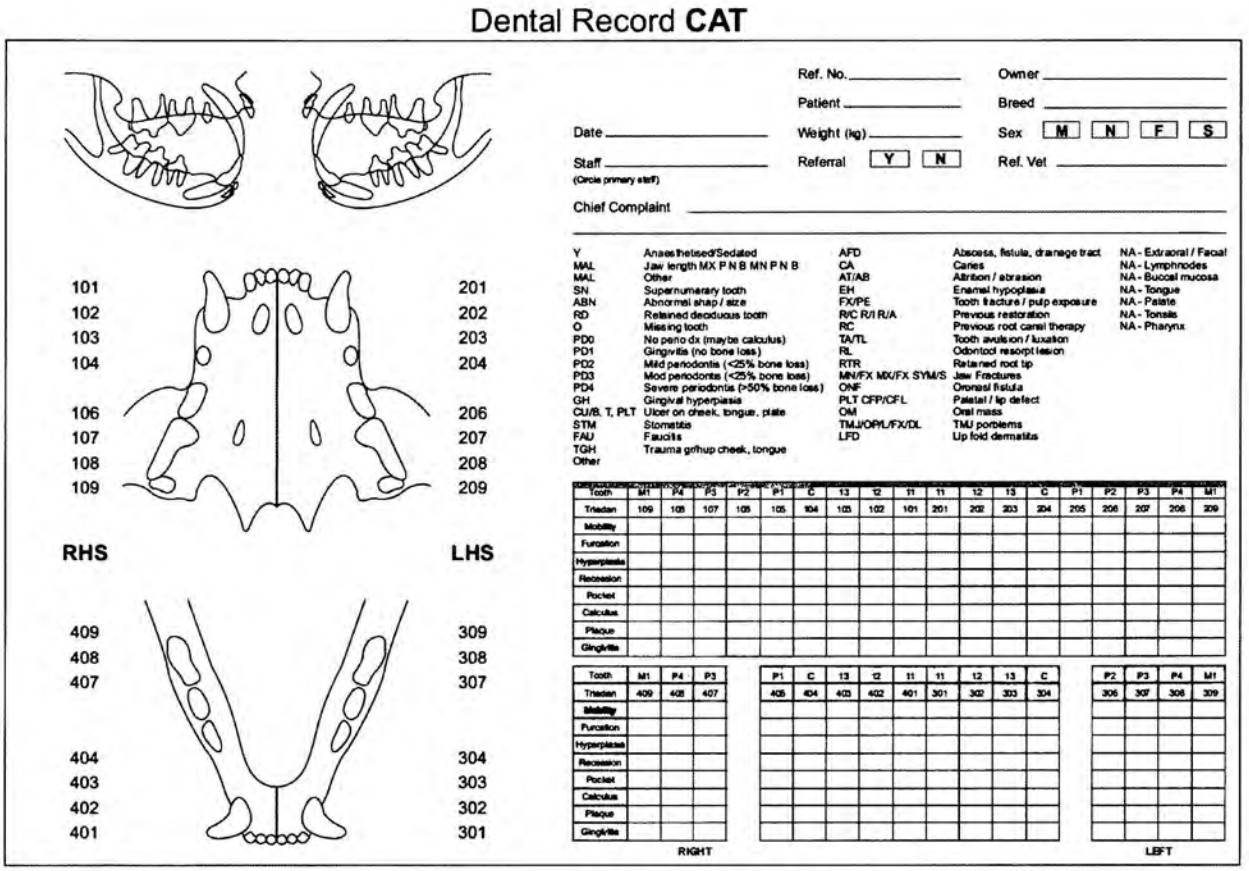

Figure 7 Document used to record the sex, age, diet, weight, and dental record of cheetahs.

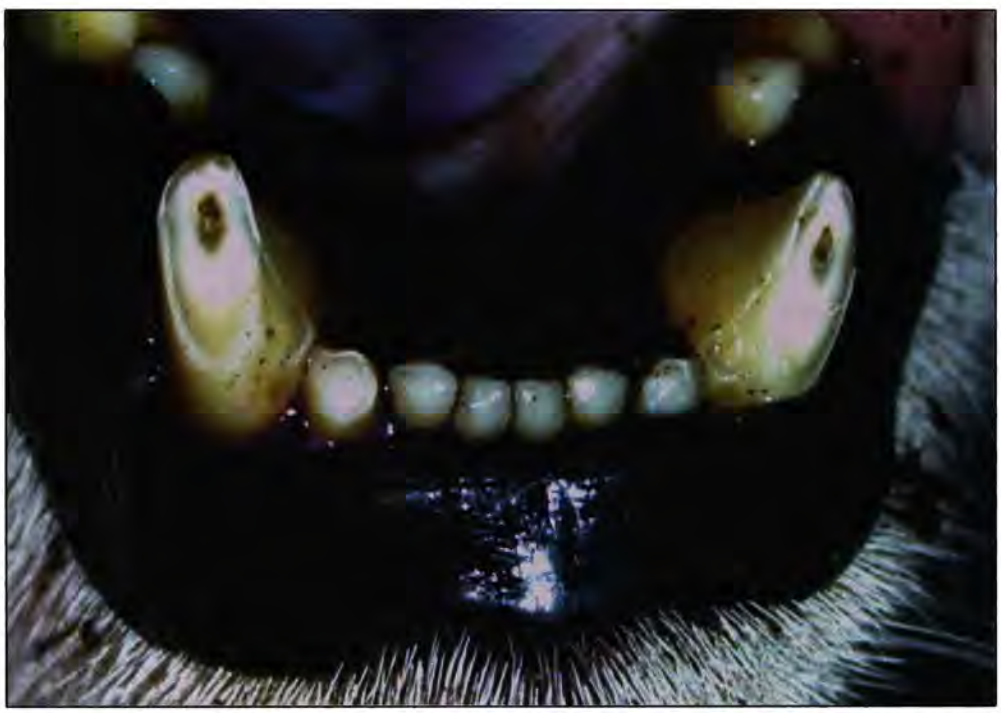

Figure 8 Complicated crown fracture in the right (404) and left (304) mandibular canine teeth of a cheetah from TAVDCC. (Courtesy of Dr. Gerhard Steenkamp)

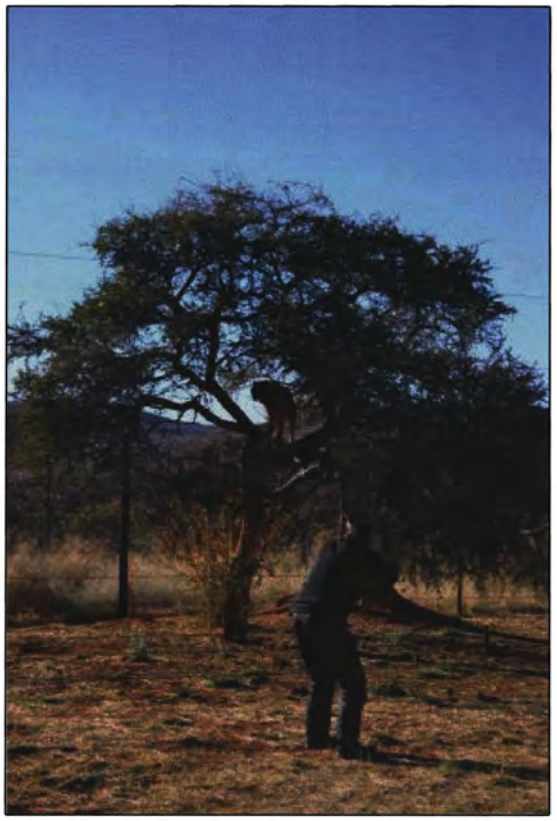

Figure 9 Cheetahs were immobilised by darting them, using a pressurised darting system.

Figure 10 Cheetahs were placed in lateral recumbency with the affected tooth uppermost.

Figure 11 A Kerr file is inserted into the RC of the canine tooth to file the dentine of the walls of the tooth. In this way a more representative sample of the microbiota is obtained.

Figure 12 All the instruments inserted into the RC were sterilised in a bead steriliser before they were employed in the sampling process to avoid any contamination of the samples.

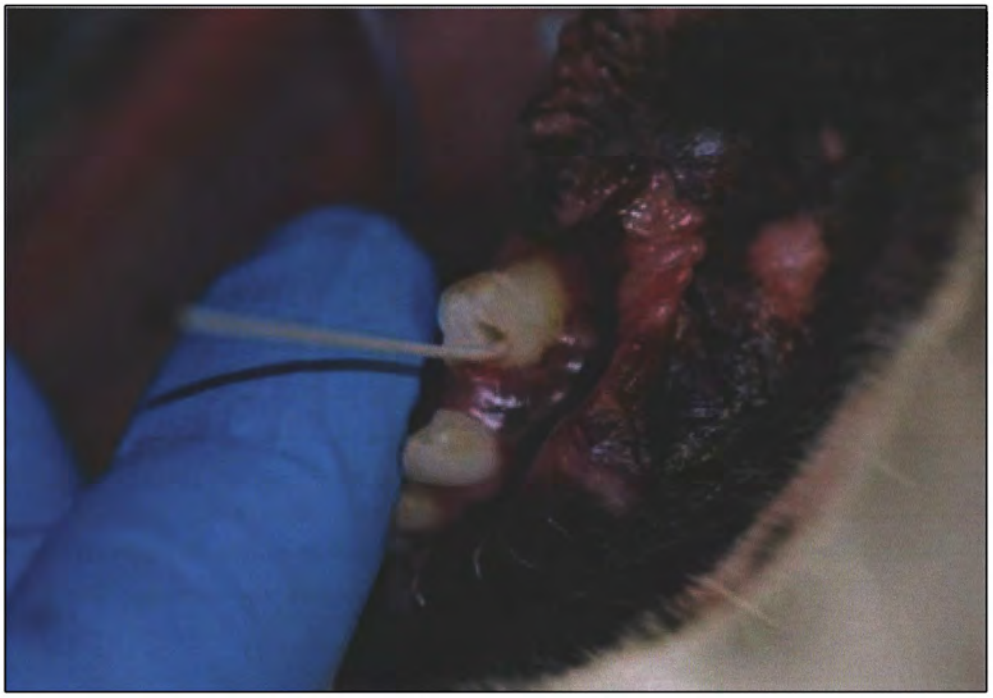

Figure 13 A paper point was placed in the RC of the canine tooth for one minute at maximum depth.

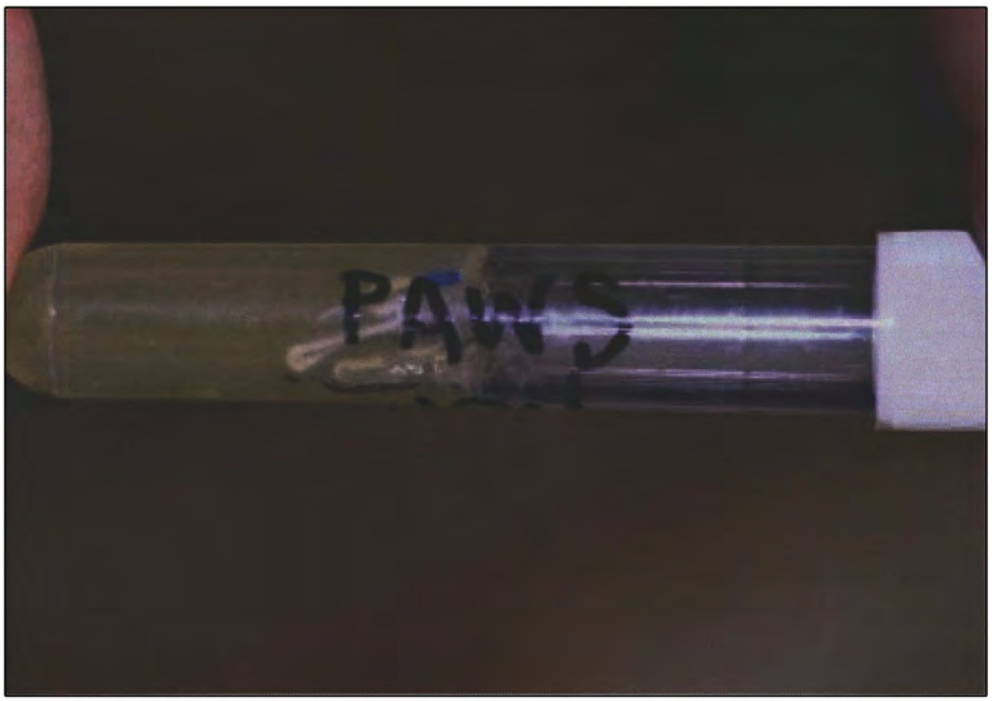

Figure 14 Paper point in a sterile tube containing anaerobic culture medium for submission to the bacteriology laboratory.

UNIVERSITY OF PRETORIA
FACULTY OF VETERINARY SCIENCE
ONDERSTEPOORT VETERINARY ACADEMIC HOSPITAL

TRANSFER FORM № 28203

From Clinic: ..... To: ☐ Tropical Diseases ☐ Pathology ☐ Other .....

STICKER OR
Patient: ..... Date: .....
Patient Number: ..... Owner Address: .....
Species: ....
Breed: ..... Sex: ..... Age: ....
Owner: ..... Owner Tel: .....
Owner number: .....

Specimen: .....
History: .....
.....
.....
Examination required:

Diagnostic Pathology

- ☐ PM Small animal
- ☐ PM Large animal
  - ☐ Required
  - ☐ Optional
  - ☐ Requested by Clinician
- ☐ PM Insurance Case
- ☐ Histopath Biopsy
- ☐ IFA
- ☐ IMP first section
- ☐ IMP additional section
- ☐ HE stained sections
- ☐ Special stains

TropicalDiseases

- ☐ Bacterlology - Aerobic ☐ Anaerobic ☐
- ☐ Antibiogram
- ☐ Fungal Culture
- ☐ Virus Isolation
- ☐ EM
- ☐ FIV/FIP
- ☐ FIV Antigen
- ☐ FeLV
- ☐ IgM IgG antibodies
- ☐ Mycoplasma Culture

Other (please specify): .....
Fees levied: R ..... Fund case: Yes ☐ No ☐
Clinician signature: ..... Print: .....
Clinician contact tel no:..... Clinician pager no: .....

Preliminary/Final result: ..... Reference No .....
.....
.....
.....
Date: ..... Signed: .....

Figure 15 All samples submitted to the Bacteriology Laboratory, Department of the Veterinary of Tropical Diseases of the University of Pretoria, were accompanied by this document.

Figure 16 Samples that were to be cultured anaerobically were processed in this anaerobic chamber, to preserve the anaerobic conditions during the culture process.

Figure 17 Positive growth of bacterial colonies on a 7 % horse blood agar plate.

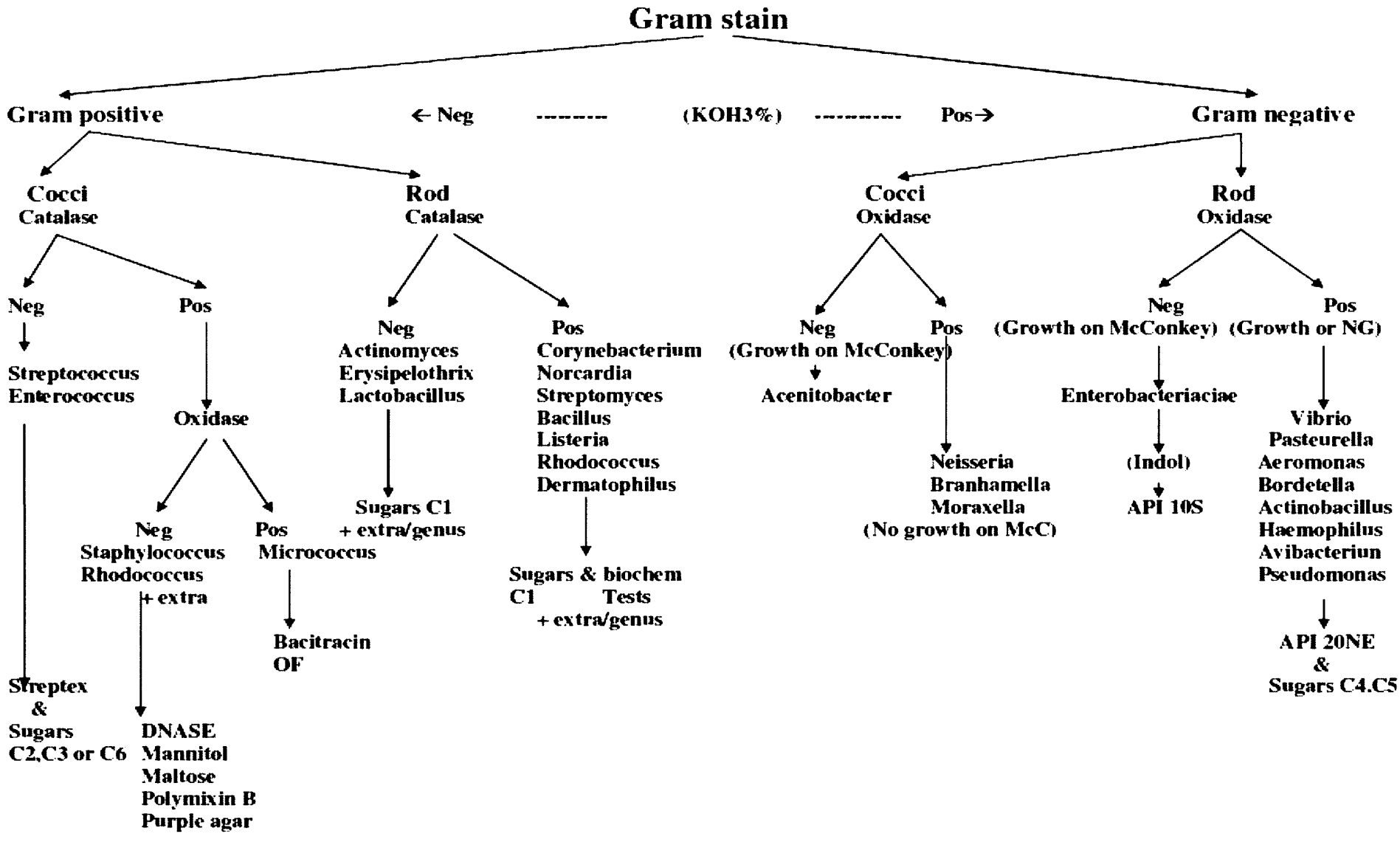

Figure 18 Bacterial colonies were identified using this algorithm. (Courtesy of the Bacteriology Section of the Department of Veterinary Tropical Diseases of the Univerity of Pretoria)

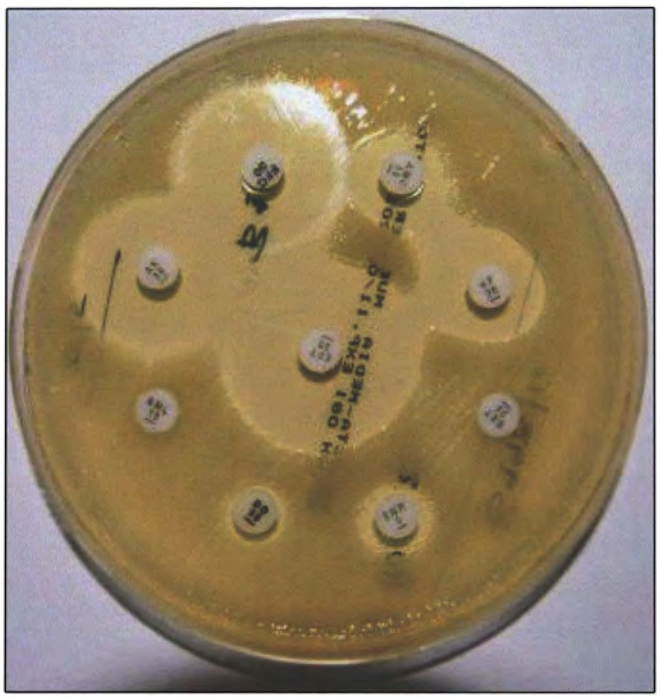

Figure 19 This picture represents the inhibition of bacterial growth around antibiotic containing discs.

Figure 20 Measurement of the halo around each antibiotic containing disc tested, using a digital vernier calliper. According to the halo´s size, bacteria were classified as sensitive, intermediate or resistant to that particular antibiotic.

Figure 21 Measurement of the clinical crown of the right maxillary canine tooth (104) in the skull of a cheetah with a steel ruler, marked in millimetres.

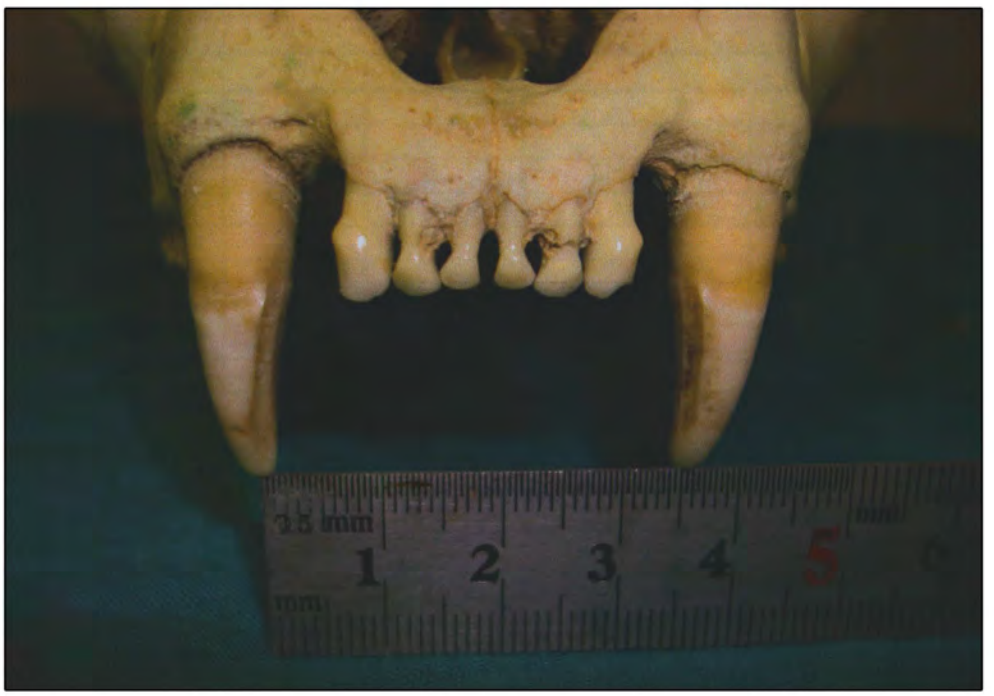

Figure 22 Measurement of the distance between the occlusal parts (crown tips) of the maxillary canine teeth in a cheetah with a steel ruler, marked in millimetres.

3.8 Tables

Table 2 Spectrum of antibiotics tested according to the protocol of the Bacteriology Laboratory. (Courtesy of the Bacteriology Laboratory, Department of Veterinary Tropical Diseases of the University of Pretoria)

Veterinary Tropical Diseases
Bacteriology Laboratory
Faculty of Veterinary Science

Page 1 of 2

Procedure number QA/BS/GE 008

Date Issued August 2009

ANTIBIOTICS SENSITIVITY TESTS

Case Number:_____

ANTIBIOTICS		S/R	S/R	S/R	S/R	R	I	S
PANEL 1 (Horses, dog, cat and fish)								
Amikacin	AK 30					14	15-16	17
Ampicillin* Gram -ve & enterococci	AML10					13* 21	14-16* 22-29	17* 30
Ceftiofur	XNL					17	18-20	21
Doxycycline	DOX30					14	15-18	19
Enrofloxacin (Baytril)	ENR5					16	17-19	20
Gentamicin	CN10					12	13-14	15
Penicillin G * Enterococci, **NO** G-ve	P10					14* 19	-* 10-27	15* 28
Sulpha/Trimethroprim	SXT					10	11-15	16
Chloramphenicol	C30							18
PANEL 2 (Dog, cat and fish)								
Cephalothin/lexin (CL)	KF30					14	15-17	18
Kanamycin	K30					13	14-17	18
Clindamycin/Lincomycin	MY					14	15-20	21
Lincospectin (MXS)	LS							20
Orbifloxacin	OBX5					16	17-19	20
Synulox *staphylococci	AMC 20/10					19* 13	- 14-17	20* 18
Tylosin	TY15					12	13-14	15
Polymixin B	*PB*							*12*
PANEL 3 (Ruminants and pigs)								
Ampicillin* Gram -ve & enterococci	AML10					13*	14-16*	17*
Ceftiofur	XNL					17	18-20	21
Enrofloxacin (Baytril)	ENR5					16	17-19	20
Florfenicol	FFC30					13	14-15	16
Kanamycin	K30					13	14-17	18
Oxytetracycline	OT30					14	15-18	19
Penicillin G * Enterococci	P10					14* 19	- 10-27	15 28
Sulpha/Trimethroprim	SXT					10	11-15	16
Tilmicosin/tylosin (TY)	TIL15					12	13-14	15
Tulathromycin	TUL30					15	16-17	18
Lincospectin	*LS*					*<20*		*20*
Neomycin	*N*					*<10*	*10-16*	*17*
Polymixin B	*PB*							*12*
Sulphamethoxazole	*RL300*					*12*	*13-16*	*17*

Table 3 Table showing the extra / resistant panel of antibiotics tested according to the protocol of the Bacteriology Laboratory. (Courtesy of the Bacteriology Laboratory, Department of Veterinary Tropical Diseases of the University of Pretoria)

ANTIBIOTICS		S/R		S/R		S/R		S/R		R	I	S
PANEL 4 (Poultry and Ostrich)												
Ampicillin* Gram -ve & enterococci	AML10									13*	14-16*	17*
Enrofloxacin (Baytril)	ENR5									16	17-19	20
Fosfomycin (Fosbac)	FOS											17
Fosbac										12	13 -15	16
Fosbac + T										12	13 -15	16
Doxycycline	DOX30									14	15-18	19
QuinAbic	QA									20	20	20
Sulpha/Trimethroprim	SXT									10	11-15	16
Lincospectin	LS									20	20	20
Lincomycin	MY									14	15-20	21
Tilmicosin/tylosin (TY)	TIL15									12	13-14	15
PANEL 5 (Bird and Exotic)												
Amikacin	AK 30									14	15-16	17
Ceftiofur	XNL									17	18-20	21
Doxycycline	DOX									20	20	20
Enrofloxacin (Baytril)	ENR5									16	17-19	20
Sulpha/Trimethroprim	SXT									10	11-15	16
Cephalothin/lexin(CL)	KF30									14	15-17	18
Synulox *staphylococci	AMC 20/10									19* 13	- 14-17	20* 18
Florfenicol	FFC30									13	14-15	16
Ceftazidime	CAZ											22
EXTRA / RESISTANT PANEL												
Carbencillin **P. aeruginosa*	CAR									13* 1 9	14-16* 20-22	17* 23
Ceftazidime	CAZ											22
Chloramphenicol	C30									12	13-17	18
Imipenem	IPM10									13	14-15	16
Piperacillin	PRL											18
Tobramycin	TOB									12	1 3-14	15
Ticarcillin **P. aeruginosa*	75									14* 14	- 15-19	15* 20
SPECIAL												
Methicillin Staphylococci only										10	11-12	13
Vancomycin Enterococci only	VAN 30									14	15-16	17
Erythromycin (*R. equi*)	15									13	14-22	23
Rifampicin (*R. equi*)	5									16	17-19	20

Chapter 4
Results

Thirty-nine animals were included in this study of which 20 were dogs and 19 were cheetahs.

4.1 Dogs

Of the 20 dogs seen in this study Staffordshire bull terrier was the most common breed presented (Figure 23). The age of the dogs ranged between 1-10 years, with the average age 4.6 years. Half of the dogs were younger than three years of age (Figure 24). The dogs were classified as male, female, neutered or spayed (Figure 25). Males were the most commonly affected (14/20), and represented nearly 70 % of the dogs studied. All the dogs were fed a commercially available dry food diet.

Of the dogs presented, 20 % (4/20) had two fractured canine teeth and only one, 5 % (1/20), had all four canine teeth fractured (Figure 26). In the majority of the animals (15/20) just one RC treatment was required. In total 27 pulps were sampled from the dogs in this study. Maxillary canine teeth were fractured in 16 (59.24 %) of the cases compared to 11 cases (40.76 %) in which a mandibular tooth was fractured. There was no predilection for fracture of left or right canine tooth of the maxilla or mandible (Figure 27).

A total of 49 cultivable isolates, belonging to 27 different microbial species and 18 different genera, were recovered from the 27 RC sampled (Table 4). Twenty (40.81 %) of those 49 cultivable isolates were Gram positive and the other 29 (59.19 %) Gram negative. All different colony types isolated from the primary cultures were subcultured and identified. Individual RC yielded a maximum of four species each. Two RC had no cultivable bacteria. A single microorganism was found in nine cases. Ten cases presented two species (*Pasteurella* spp. and *Enteric* group 8, *Staphylococcus aureus* and *Weeksella virosa*; *Pasteurella pneumotropica* and *Enterococcus* spp.; *Staphylococcus intermedius* and *Moraxella* spp.; *Staphylococcus* spp. and *Pasteurella multocida*; *Corynebacterium* spp. and *Moraxella* spp.; *Enterococcus* spp. and *Corynebacterium* spp.; *Aeromonas salmonicida* and *Pasteurella multocida*; *Actinomyces* spp and *Moraxella* spp.; *Aeromonas salmonicida* and *Moraxella* spp.) and six cases were polymicrobial infections consisting of three or more species per canal. In those animals, which required more

than one RCT, the bacteria isolated from the different RC showed the results represented on Table 5.

Of the bacterial isolates, 4.08 % (2/49) were strict anaerobes, *Clostridium acetobulitycum* (2.04 %), and *Prevotella melalinogenica* (2.04 %). Aerobic bacteria made up 18.36 % (9/49) of the bacteria isolated. Facultative anaerobic bacteria with 77.56 % (38/49) were the most common bacteria isolated (Figure 28). Bacteria, which presented with an incidence higher than 6 % are shown in Figure 29.

The most effective bactericidal antibiotics were Enrofloxacin (85.21 %), Gentamicin (92.39 %) and Chloramphenicol (89.13 %). Penicillin G (47.28 %), Lincomycin (13.04 %) and Lincospectin (39.13 %) all showed poor results (Table 7). All the results of the antibiotics tested against all the microbes in dogs are represented in Table 6. The bacterial isolates that showed the highest resistance against the majority of the antibiotics tested in this study were *Staphylococcus intermedius*, CDC group Ve-2, and *Pseudomonas aeruginosa* (Table 5). It was impossible to test the sensitivity of *Lactobacillus* spp. against any of the antibiotics as it grew too slow.

4.2 Cheetahs

Of the 19 cheetahs in this study, three (15.79 %) were treated in the Dentistry and Maxillofacial Surgery Clinic of the OVAH, University of Pretoria, Pretoria, South Africa. The other 16 (84.21 %) were treated in the clinic of AF, Otjiwarongo, Namibia.

The ages of the cheetahs ranged from 3.5 years to 15 years, with an average age of 6.94 years (Figure 30). Female cheetahs were nearly twice as likely to present with CCF (63.16 %) compared to males (36.84 %) (Figure 31). All the females included in this study from AF, are animals that are treated yearly with contraceptive implants, as it is prohibited by law to breed large carnivores in captivity in Namibia.

The feeding regime of the cheetahs at the AF was as follows:

- Two of the males and one female were fed meat every day, excluding Wednesdays and Sundays.

- Five of the males and eight females were fed meat on Monday and Friday; and 500 grams of IAMS® cat food (soaked in water) per animal on Tuesday, Thursday and Saturday.
- The meat that is fed to the animals is usually from horses or donkeys which are cut into 1.5-2 kg pieces.

At TAVDCC the cheetahs are fed horse meat and whole chickens 2-3 times a week.

In this study maxillary canine teeth were more frequently fractured (62 %) than mandibular canine teeth (38 %). When comparing fractures of the left or right maxillary canine teeth, the numbers seem to be comparable while those of the fractures affecting mandibular canine teeth are equal (Figure 32).

A total of 59 cultivable isolates, belonging to 19 different microbial species and 13 different genera, were recovered from the 36 RC sampled (Table 8). Thirty-two (54.49 %) of these isolates are Gram positive bacteria and the other 27 (45.71 %) of them Gram negative. All different colony types isolated from the primary cultures were subcultured and identified. Individual RC yielded a maximum of six species each. Four RC had no cultivable bacteria. A single microorganism was found in 17 cases. Nine cases presented two species (*Pasteurella multocida* and *Aeromonas salmonicida* subsp. *achromogenes*, *Bacillus* spp. and *Corynebacterium* spp.; *Pseudomonas aeruginosa* and *Bacillus* spp.; *Pseudomonas aeruginosa* and *Enterococcus* spp.; *Pseudomonas aeruginosa* and *Moraxella* spp.; *Clostridium sordelli* and *Moraxella* spp.; *Moraxella* spp. and *Moraxella lacunata*; *Bacillus* spp. and *Corynebacterium* spp.; *Bacillus* spp. and *Pasteurella multocida*; *Aeromonas salmonicida* and *Moraxella* spp.) and six cases were polymicrobial infections consisting of three or more species per canal.

Differences between those animals, which were fed only meat, and those eating meat and a commercial diet are represented in Table 9. In those cheetahs, which had more than one RC treated, the difference between them is presented in Table 10.

Of all the bacterial species isolated, 8.47 % (5/59) were strict anaerobes and 28.81 % (17/59) strict aerobes. Facultative anaerobic species accounted for the remainder of the isolates (62.72 %; 37/59) (Figure 33). The identity of the anaerobic bacteria was *Clostridium sordelli* (5.08 %), and *Clostridium septicum* (3.38 %). All the different bacteria isolated from the

necrotic pulps of cheetahs are represented in the Table 8. Bacteria with an incidence higher than 5.25 % are represented in Figure 34.

All the bacteria cultured were subjected to an antibiogram panel containing 15 different antibiotics. The efficacy of the antibiotics against the bacteria is reported in Table 11. Of all the antibiotics tested Enrofloxacin (91.96 %) was the most effective and Lincomycin (31.57 %) the least effective (Table 12). The bacterial isolates that showed the higher resistance against the majority of the antibiotics tested in this study were *Acinetobacter calco* var. *Anitratus*, *Moraxella lacunata*, *Pseudomonas aeruginosa* and *Vibrio parahaemolyticus*.

Maxillary canine teeth have a clinical crown length ranging from 16-23 mm, and a mean of 19.6 mm, whereas the mandibular canine teeth's clinical crown length ranged from 12-18 mm, with a mean of 15.4 mm (Table 13). The inter maxillary canine teeth distance ranged from 28-43 mm, and a mean of 36.45 mm. Mandibular canine teeth had an inter canine distance that ranged from 21-33 mm, with a mean of 28.35 mm (Table 13).

4.3 Nucleic acid-base detection

A total of eight samples, comprising six from cheetahs and two from dogs, were analysed using culture techniques and an initial screening with the 16S rRNA-specific PCR. In dogs, Gram negative and Gram positive bacteria were equally represented with a 50 % (3/6) of all the bacteria detected. Anaerobic bacteria were predominant and were represented by 83.3 % (5/6) of the bacteria detected, while aerobic bacteria comprised 16.6 % (1/6). On the other hand, in cheetahs, the bacteria obtained by PCR method showed prevalence rates of anaerobic bacteria of 60.8 % (14/23), facultative anaerobic bacteria of 30.2 % (7/23) and aerobic bacteria of 8.6 % (2/23).

The bacteria found in the dog and cheetah samples which were identified from the BLAST (Basic Local Alignment Search Tool) searches are represented in Table 14. Where a sequence appeared in more than one sample, only one clone name is given. Many of the clone sequences were similar to sequences from bacterial species, which have been reported from human infected RC, such as *Pseudoramibacter alactolyticus* (69), *Tisierella praecuata* (33) and *Fusobacterium necrophorum* (33). However, other clone sequences were similar with sequences, which were only identified to the genus level. Some of these belong to genera, which had previously been isolated from RC infections in human. For example, *Bacteroides* spp., *Porphyromonas* spp. (8).

However, other clone sequences were similar to those of unidentified bacteria, such as clone H9PJET, which matched those of a bacterial isolate from the rumen.

A comparison between standard bacterial culture techniques and 16S rRNA PCR for detection of bacteria in necrotic RC of dogs and cheetahs was performed. There was a greater number of positive results of bacteria identified for dog and cheetah samples by the PCR assay than by culture techniques (Table 15), although a larger sample size would be necessary to determine whether this was a significant difference. The results from culture analysis and 16S RNA PCR, displayed a relatively low similarity in the species, in both dogs and cheetahs.

4.4 Figures

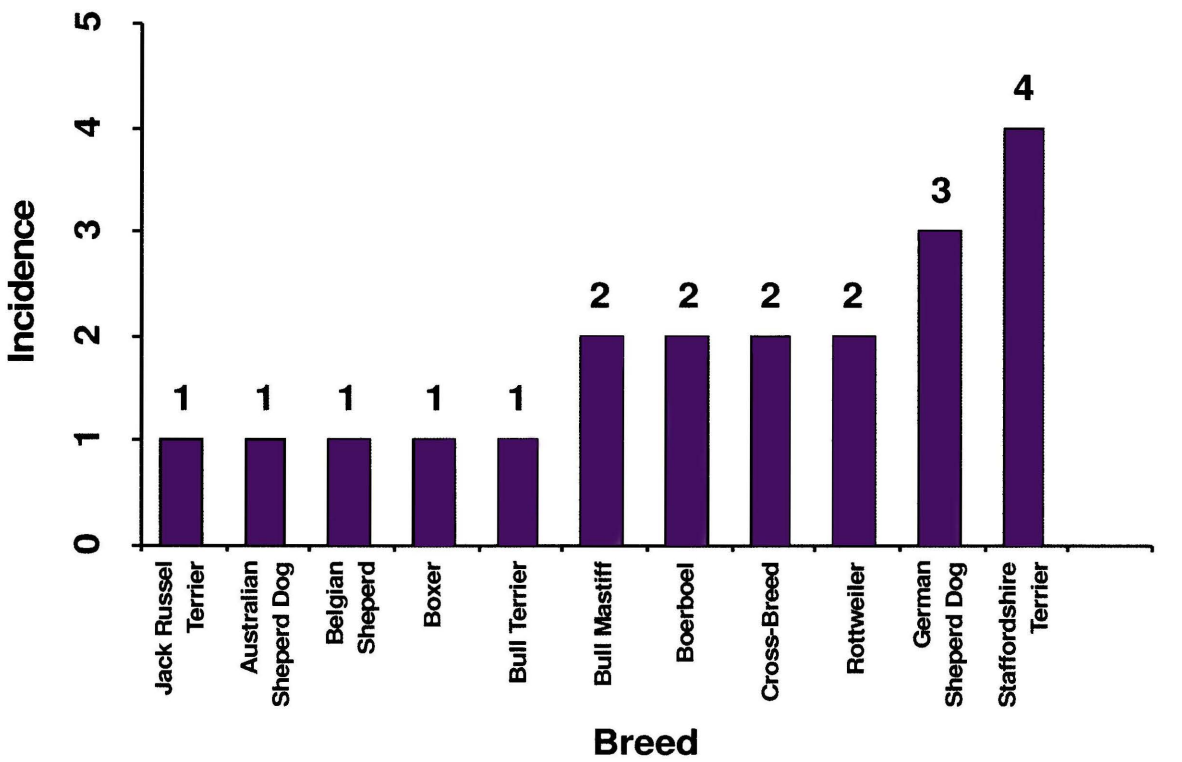

Figure 23 Breed incidence of the dogs included in the study.

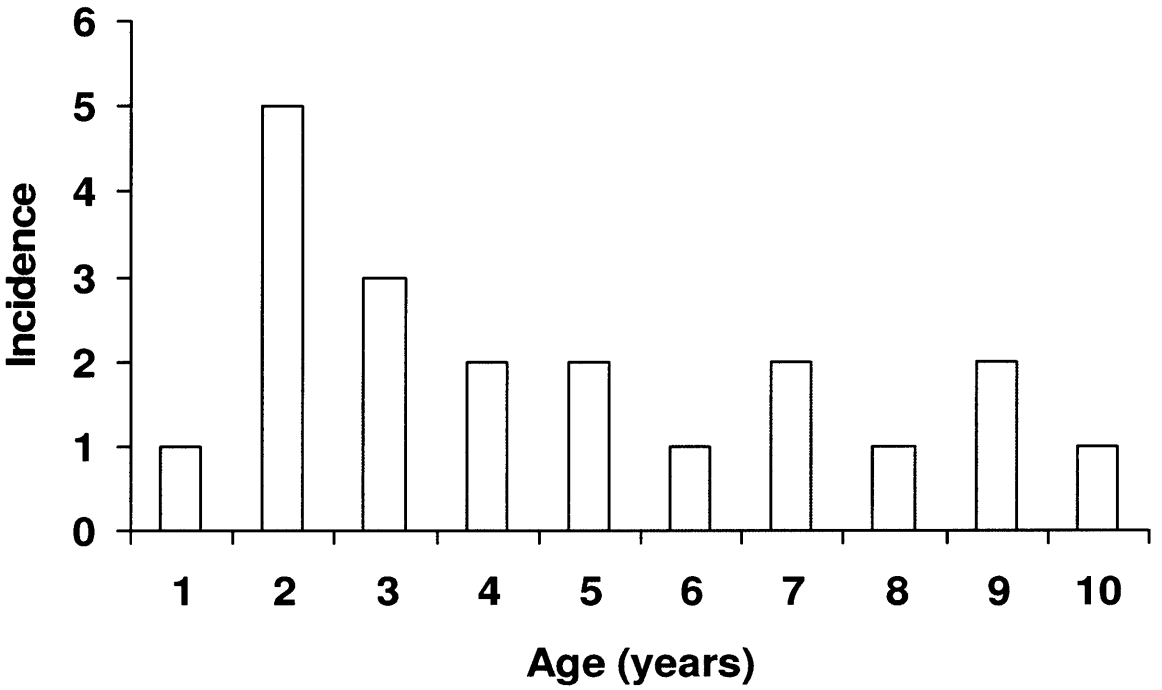

Figure 24 Age distribution of the dogs included in this study. Note the higher incidence in animals younger than five years of age.

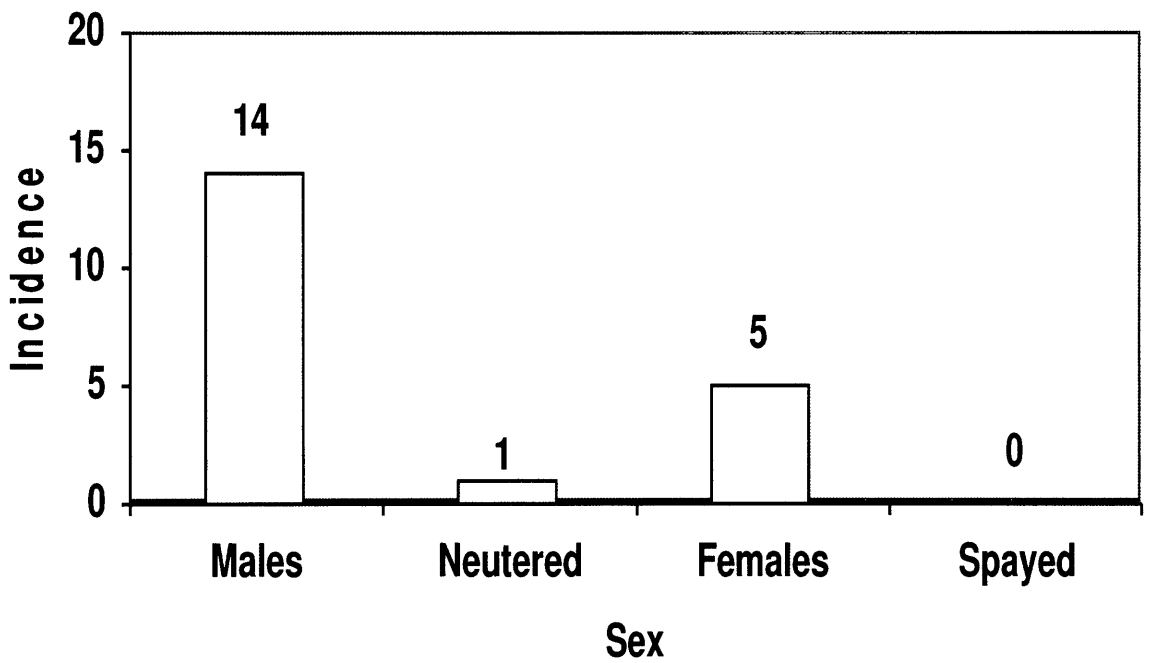

Figure 25 Sex distribution of the dogs included in this study. Note the higher incidence of CCF in males.

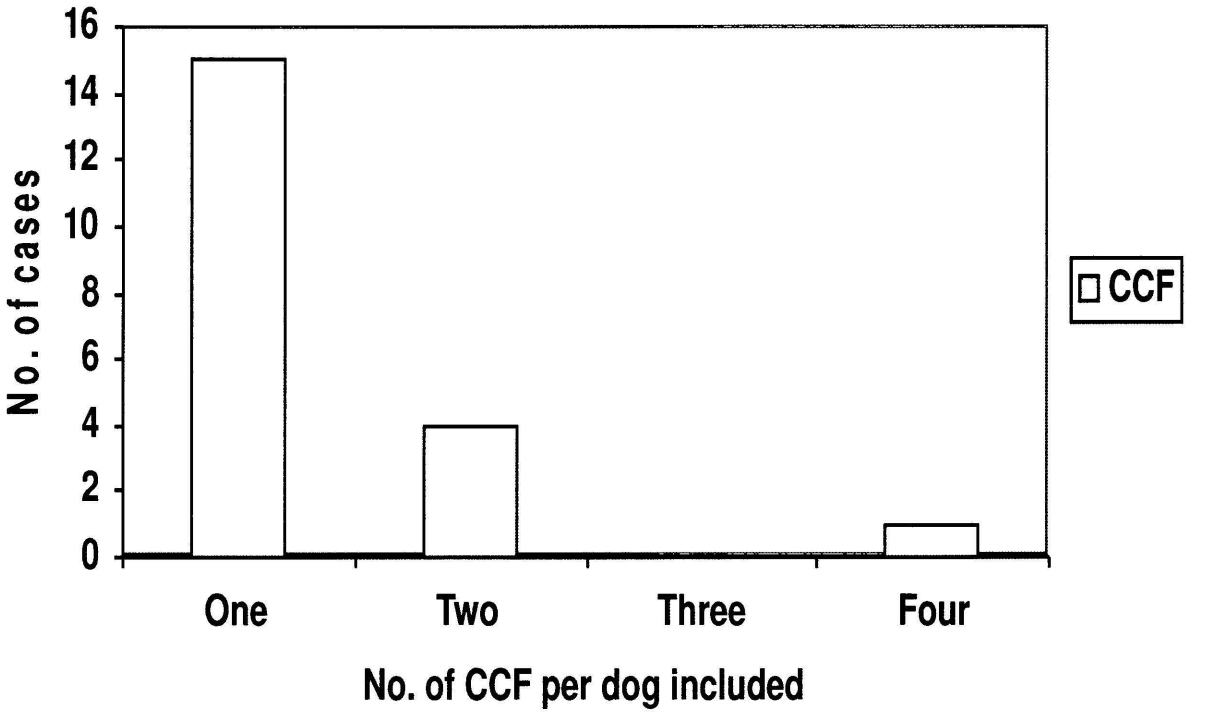

Figure 26 Number of canine teeth with CCF per dog included in the study.

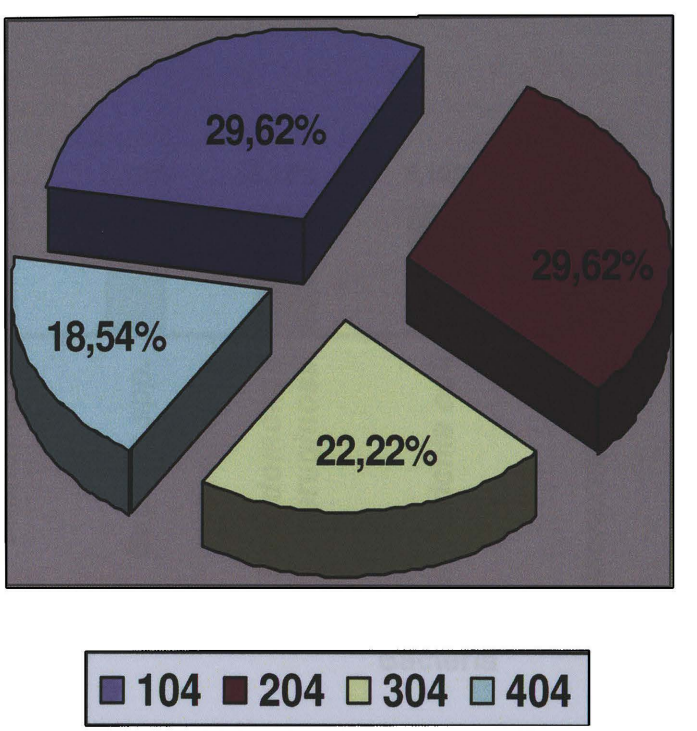

Figure 27 Distribution of CCF incidence of the canine teeth of the dogs.

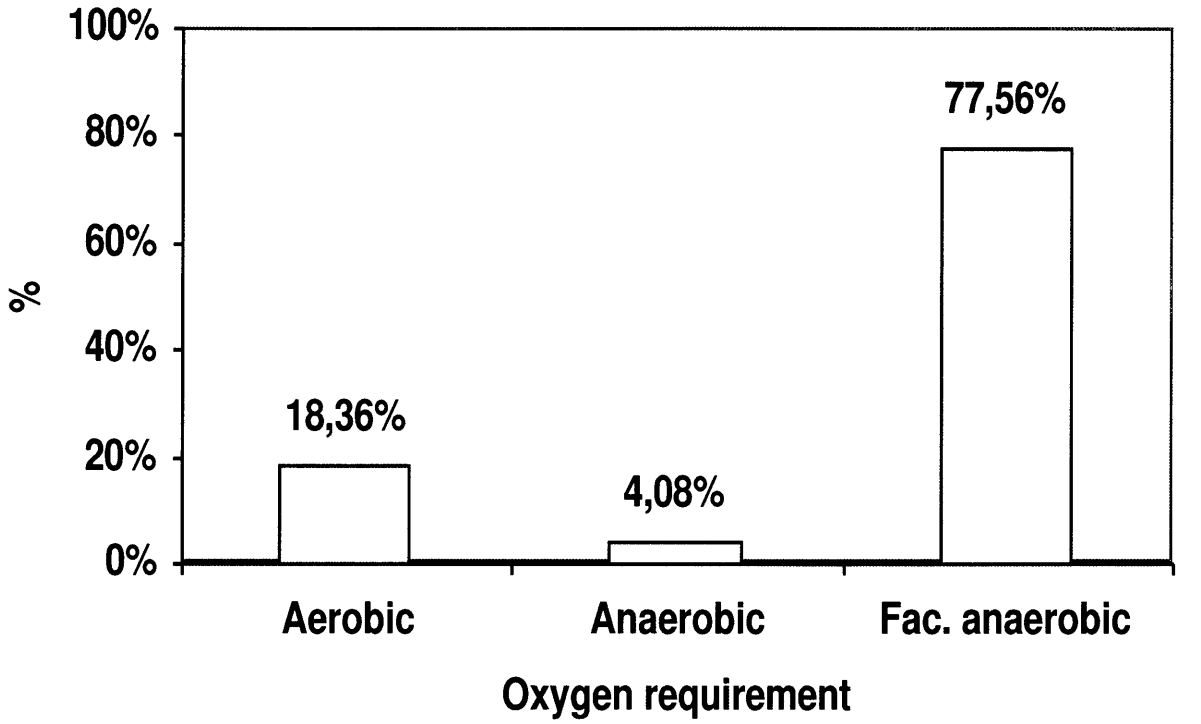

Figure 28 Percentage of the different bacteria according to the oxygen requirement.

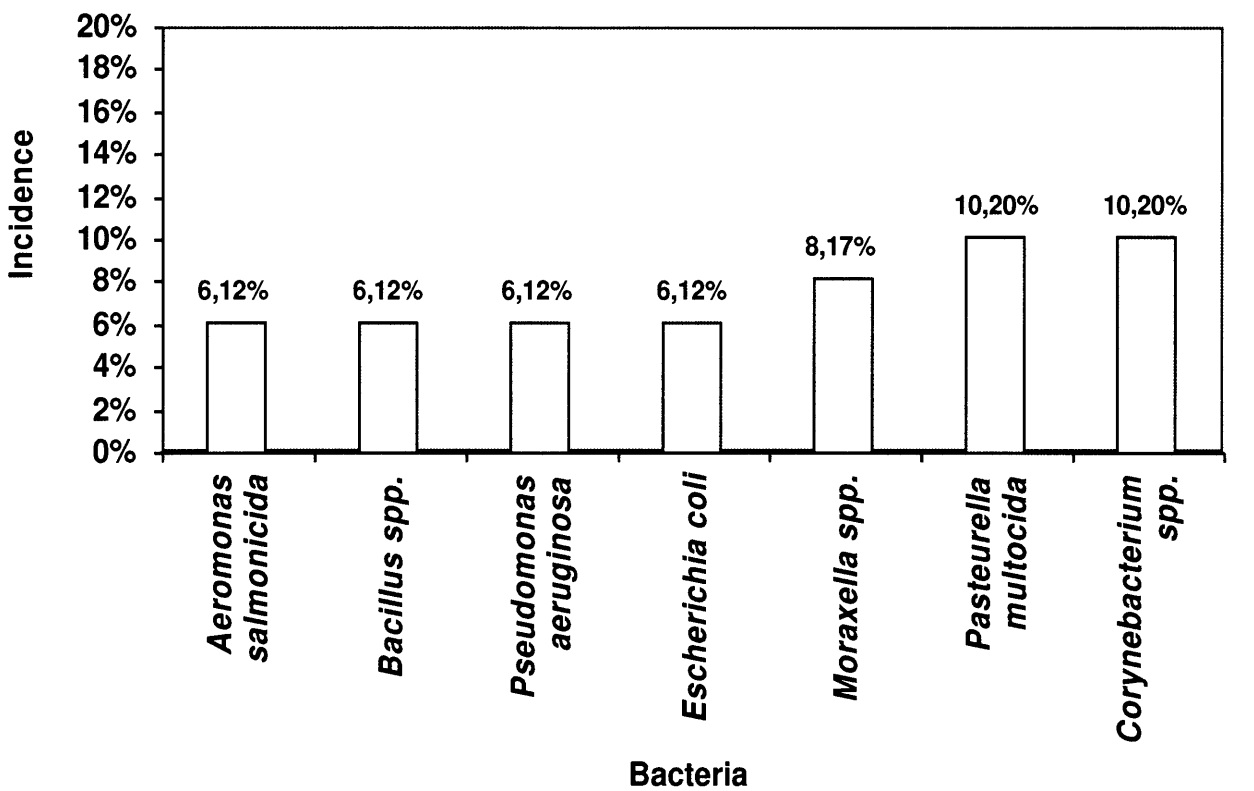

Figure 29 Bacteria isolated with an incidence higher than 6 %.

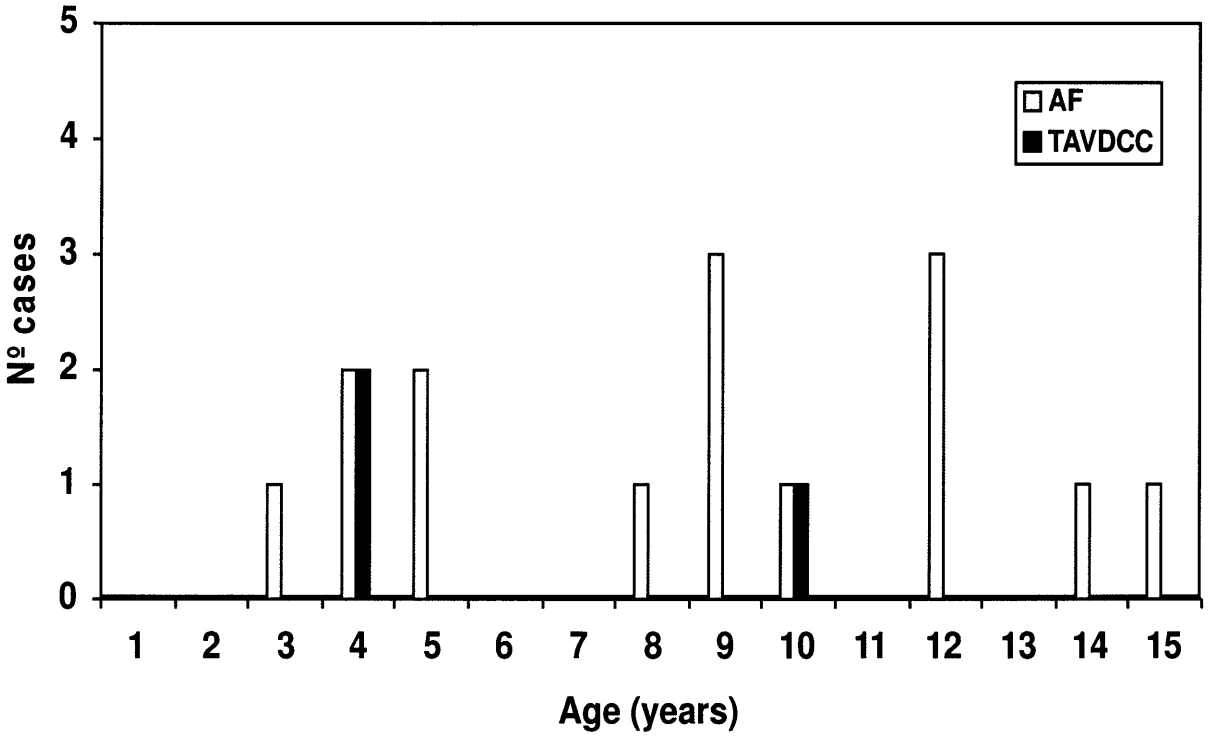

Figure 30 Age distribution of the cheetahs in the study.

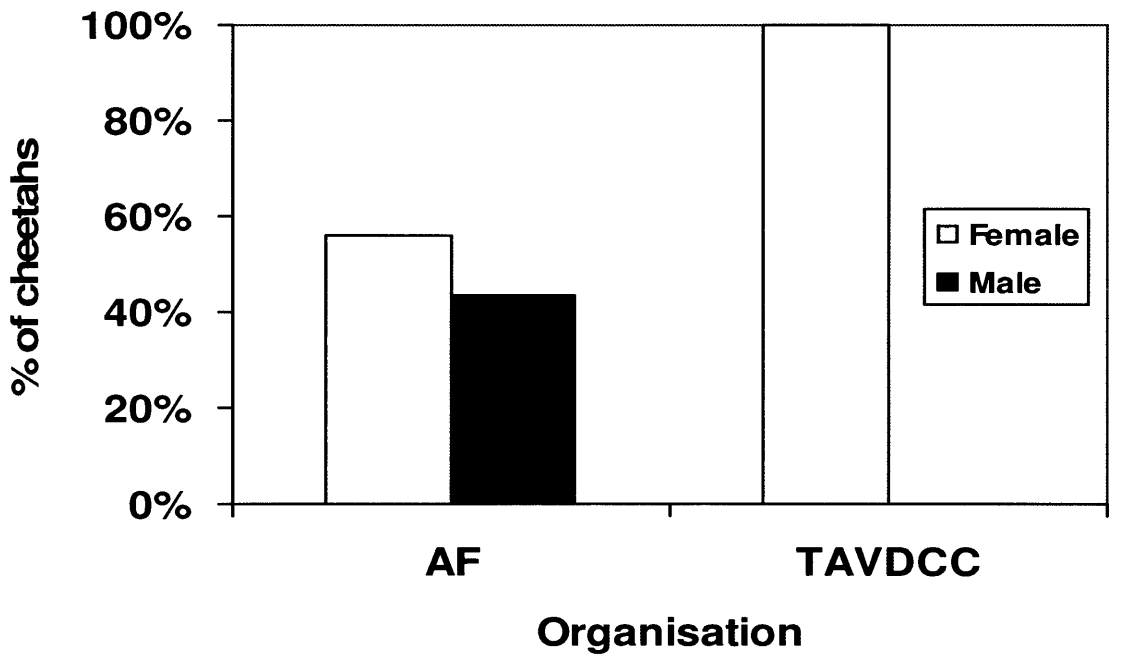

Figure 31 Sex distribution of all cheetahs in the study. Note the higher incidence of CCF in females.

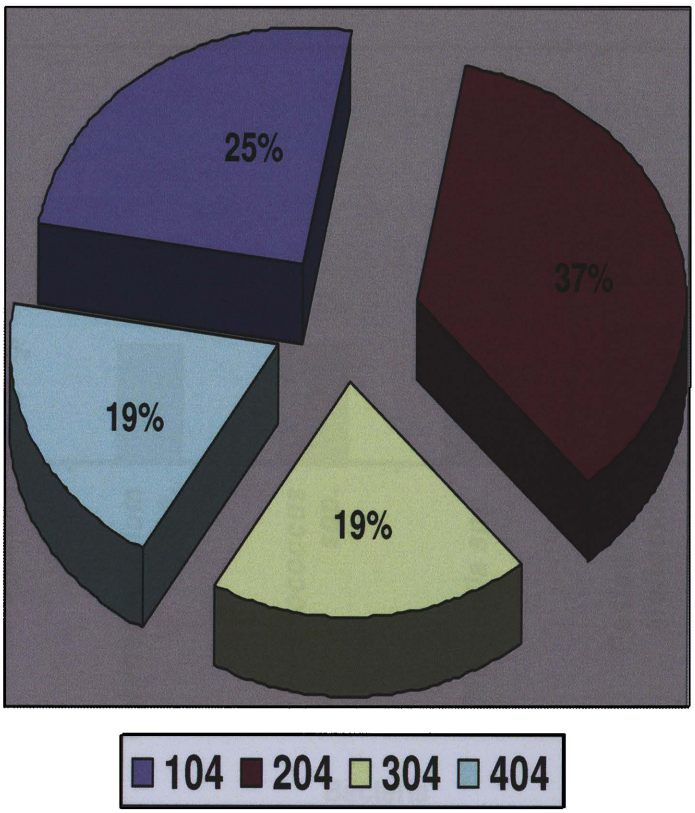

Figure 32 Distribution of the CCF incidence in the canine teeth of cheetahs. Note the marked incidence in the maxillary canine teeth (104 and 204).

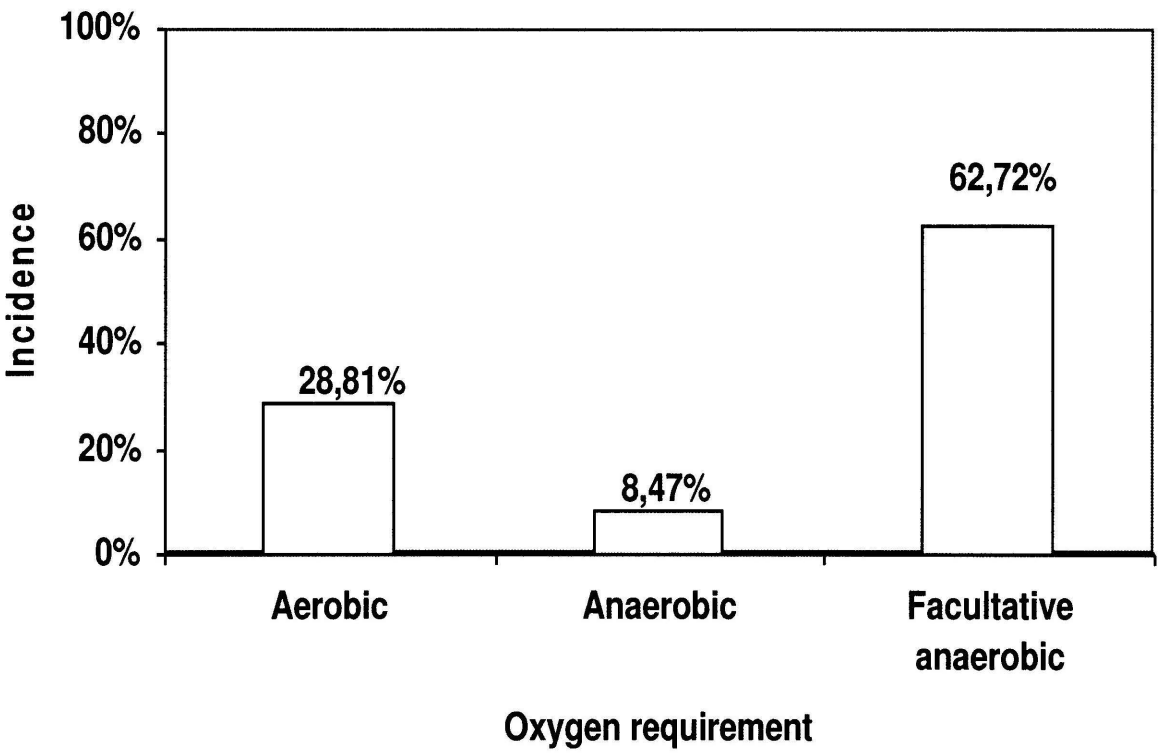

Figure 33 Percentage of the different bacteria according to their oxygen requirement isolated from cheetahs.

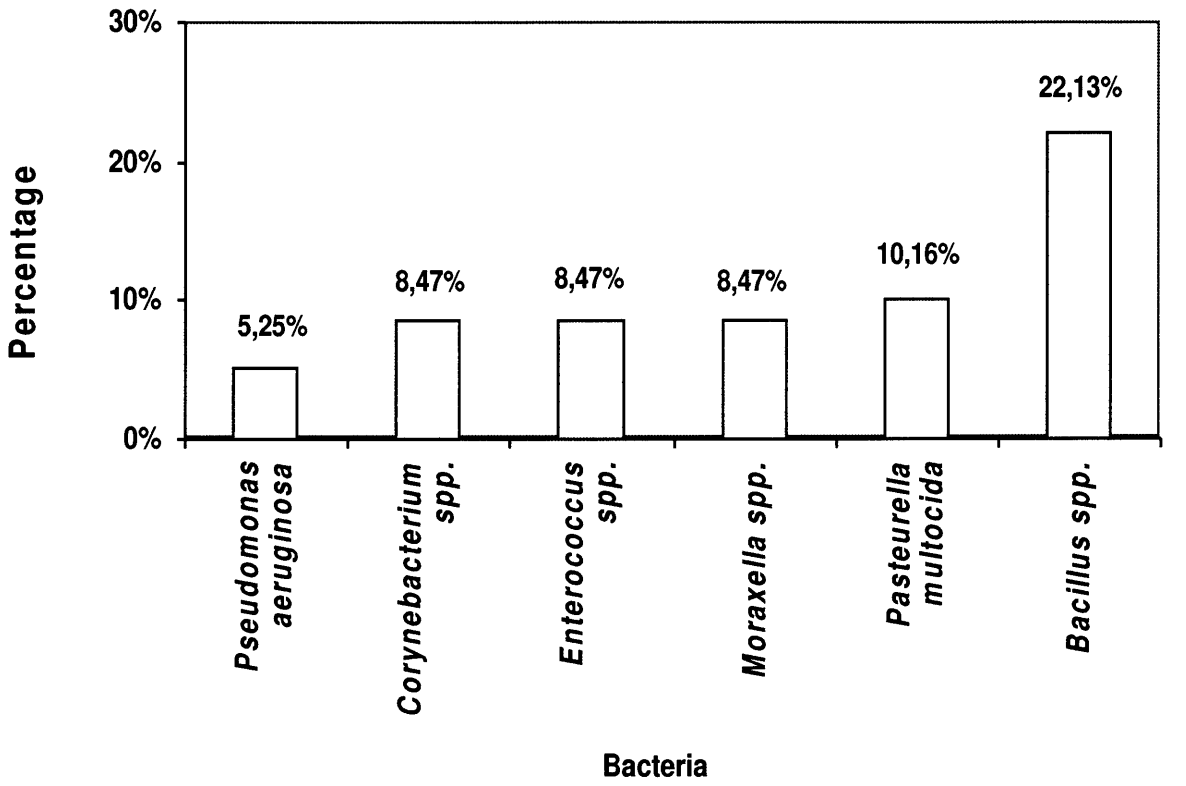

Figure 34 Representation of the bacteria isolated from the necrotic pulps of cheetahs with an incidence higher than 5.25 %.

4.5 Tables

Table 4 Bacteria isolated from 27 pulps exposed due to CCF in the canine teeth of dogs (N = 49).

	Isolates	Total isolates (%)
Gram-positive		
Facultative Anaerobic		
Actinomyces spp.	2	4.08
Bacillus spp.	3	6.12
Bacillus cereus	1	2.04
Corynebacterium spp.	5	10.20
Corynebacterium spp. No 1	1	2.04
Corynebacterium spp. No 2	1	2.04
Enterococcus spp.	2	4.08
Lactobacillus spp.	1	2.04
Staphylococcus spp.	1	2.04
Staphylococcus aureus	1	2.04
Staphylococcus intermedius	1	2.04
Anaerobic		
Clostridium acetobulyticum	1	2.04
	20	**40.81**
Gram-negative		
Aerobic		
Pseudomonas aeruginosa	3	6.12
Pseudomonas alcaligenes	1	2.04
CDC group VE-2	1	2.04
Moraxella spp.	4	8.17
Facultative Anaerobic		
Aeromonas salmonicida	3	6.12
Enteric group 8	1	2.04
Enterobacter cloacae	1	2.04
Escherichia coli	3	6.12
Pasteurella spp.	2	4.08
Pasteurella canis	1	2.04
Pasteurella multocida	5	10.20
Pasteurella pneumotropica	1	2.04
Proteus mirabilis	1	2.04
Weeksella virosa	1	2.04
Anaerobic		
Prevotella melalinogenica	1	2.04
	29	**59.19 %**
Total isolates	**49**	**100 %**

Table 5 Bacteria isolated from those dogs in which more than one CCF was sampled.

	Canine teeth sampled			
Animal	**104**	**204**	**304**	**404**
1		*Enterococcus* spp. *Corynebacterium* spp.	*Corynebacterium* spp. *Moraxella* spp.	
2			*Aeromonas salmonicida Pasteurella multocida.*	*Actinomyces* spp. *Moraxella* spp.
3		*Corynebacterium* spp.	*Corynebacterium* spp.	
4	*Corynebacterium* spp. No. 1 *Corynebacterium* spp. No. 2 *Actinomyces* spp. *Pasteurella canis*	*Bacillus* spp.		
5	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa Pseudomonas alcaligenes Escherichia coli*	*Corynebacterium* spp.	*Pseudomonas aeruginosa Pasteurella multocida Escherichia coli*

Table 7 Efficacy of the different antibiotics tested against all the aerobic and facultative anaerobic bacteria isolated in dogs.

Antibiotics	Efficacy
Gentamicin	92.39 %
Chloramphenicol	89.13 %
Enrofloxacin	85.21 %
Orbifloxacin	76.08 %
Amoxycillin-Clavulanic Acid	73.91 %
Doxycycline / Oxitetracycline	72.82 %
Kanamycin	69.74 %
Amikacin	69.56 %
Sulpha / Trimethropim	65.21 %
Cephalothin / Lexin	60.86 %
Tylosin tartrate	60.13 %
Amoxicillin / Ampicillin	55.43 %
Penicilin G	47.28 %
Lincospectin	39.13 %
Lincomycin	13.04 %

Table 6 Antibiogram and the bacterial profile in dogs.

Bacteria Profile																								
Antibiotics	***Actinomyces* spp. (n=2)**	***Bacillus* spp. (n=3)**	***Bacillus cereus* (n=1)**	***Corynebacterium* spp. (n=5)**	***Corynebacterium* spp. No 1 (n=1)**	***Corynebacterium* spp. No 2 (n=1)**	***Enterococcus* spp. (n=2)**	***Lactobacillus* spp. (n=1)**	***Staphylococcus* spp. (n=1)**	***Staphylococcus aureus* (n=1)**	***Staphylococcus intermedius* (n=1)**	***Aeromonas salmonicida* (n=3)**	**CDC group VE-2 (n=1)**	**Enteric group 8 (n=1)**	***Enterobacter cloacae* (n=1)**	***Escherichia coli* (n=3)**	***Moraxella* spp. (n=4)**	***Pasteurella* spp. (n=2)**	***Pasteurella canis* (n=1)**	***Pasteurella multocida* (n=5)**	***Pasteurella pneumotropica (n=1***	***Proteus mirabilis* (n=1)**	***Pseudomonas aeruginosa (n=3)***	***Pseudomonas alcaligenes* (n=1)**	***Weeksella virosa* (n=1)**
Amikacin	50	100	100	80	100	100	100		100	50	100	66.66	50	50	100	33.33	87.5	100	100	70	100	0	0	100	100
Amoxycillin / Ampicillin	50	66.66	0	20	100	0	50		100	100	0	0	0	100	0	0	50	100	0	60	100	0	0	0	100
Doxycycline / Oxitetracycline	100	100	100	0	50	0	0		100	100	100	66.66	0	100	100	100	75	50	100	90	100	100	100	100	0
Enrofloxacin	100	50	0	60	50	50	100		0	0	0	66.66	0	50	0	33.33	100	25	100	80	100	100	0	100	0
Gentamicin	100	100	100	100	100	50	25		100	100	100	83.33	100	100	100	100	75	75	100	70	100	100	50	100	100
Penicilin G	100	83.33	100	100	100	50	75		100	100	100	83.33	50	100	100	100	87.5	100	100	90	100	100	100	100	100
Sulpha / Trimethropim	100	100	0	20	100	0	100		100	0	100	66.66	0	100	100	66.66	75	75	100	90	100	100	0	100	0
Cephalothin / Lexin	100	100	0	100	100	100	100		100	100	0	16.66	0	0	0	0	62.5	100	100	60	100	100	0	100	0
Kanamycin	100	66.66	50	25	100	50	25		100	100	100	66.66	50	100	100	100	83.33	75	100	90	100	100	0	100	50
Lyncomycin	100	33.33	0	20	0	50	25		0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0
Lincospectin	100	100	100	40	100	50	50		50	0	100	100	0	100	100	100	87.5	75	100	100	100	100	0	100	100
Orbifloxacin	100	100	50	100	100		100		100	100	0	66.66		50	0	83.33	100	75	100	80	100	100	0		100
Amoxycillin-Clavulanic Acid	100	83.33	100	100	100	100	100		100	100	0	50	0	0	0	0	75	75	100	60	100	0	0	0	100
Tylosin Tartrate	100	100	100	100	100	100	100		100	100	0	100	0	100	100	100	100	100	100	100	100	100	0	100	
Chloramphenicol	37.5	100	0	70	50	50	100		0	0	0	33.33	0	0	0	0	50	25	100	80	100	50	0	50	50

Table 8 Bacterial isolates from 36 pulps exposed due to CCF in the canine teeth of cheetahs (N = 59).

	Isolates	Total isolates (%)
Gram-positive		
Facultative anaerobic		
Actinomyces spp.	1	1.69 %
Actinomyces hordeovulnaris	1	1.69 %
Bacillus spp.	13	22.13 %
Corynebacterium spp.	5	8.47 %
Enterococcus spp.	5	8.47 %
Lactobacillus spp.	1	1.69 %
Streptococcus anginosus	1	1.69 %
Anaerobic		
Clostridium septicum	2	3.38 %
Clostridium sordelli	3	5.08 %
	32	**54.29 %**
Gram-negative		
Aerobic		
Acinetobacter calco var. Anitratus	1	1.69 %
Moraxella spp.	5	8.47 %
Moraxella lacunata	1	1.69 %
Pseudomonas aeuroginosa	9	15.25 %
Stenotrophomonas maltophila	1	1.69 %
Facultative Anaerobic		
Aeromonas salmonicida	1	1.69 %
Pasteurella spp.	1	1.69 %
Pasteurella multocida	6	10.16 %
Vibrio spp.	1	1.69 %
Vibrio parahaemolyticus	1	1.69 %
	27	**45.71 %**
Total isolates	**59**	**100 %**

Table 9 Representation of the bacteria isolated from 36 pulps exposed due to CCF in the canine teeth of cheetahs (N = 59), according to the diet cheetahs were fed during the period of the study.

	Meat & IAMS® **Total isolates (%)**	**Meat** **Total isolates (%)**
Gram-positive		
Facultative anaerobic		
Actinomyces spp.		1 (5.88)
Actinomyces hordeovulnaris		1 (5.38)
Bacillus spp.	9 (21.4)	4 (23.52)
Corynebacterium spp.	3 (7.14)	2 (11.76)
Enterococcus spp.	5 (11.9)	
Lactobacillus spp.		1 (5.88)
Streptococcus anginosus		1 (5.88)
Anaerobic		
Clostridium septicum	1 (2.38)	1 (5.88)
Clostridium sordelli	2 (4.76)	1 (5.88)
	20 (47.64)	12 (70.56)
Gram-negative		
Aerobic		
Acinetobacter calco var. Anitratus	1 (2.38)	
Moraxella spp.	4 (9.52)	1 (5.88)
Moraxella lacunata	1 (2.38)	
Pseudomonas aeuroginosa	9 (21.4)	
Stenotrophomonas maltophila	1 (2.38)	
Facultative Anaerobic		
Aeromonas salmonicida		1 (5.88)
Pasteurella spp.	1 (2.38)	
Pasteurella multocida	4 (9.52)	2 (11.76)
Vibrio spp.		1 (5.88)
Vibrio parahaemolyticus	1 (2.38)	
	22 (52.36)	5 (29.4)
Total isolates	**42**	**17**

Table 10 Bacteria isolated from those dental pulps of cheetahs in which more than one CCF was sampled.

	Canine teeth sampled			
Animal	**104**	**204**	**304**	**404**
1		*Lactobacillus* spp.	*Pasteurella multocida* *Aeromonas salmonicida*	*Bacillus* spp.
2	*Acinetobacter calco var. Anitratus* *Bacillus* spp. *Pasteurella* spp.	*Bacillus* spp. *Corynebacterium* spp.		
3		*Bacillus* spp.	*Bacilllus* spp. *Vibrio parahaemolyticus* *Pasteurella multocida* *Moraxella* spp. *Clostridium septicum.*	*Bacillus* spp. *Pseudomonas aeruginosa.*
4	*Pseudomonas aeruginosa* *Enterococcus* spp.	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	*Bacillus* spp.
5	*Stenotrophomonas maltophila*	*Pseudomonas aeruginosa* *Moraxella* spp.		
6	*Enterococcus* spp.	*Enterococcus* spp.	*Enterococcus* spp. *Pasteurella multocida* *Clostridium sordelli*	*Pseudomonas aeruginosa*
7	No growth	*Pseudomonas aeruginosa*	No growth	*Clostridium sordelli*
8	*Bacillus* spp. *Corynebacterium* spp.	*Bacillus* spp.		
9		*Corynebacterium* spp.		No growth.

Table 11 Antibiogram and the bacterial profile in cheetahs.

Bacteria Profile																	
Antibiotics	***Actinomyces* spp. (n=1)**	***Actinomyces hordeovulnaris* (n=1)**	***Bacillus* spp. (n=13)**	***Corynebacterium* spp. (n=5)**	***Enterococcus* spp. (n=5)**	***Lactobacillus* spp. (n=1)**	***Streptococcus anginosus* (n=1)**	***Acinetobacter calco var. Anitratus* (n=1)**	***Aeromonas salmonicida* (n=1)**	***Moraxella* spp. (n=5)**	***Moraxella lacunata* (n=1)**	***Pasteurella* spp. (n=1)**	***Pasteurella multocida* (n=6)**	***Pseudomonas aeuroginosa* (n=9)**	***Stenotrophomonas maltophila* (n=1)**	***Vibrio* spp. (n=1)**	***Vibrio parahaemolyticus* (n=1)**
Amikacin	100	100	96.15	100	20	0	100	100	100	50	0	100	41.66	100	100	100	50
Amoxycillin / Ampicillin	50	100	76.92	60	100	100	100	0	100	100	50	100	66.66	0	100	100	0
Doxycycline / Oxitetracycline	100	0	96.1	100	100	100	100	100	100	100	50	100	91.66	0	100	100	100
Enrofloxacin	100	100	100	100	80	100	100	100	100	100	50	100	100	33.33	100	100	100
Gentamicin	100	100	100	100	30	50	100	100	100	80	50	100	58.33	100	100	100	100
Penicillin G	50	100	92.30	60	100	50	100	0	100	70	50	100	58.33	0	100	50	0
Sulpha / Trimethropim	100	100	88.46	80	80	100	100	100	100	100	0	100	100	0	100	50	100
Cephalothin / Lexin	100	100	100	100	90	100	100	0	100	100	50	100	83.33	0	100	100	0
Kanamycin	100	100	100	90	20	0	100	100	100	60	0	100	41.66	0	100	100	50
Lyncomycin	50	100	38.46	20	20	0	100	0	100	0	0	100	8.33	0	0	0	0
Lincospectin	100	100	38.46	40	60	100	100	100	100	60	0	100	25	0	100	100	0
Orbifloxacin	100	100	76.92	90	50	100	100	100	100	100	50	100	100	0	100	100	100
Amoxycillin-Clavulanic Acid	100	100	96.15	100	100	100	100	0	100	100	100	100	66.66	0	100	100	100
Tylosin Tartrate	100	100	96.15	90	90	100	100	0	100	80	50	100	41.66	0	100	0	0
Chloramphenicol	100	100	100	100	80	100	100	0	100	100	100	100	100	0	0	100	100

Table 12 Efficacy of the different antibiotics tested against all the aerobic and facultative anaerobic bacteria isolated from the cheetahs.

Antibiotics	Efficacy
Enrofloxacin	91.96 %
Gentamicin	86.37 %
Orbifloxacin	86.28 %
Amoxycillin-Clavulanic Acid.	86.04 %
Doxycycline / Oxitetracycline	84.57 %
Sulpha / Trimethropim	82.26 %
Chloramphenicol	81.17 %
Cephalothin / Lexin	77.84 %
Amikacin	73.98 %
Amoxycillin / Ampicillin	70.79 %
Kanamycin	68.33 %
Tylosin Tartrate	67.51 %
Lincospectin	66.08 %
Penicilin G	63.56 %
Lincomycin	31.57 %

Table 13 Results of the measurements of the clinical crown length of the canine teeth, and the distance between the occlusal part of both maxillary canine teeth (104-204) and mandibular canine teeth (304-404) performed in twenty cheetah skulls (all measurements in millimetres).

Skull label	104	204	304	404	Distance between 104-204	Distance between 304-404
G00364	22	21	16	16	42	33
248/10	21	21	16	17	33	21
F278	18	19	14	14	35	30
No ID	19	19	15	15	40	28
M264	21	20	16	16	38	30
PM10/146	19	20	14	15	42	32
5	20	20	15	15	36	31
M403	22	22	16	16	34	28
No ID	21	21	17	17	33	27
M184	18	18	15	15	37	30
No ID	22	22	18	17	36	26
M382	19	19	16	16	35	23
No ID	19	20	17	17	43	30
M 2004 wild caught	23	22	16	17	40	32
F183	16	16	12	12	37	29
F303	17	18	14	15	33	28
04/156	19	19	15	15	35	29
No ID	18	17	14	14	35	28
No ID	20	20	15	15	37	26
F 4yrs	19	18	15	15	28	26
Mean	**19.65**	**19.6**	**15.3**	**15.45**	**36.45**	**28.35**
Length Range	**16-23**	**16-22**	**12-18**	**12-17**	**28-43**	**21-33**

Table 14 Representation of the patient data and nucleic acid-base detection results.

Sample	Transfer No	Patient	Tooth	Species	PCR		
					Clone	Genus or species match	Acc. No
1	28237	Paws	104	Cheetah	F3PJET	*Clostridiale bacteruim*	EU289058
					R6B2275F	*Cardiobacteruim* spp.	Y827877
					6C2275F	*Bacteruim enrichment*	HQ122965
					6G227F	*Clostridiales bacteruim*	EU289058
					A2PJETR	*Tissierella praeacuta*	GQ461814
					BOSA2	*Clostridiales bacteruim*	EU289058
					BOSC2	*Uncultured Synergistetes*	AB522155
					BOSG2	*Clostridiales bacteruim*	EU289058
					D2PJETR	*Caloranaerobacter azorensis*	NR028919
					E2PJETR	*Bacteroidetes bacteruim*	CU922596
					F2PHETR	*Uncultured Clostridiales*	EU289058
2	28234	Selkie	404	Cheetah	BOSH3	*Uncultured bacteruim*	HQ400334
					5E3F0618	*Ehrlichia coli*	AP012030
					6C327F	*Uncultured Eubacteruim*	AM419990
					6E327F	*Uncultured bacteruim*	GQ016861
					B3PJETRE	*Uncultured Eubacteruim*	AM419990
					BOSB3PJET	*Pseudoramibacteruim alactolyticus*	BO36759
					BOSC3PJET	*Delfti tsuruhatensis str*	EF440614
					BOSD3PJET	*Uncultured bacteruim*	EU775855
					BOSE3PJET	*Propionibacteruim* sp. *aura*	GQ4226728
					BOSF3PJET	*Leuconostoc mesenteroides str*	FJ65776
3	28234	Selkie	204	Cheetah	C6PJETR	*Syntrophomonas curvata*	MR025752
					3F627FG	*Pseudoramibacteruim alactolyticus*	B036759
					3G627FH	*Uncultured bacteria camel*	HQ008629
					3D6B0205	*Uncultured rumen bacteruim*	HQ400334
					3E6C0208	*Uncultured bacteruin camel*	HQ008603
					4H6F0217	*Uncultured bacteruim camel*	HQ008629
					5E6PJET	*Uncultured rumen bacteria*	HQ400334
					5F6PJET	*Uncultured bacteruim*	HM248358
					5H6PJETR	*Uncultured bacteruim*	HM248358
					A6PJETR	*Streptococcus gallolycticus*	EU163484
					BOSA6PJET	*Uncultured bacteruim*	GQ016861
					BOSB6PJET	*Uncultured bacteruim*	FJ032552
					BOSD6PJET	*Uncultured Bacteruim*	EU458979
4	28235	Charley	104	Cheetah	F7PJET	*Paenibacillus barcinonensis*	DQ870733
					B11PJET	*Uncultured bacteruim*	HQ728208
					B12PJETR	*Bacillus* sp.	AB425363
					BOSF6PJET	*Uncultured Delftia*	GU563748
					BOSH6PJET	*Uncultured bacteruim*	HQ008619
					C7PJET	*Uncultured bacteruim*	HM272655
					D75PJET	*Uncultured rumen bacteria*	GQ327262
					D7PJETR	*Uncultured bacteruim*	FN985404
					E75PJET	*Uncultured bacteruim*	HM366499
					E11PJETR	*Uncultured bacteruim*	EU748123
					F75PJETR	*Uncultured bacteruim*	HM272655

Table 14 continued

Sample	Transfer No	Patient	Tooth	Species	PCR		
					Clone	Genus or species match	Acc. No
5	28235	Charley	204	Cheetah	H9PJET	*Uncultured rumen bacteruim*	HQ400334
					3H427FF	*Uncultured Bacillus* sp.	EF636830
					5C427FD	*Tissierella praeacuta*	GQ461814
					5F227F	*Tissierella praeacuta*	GQ461814
					A5PETRD	*Tissierella praeacuta*	GQ461814
					B4PJETRF0217	*Bacteroides*	EU136689
					B6PJETRB0606	*Clostriduim hastiforme*	X80841
					C4PJETRG0202	*Fusobacterium russi*	M58681
					C5PJETR	*Uncultured bacteruim camel*	HQ008619
					D10PJET	*Uncultured bacteruim*	EU458979
					E4PJETRH	*Bacteroides suis*	AB542771
					F10PJETRF	*Uncultured Peptostreptococcus acaea*	EU289040
6	28236	Tongs	204	Cheetah	E7PJET	*Porphyromonas* sp.	EU012331
					1G115E1	*Lactobacillus curvateos*	AB494734
					711B27	*Uncultured bacteria*	FJ959685
					711B31	*Uncultured Eubacteriaceae*	AM419965
					712A27	*Uncultured bacterium*	FM873231
					712B27	*Delftia tsuruhatensis*	HM003215
					715F27	*Uncultured bacteria*	FJ959686
					712H27	*Fusobacterium russi*	M58681
					BOSA5PJ	*Uncultured bacteruim*	EU844467
					BOSC5PJET	*Uncultured bacteruim*	FJ55776
					BOSE5PJET	*Uncultured bacteruim*	HM336345
					BOSF5PJET	*Uncultured bacteruim*	EU844467
					C118	*Fusobacterium necrophorum*	AB525413
7	28232	Tosca	304	Canine	E118	*Uncultured bacteruim*	Q308572
					5B8	*Uncultured bacteruim*	EU681991
					5F8	*Uncultured bacteria*	FJ959656
					EH8	*Uncultured*	HM272655
					B118	*Fusobacterium necrophorum*	AB525413
					BOSD7	*Uncultured Bacteruim*	HM272655
					BOSE7	*Uncultured Bacteruim*	HM272655
					BOSF7	*Uncultured Bacteruim*	HM272655
					3G8G0220	*Uncultured bacteruim*	HM272655
					C118	*Fusobacterium necrophorum*	AB525413
8	28233	Jabu	204	Canine	F1PJET	*Uncultured bacteruim*	HM341046
					5E1C0609	*Clostridium sp*	FJ384368
					5G1D0612	*Uncultured bacteria*	HM272655
					6B127F	*Clostridium* sp.	FJ159526
					6C127F	*Uncultured bacterium*	HM336453
					BOSA1	*Uncultured bacteruim*	CU915048
					BOSB1	*Uncultured Delftia* sp.	GU563745
					BOSD1	*Uncultured bacteruim*	FJ024720
					BOSF1	*Achromobacter* sp.	HQ619222
					BOSG1	*Filifactor villosus*	F537211
					BOSH1	*Clostridium bifermentans*	AB538434

Table 15 Comparative results of standard culture and 16S rRNA PCR for detection of bacteria in RC with necrotic pulps of canine teeth in those dogs and cheetahs where both methods were applied.

Cheetahs	PCR	Culture
Paws 104	*Clostridiales bacteruim* (Freq. 4) G+ Anaerobic *Cardiobacterium* spp. G- Fac. Anaerobic *Bacteruim enrichment* *Tisierella Praeacuta* G- Anaerobic *Uncultured Synergistetes* G- Anaerobic *Caloranaerobacter azorensis* G- Anaerobic *Bacteroridetes bacteruim* G- Anaerobic *Uncultured Clostridiales* G+ Anaerobic	*Moraxella* spp. G- Aerobic *Moraxella lacunata* G- Aerobic
Selkie 404	*Uncultured Bacteruim* (Freq. 3) *Ehrlichia coli* GI disorders in foal *Uncultured Eubacterium* (Freq. 2) G- Anaerobic *Pseudoramibacterium alactolyticus* G+ Anaerobic *Delftia tsuruhatensis str* G- Aerobic *Propionibacterium* sp. *aura* G+ Anaerobic *Leuconostoc mesenteroides str* G+ Fac. Anaerobic	No growth after 72 h of incubation
Selkie 204	*Syntrophomonas curvata* G+ Anaerobic *Pseudoramibacterium alactolyticus* G+ Anaerobic *Uncultured bacteruim camel* (Freq. 3) *Uncultured rumen bacteria* (Freq. 2) *Uncultured bacteruim* (Freq. 5) *Streptococcus gallolycticus (S. Bovis* type I) G+ Fac. Anaerobic	*Corynebacterium* spp. G+ Fac. Anaerobic
Charley 104	*Paenibacillus barcinonensis* G+ Fac. Anaerobic *Uncultured Bacteruim* (Freq. 7) *Bacillus* spp. G+ Fac. Anaerobic *Uncultured Delftia* G- Aerobic *Uncultured rumen bacteria*	*Bacillus* spp. G+ Fac. Anaerobic *Corynebacterium* spp. G+ Fac. Anaerobic
Charley 204	*Uncultured rumen bacteria* *Uncultured Bacillus* spp. G+ Fac. Anaerobic *Tisierella praeacuta* G- Anaerobic (Freq. 3) *Bacteroides* G- Anaerobic *Clostriduim hastiforme (*Synonym *Tisierella praeacuta)* G- Anaerobic *Fusobacterium russi* G- Anaerobic *Uncultured bacteruim camel* *Uncultured bacteruim* *Bacteroides suis* G- Anaerobic *Uncultured Peptostreptococcus acaea* G+ Anaerobic	*Bacillus* spp. G+ Fac. Anaerobic
Tongs 204	*Porphyromonas* spp. G- Anaerobic *Lactobacillus curvateos* G+ Fac. Anaerobic *Uncultured bacteruim* (Freq. 7) *Uncultured Eubacteriaceae* *Delftia tsuruhatensis* G- Aerobic *Fusobacterium russi* G- Anaerobic *Fusobacterium necrophorum* G- Anaerobic	*Bacillus* spp. G+ Fac. Anaerobic *Pasteurella multocida* G- Fac. Anaerobic
Dogs	**PCR**	**Culture**
Tosca 304	*Uncultured bacteruim* (Freq. 8) *Fusobacterium necrophorum* (Freq. 2) G- Anaerobic	*Actinomyces* spp. G+ Fac. Anaerobic
Jabu 204	*Uncultured bacteruim* (Freq. 5) *Clostridium* spp. (Freq. 2) G+ Anaerobic *Uncultured Delftia* G- Aerobic *Achromobacter* spp. G- Anaerobic *Filifactor villosus synonym Clostridium villosum* G+ Anaerobic *Clostridium bifermentans* G+ Anaerobic	*Enterobacter cloacae* G+ Fac. Anaerobic *Pasteurella multocida* G- Fac. Anaerobic *Aeromonas salmonicida* G- Fac. Anaerobic *Clostridium acetobulyticum* G+ Anaerobic

Chapter 4
Results

Thirty-nine animals were included in this study of which 20 were dogs and 19 were cheetahs.

4.1 Dogs

Of the 20 dogs seen in this study Staffordshire bull terrier was the most common breed presented (Figure 23). The age of the dogs ranged between 1-10 years, with the average age 4.6 years. Half of the dogs were younger than three years of age (Figure 24). The dogs were classified as male, female, neutered or spayed (Figure 25). Males were the most commonly affected (14/20), and represented nearly 70 % of the dogs studied. All the dogs were fed a commercially available dry food diet.

Of the dogs presented, 20 % (4/20) had two fractured canine teeth and only one, 5 % (1/20), had all four canine teeth fractured (Figure 26). In the majority of the animals (15/20) just one RC treatment was required. In total 27 pulps were sampled from the dogs in this study. Maxillary canine teeth were fractured in 16 (59.24 %) of the cases compared to 11 cases (40.76 %) in which a mandibular tooth was fractured. There was no predilection for fracture of left or right canine tooth of the maxilla or mandible (Figure 27).

A total of 49 cultivable isolates, belonging to 27 different microbial species and 18 different genera, were recovered from the 27 RC sampled (Table 4). Twenty (40.81 %) of those 49 cultivable isolates were Gram positive and the other 29 (59.19 %) Gram negative. All different colony types isolated from the primary cultures were subcultured and identified. Individual RC yielded a maximum of four species each. Two RC had no cultivable bacteria. A single microorganism was found in nine cases. Ten cases presented two species (*Pasteurella* spp. and *Enteric* group 8, *Staphylococcus aureus* and *Weeksella virosa*; *Pasteurella pneumotropica* and *Enterococcus* spp.; *Staphylococcus intermedius* and *Moraxella* spp.; *Staphylococcus* spp. and *Pasteurella multocida*; *Corynebacterium* spp. and *Moraxella* spp.; *Enterococcus* spp. and *Corynebacterium* spp.; *Aeromonas salmonicida* and *Pasteurella multocida*; *Actinomyces* spp and *Moraxella* spp.; *Aeromonas salmonicida* and *Moraxella* spp.) and six cases were polymicrobial infections consisting of three or more species per canal. In those animals, which required more

than one RCT, the bacteria isolated from the different RC showed the results represented on Table 5.

Of the bacterial isolates, 4.08 % (2/49) were strict anaerobes, *Clostridium acetobulitycum* (2.04 %), and *Prevotella melalinogenica* (2.04 %). Aerobic bacteria made up 18.36 % (9/49) of the bacteria isolated. Facultative anaerobic bacteria with 77.56 % (38/49) were the most common bacteria isolated (Figure 28). Bacteria, which presented with an incidence higher than 6 % are shown in Figure 29.

The most effective bactericidal antibiotics were Enrofloxacin (85.21 %), Gentamicin (92.39 %) and Chloramphenicol (89.13 %). Penicillin G (47.28 %), Lincomycin (13.04 %) and Lincospectin (39.13 %) all showed poor results (Table 7). All the results of the antibiotics tested against all the microbes in dogs are represented in Table 6. The bacterial isolates that showed the highest resistance against the majority of the antibiotics tested in this study were *Staphylococcus intermedius*, CDC group Ve-2, and *Pseudomonas aeruginosa* (Table 5). It was impossible to test the sensitivity of *Lactobacillus* spp. against any of the antibiotics as it grew too slow.

4.2 Cheetahs

Of the 19 cheetahs in this study, three (15.79 %) were treated in the Dentistry and Maxillofacial Surgery Clinic of the OVAH, University of Pretoria, Pretoria, South Africa. The other 16 (84.21 %) were treated in the clinic of AF, Otjiwarongo, Namibia.

The ages of the cheetahs ranged from 3.5 years to 15 years, with an average age of 6.94 years (Figure 30). Female cheetahs were nearly twice as likely to present with CCF (63.16 %) compared to males (36.84 %) (Figure 31). All the females included in this study from AF, are animals that are treated yearly with contraceptive implants, as it is prohibited by law to breed large carnivores in captivity in Namibia.

The feeding regime of the cheetahs at the AF was as follows:

- Two of the males and one female were fed meat every day, excluding Wednesdays and Sundays.

- Five of the males and eight females were fed meat on Monday and Friday; and 500 grams of IAMS® cat food (soaked in water) per animal on Tuesday, Thursday and Saturday.
- The meat that is fed to the animals is usually from horses or donkeys which are cut into 1.5-2 kg pieces.

At TAVDCC the cheetahs are fed horse meat and whole chickens 2-3 times a week.

In this study maxillary canine teeth were more frequently fractured (62 %) than mandibular canine teeth (38 %). When comparing fractures of the left or right maxillary canine teeth, the numbers seem to be comparable while those of the fractures affecting mandibular canine teeth are equal (Figure 32).

A total of 59 cultivable isolates, belonging to 19 different microbial species and 13 different genera, were recovered from the 36 RC sampled (Table 8). Thirty-two (54.49 %) of these isolates are Gram positive bacteria and the other 27 (45.71 %) of them Gram negative. All different colony types isolated from the primary cultures were subcultured and identified. Individual RC yielded a maximum of six species each. Four RC had no cultivable bacteria. A single microorganism was found in 17 cases. Nine cases presented two species (*Pasteurella multocida* and *Aeromonas salmonicida* subsp. *achromogenes*, *Bacillus* spp. and *Corynebacterium* spp.; *Pseudomonas aeruginosa* and *Bacillus* spp.; *Pseudomonas aeruginosa* and *Enterococcus* spp.; *Pseudomonas aeruginosa* and *Moraxella* spp.; *Clostridium sordelli* and *Moraxella* spp.; *Moraxella* spp. and *Moraxella lacunata*; *Bacillus* spp. and *Corynebacterium* spp.; *Bacillus* spp. and *Pasteurella multocida*; *Aeromonas salmonicida* and *Moraxella* spp.) and six cases were polymicrobial infections consisting of three or more species per canal.

Differences between those animals, which were fed only meat, and those eating meat and a commercial diet are represented in Table 9. In those cheetahs, which had more than one RC treated, the difference between them is presented in Table 10.

Of all the bacterial species isolated, 8.47 % (5/59) were strict anaerobes and 28.81 % (17/59) strict aerobes. Facultative anaerobic species accounted for the remainder of the isolates (62.72 %; 37/59) (Figure 33). The identity of the anaerobic bacteria was *Clostridium sordelli* (5.08 %), and *Clostridium septicum* (3.38 %). All the different bacteria isolated from the

necrotic pulps of cheetahs are represented in the Table 8. Bacteria with an incidence higher than 5.25 % are represented in Figure 34.

All the bacteria cultured were subjected to an antibiogram panel containing 15 different antibiotics. The efficacy of the antibiotics against the bacteria is reported in Table 11. Of all the antibiotics tested Enrofloxacin (91.96 %) was the most effective and Lincomycin (31.57 %) the least effective (Table 12). The bacterial isolates that showed the higher resistance against the majority of the antibiotics tested in this study were *Acinetobacter calco* var. *Anitratus*, *Moraxella lacunata*, *Pseudomonas aeruginosa* and *Vibrio parahaemolyticus*.

Maxillary canine teeth have a clinical crown length ranging from 16-23 mm, and a mean of 19.6 mm, whereas the mandibular canine teeth's clinical crown length ranged from 12-18 mm, with a mean of 15.4 mm (Table 13). The inter maxillary canine teeth distance ranged from 28-43 mm, and a mean of 36.45 mm. Mandibular canine teeth had an inter canine distance that ranged from 21-33 mm, with a mean of 28.35 mm (Table 13).

4.3 Nucleic acid-base detection

A total of eight samples, comprising six from cheetahs and two from dogs, were analysed using culture techniques and an initial screening with the 16S rRNA-specific PCR. In dogs, Gram negative and Gram positive bacteria were equally represented with a 50 % (3/6) of all the bacteria detected. Anaerobic bacteria were predominant and were represented by 83.3 % (5/6) of the bacteria detected, while aerobic bacteria comprised 16.6 % (1/6). On the other hand, in cheetahs, the bacteria obtained by PCR method showed prevalence rates of anaerobic bacteria of 60.8 % (14/23), facultative anaerobic bacteria of 30.2 % (7/23) and aerobic bacteria of 8.6 % (2/23).

The bacteria found in the dog and cheetah samples which were identified from the BLAST (Basic Local Alignment Search Tool) searches are represented in Table 14. Where a sequence appeared in more than one sample, only one clone name is given. Many of the clone sequences were similar to sequences from bacterial species, which have been reported from human infected RC, such as *Pseudoramibacter alactolyticus* (69), *Tisierella praecuata* (33) and *Fusobacterium necrophorum* (33). However, other clone sequences were similar with sequences, which were only identified to the genus level. Some of these belong to genera, which had previously been isolated from RC infections in human. For example, *Bacteroides* spp., *Porphyromonas* spp. (8).

However, other clone sequences were similar to those of unidentified bacteria, such as clone H9PJET, which matched those of a bacterial isolate from the rumen.

A comparison between standard bacterial culture techniques and 16S rRNA PCR for detection of bacteria in necrotic RC of dogs and cheetahs was performed. There was a greater number of positive results of bacteria identified for dog and cheetah samples by the PCR assay than by culture techniques (Table 15), although a larger sample size would be necessary to determine whether this was a significant difference. The results from culture analysis and 16S RNA PCR, displayed a relatively low similarity in the species, in both dogs and cheetahs.

4.4 Figures

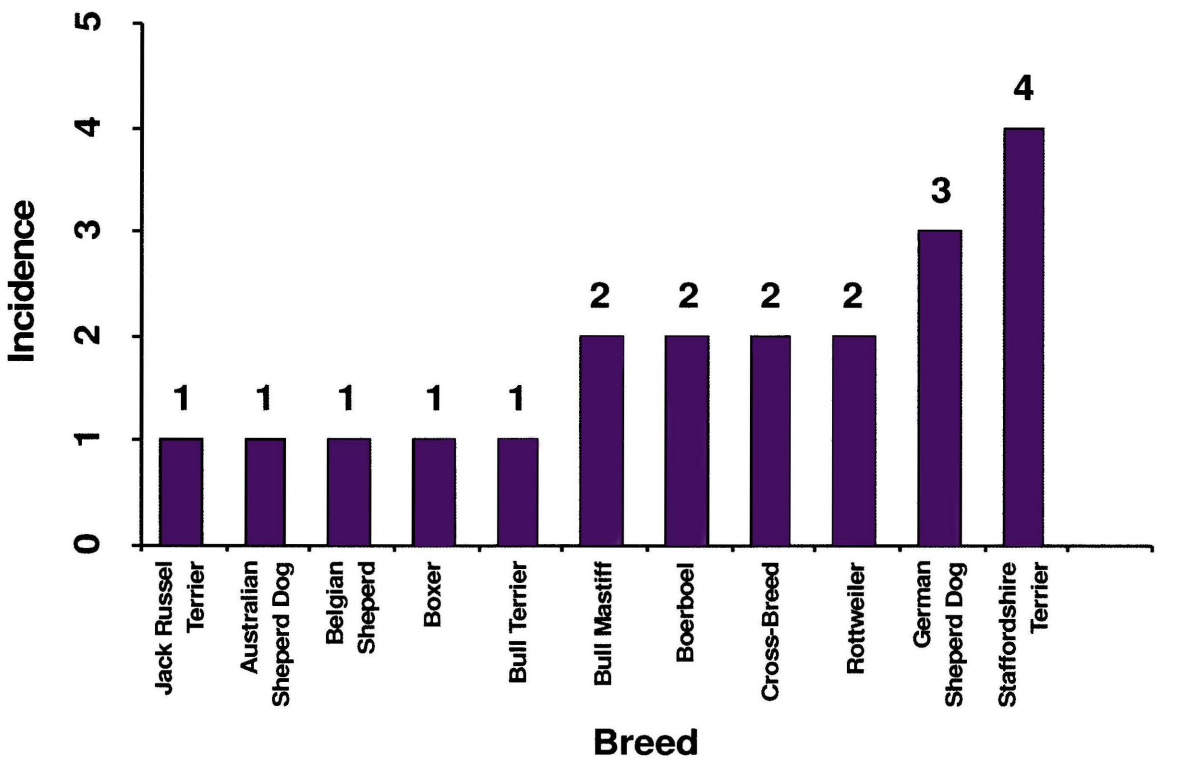

Figure 23 Breed incidence of the dogs included in the study.

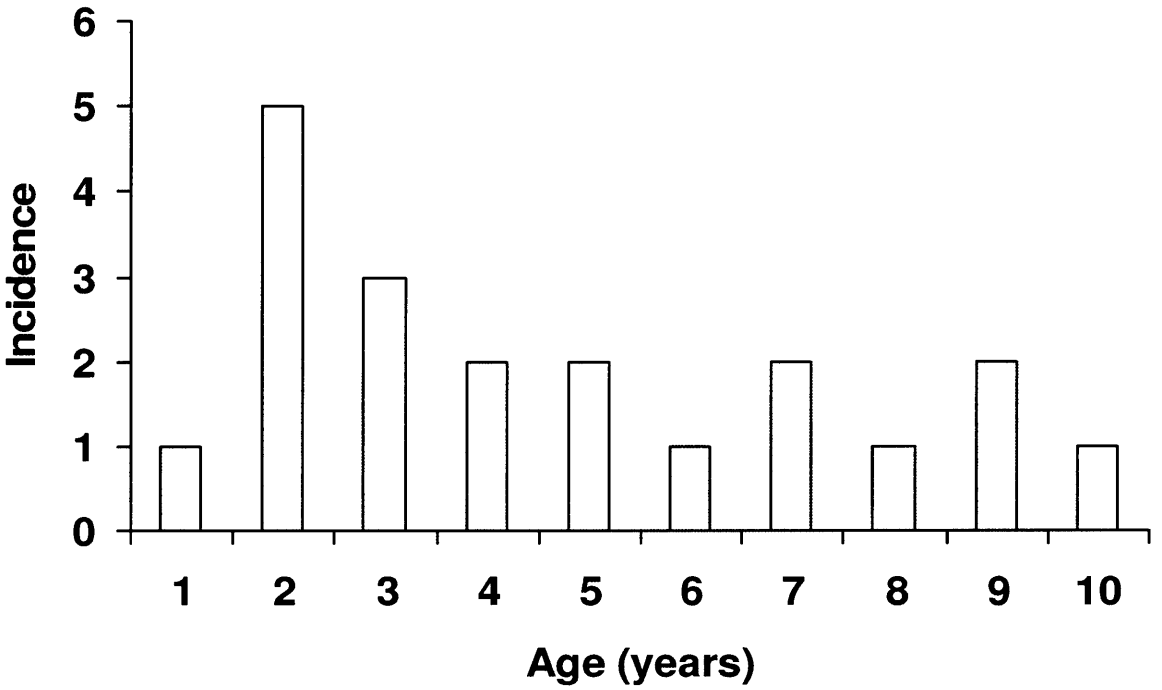

Figure 24 Age distribution of the dogs included in this study. Note the higher incidence in animals younger than five years of age.

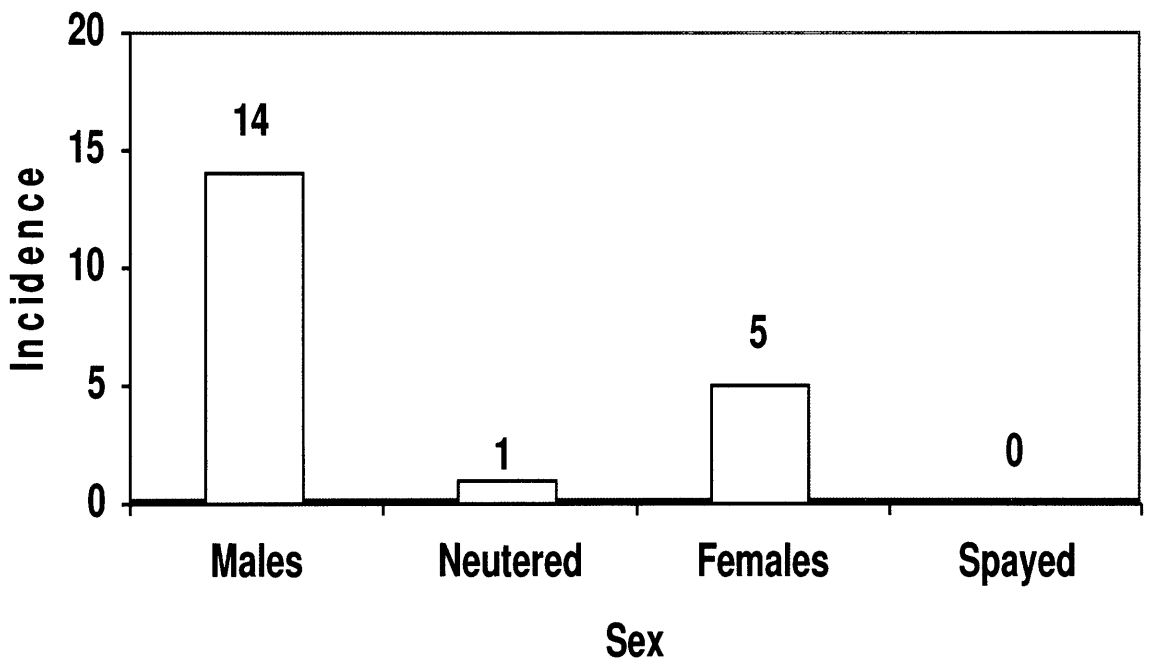

Figure 25 Sex distribution of the dogs included in this study. Note the higher incidence of CCF in males.

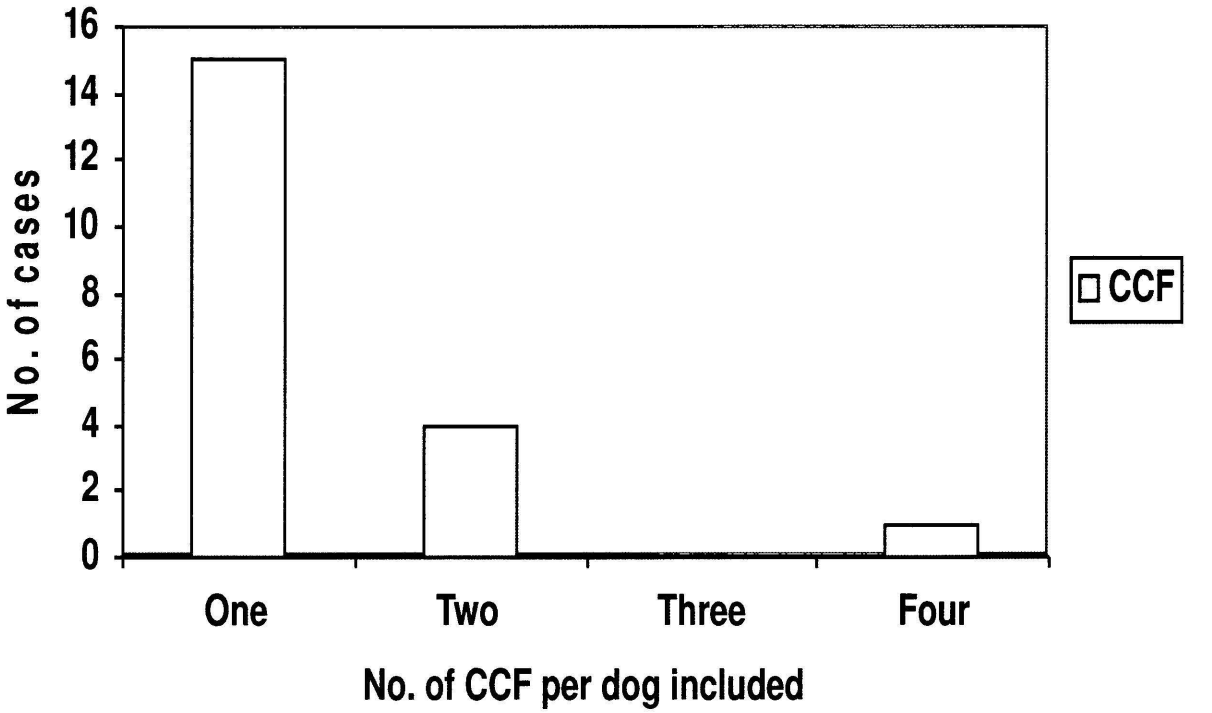

Figure 26 Number of canine teeth with CCF per dog included in the study.

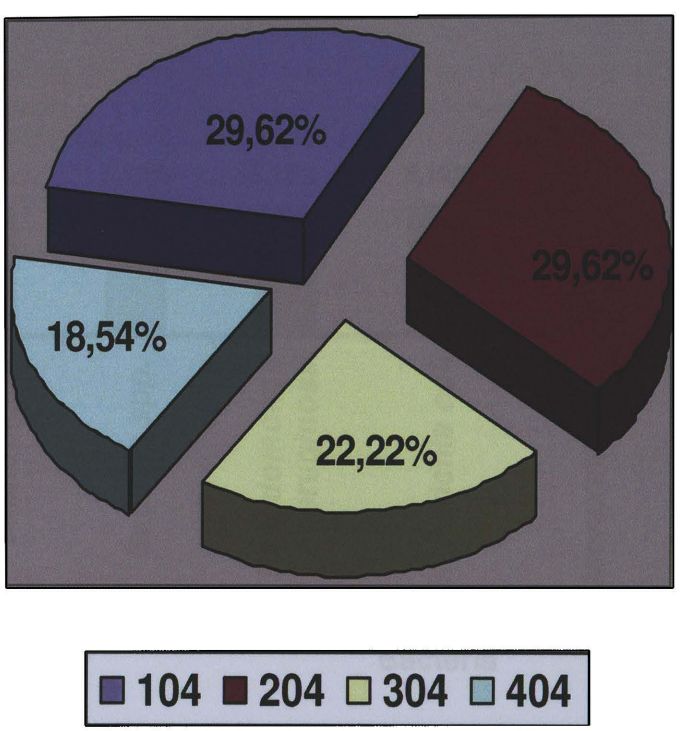

Figure 27 Distribution of CCF incidence of the canine teeth of the dogs.

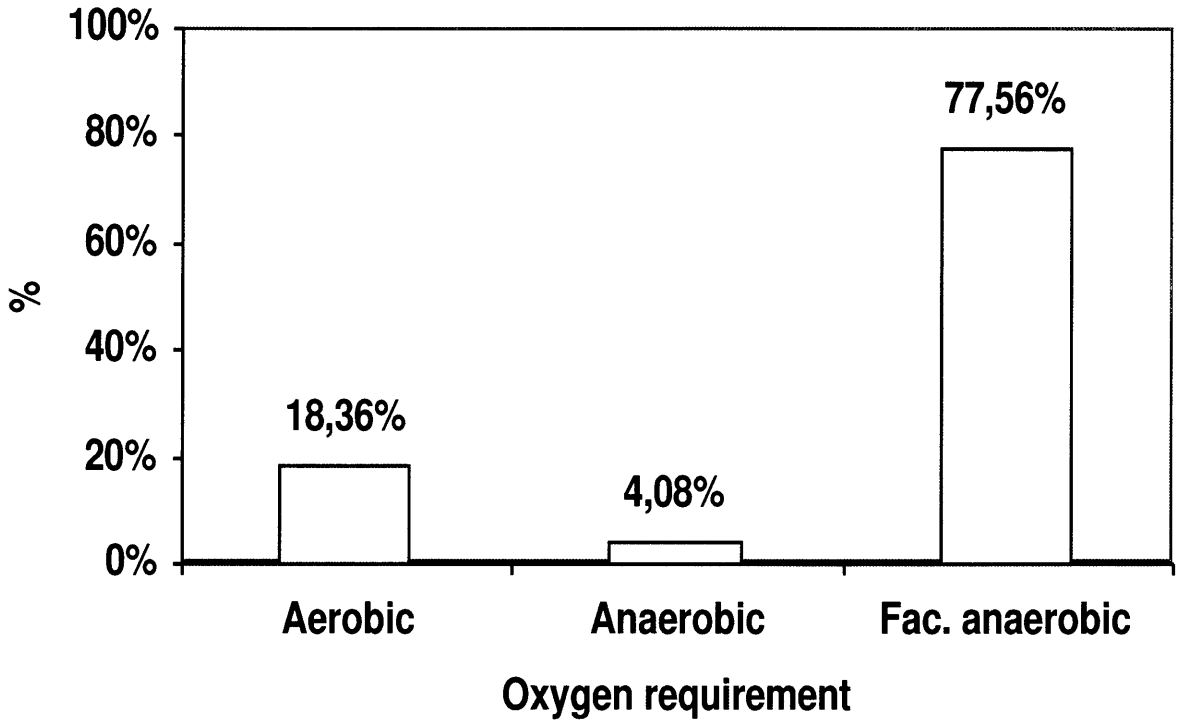

Figure 28 Percentage of the different bacteria according to the oxygen requirement.

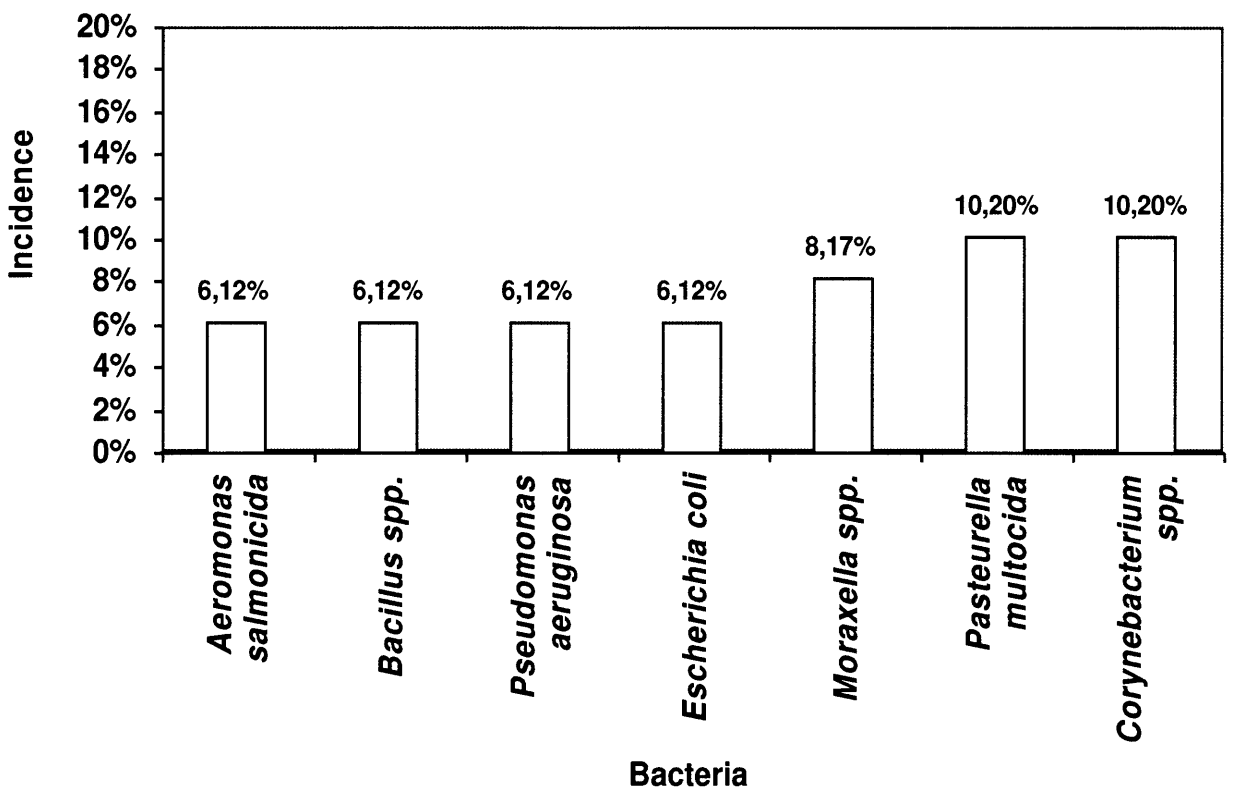

Figure 29 Bacteria isolated with an incidence higher than 6 %.

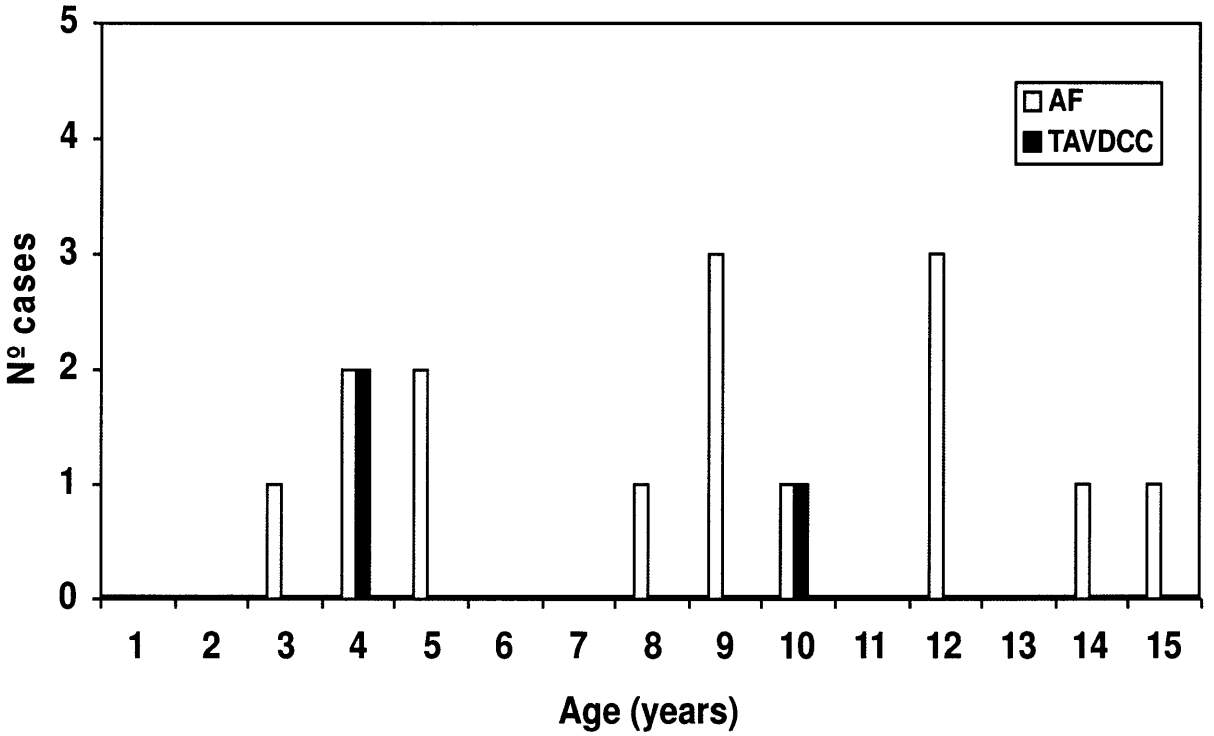

Figure 30 Age distribution of the cheetahs in the study.

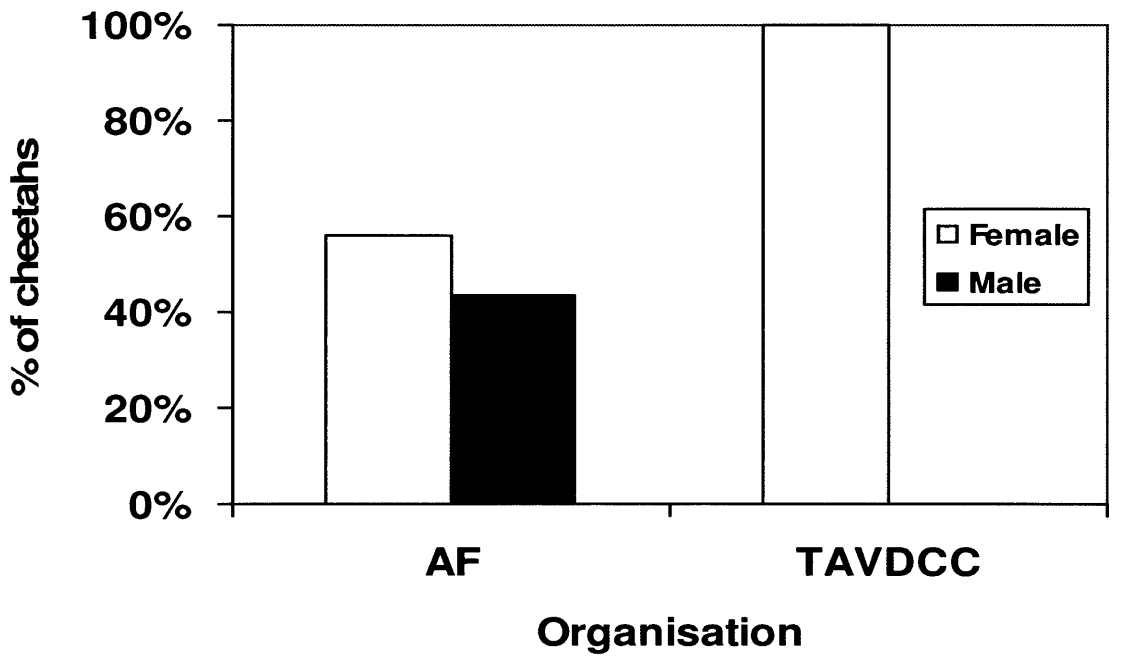

Figure 31 Sex distribution of all cheetahs in the study. Note the higher incidence of CCF in females.

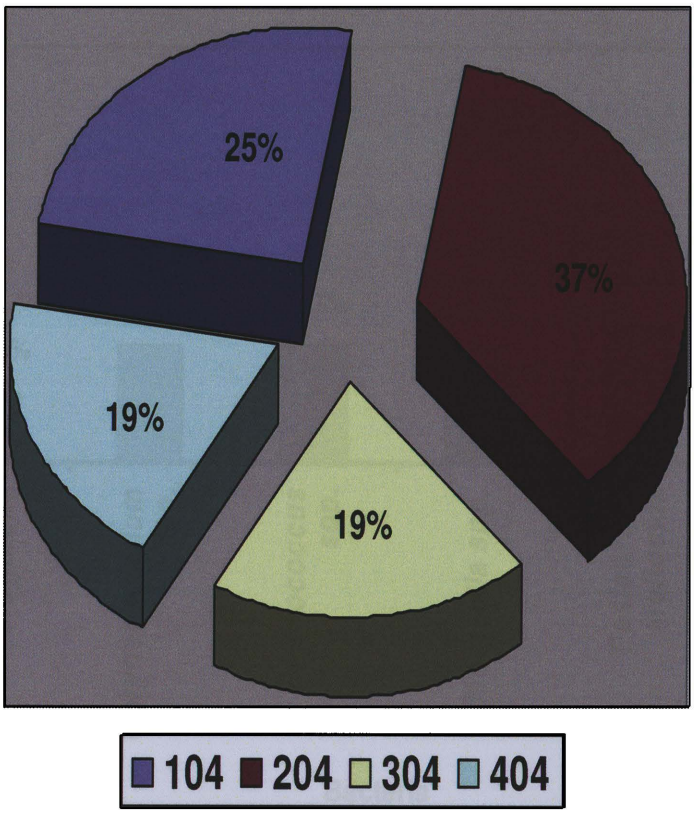

Figure 32 Distribution of the CCF incidence in the canine teeth of cheetahs. Note the marked incidence in the maxillary canine teeth (104 and 204).

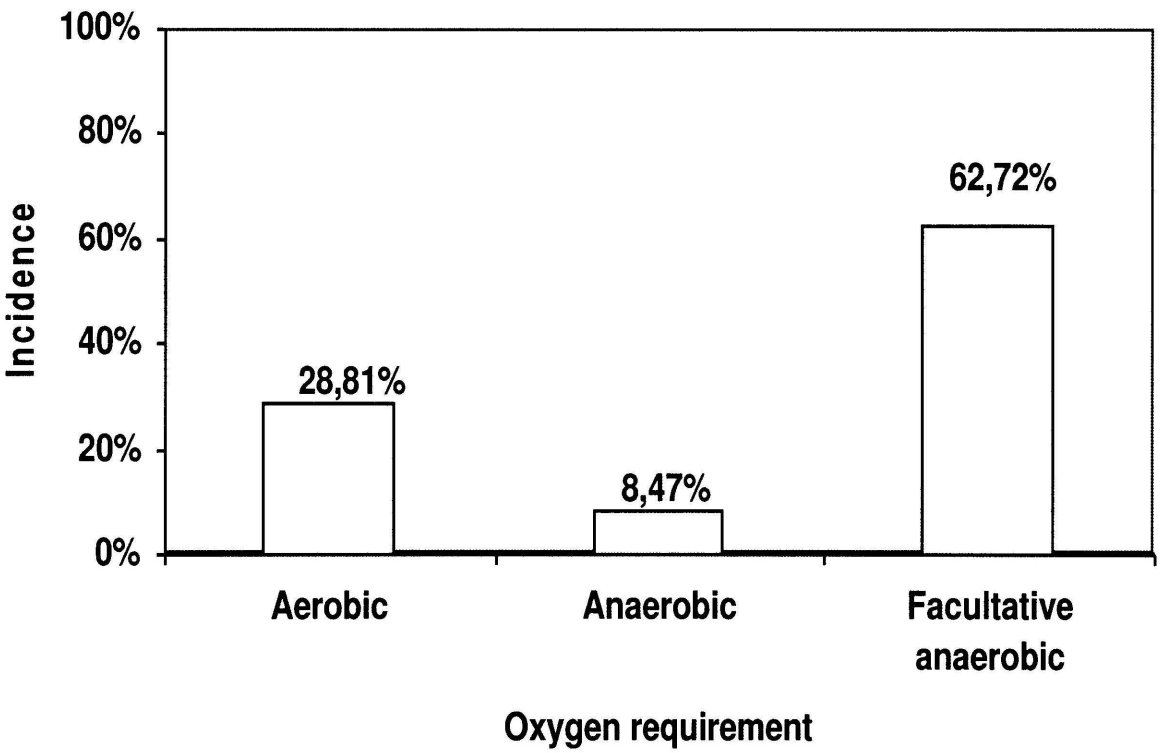

Figure 33 Percentage of the different bacteria according to their oxygen requirement isolated from cheetahs.

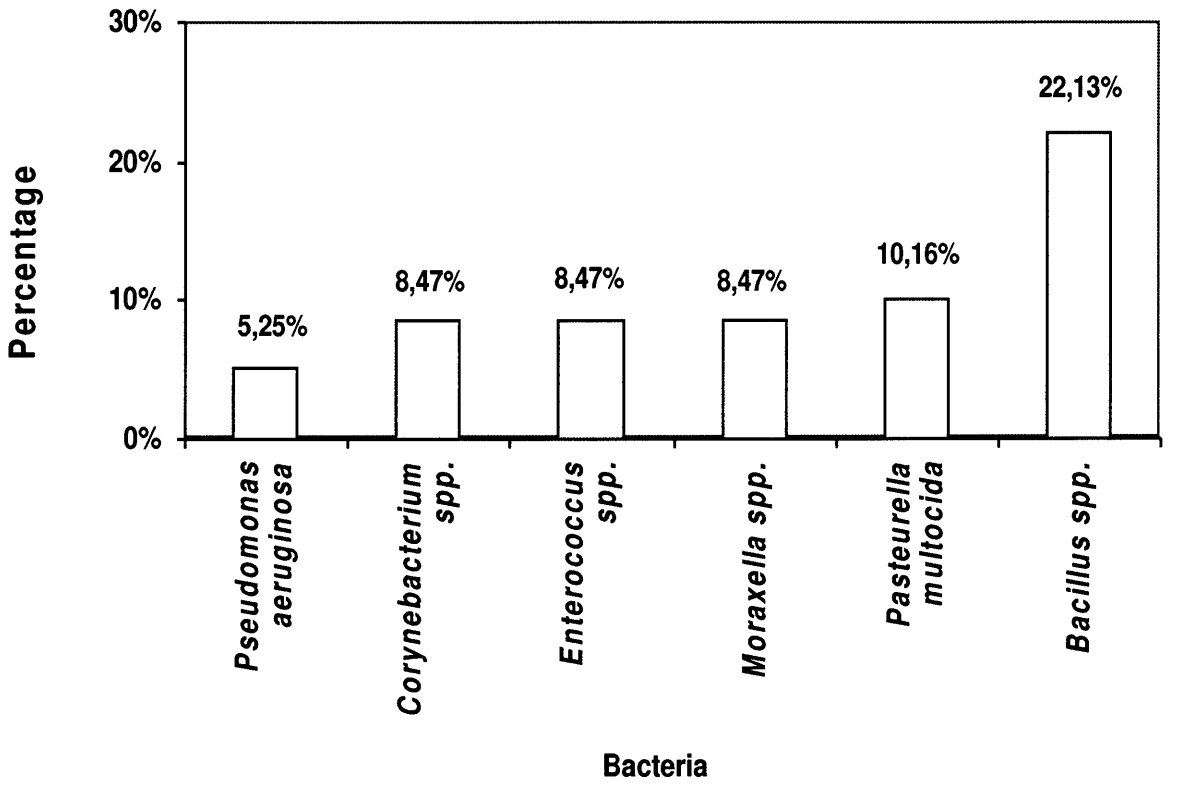

Figure 34 Representation of the bacteria isolated from the necrotic pulps of cheetahs with an incidence higher than 5.25 %.

4.5 Tables

Table 4 Bacteria isolated from 27 pulps exposed due to CCF in the canine teeth of dogs (N = 49).

	Isolates	Total isolates (%)
Gram-positive		
Facultative Anaerobic		
Actinomyces spp.	2	4.08
Bacillus spp.	3	6.12
Bacillus cereus	1	2.04
Corynebacterium spp.	5	10.20
Corynebacterium spp. No 1	1	2.04
Corynebacterium spp. No 2	1	2.04
Enterococcus spp.	2	4.08
Lactobacillus spp.	1	2.04
Staphylococcus spp.	1	2.04
Staphylococcus aureus	1	2.04
Staphylococcus intermedius	1	2.04
Anaerobic		
Clostridium acetobulyticum	1	2.04
	20	**40.81**
Gram-negative		
Aerobic		
Pseudomonas aeruginosa	3	6.12
Pseudomonas alcaligenes	1	2.04
CDC group VE-2	1	2.04
Moraxella spp.	4	8.17
Facultative Anaerobic		
Aeromonas salmonicida	3	6.12
Enteric group 8	1	2.04
Enterobacter cloacae	1	2.04
Escherichia coli	3	6.12
Pasteurella spp.	2	4.08
Pasteurella canis	1	2.04
Pasteurella multocida	5	10.20
Pasteurella pneumotropica	1	2.04
Proteus mirabilis	1	2.04
Weeksella virosa	1	2.04
Anaerobic		
Prevotella melalinogenica	1	2.04
	29	**59.19 %**
Total isolates	**49**	**100 %**

Table 5 Bacteria isolated from those dogs in which more than one CCF was sampled.

	Canine teeth sampled			
Animal	**104**	**204**	**304**	**404**
1		*Enterococcus* spp. *Corynebacterium* spp.	*Corynebacterium* spp. *Moraxella* spp.	
2			*Aeromonas salmonicida Pasteurella multocida.*	*Actinomyces* spp. *Moraxella* spp.
3		*Corynebacterium* spp.	*Corynebacterium* spp.	
4	*Corynebacterium* spp. No. 1 *Corynebacterium* spp. No. 2 *Actinomyces* spp. *Pasteurella canis*	*Bacillus* spp.		
5	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa Pseudomonas alcaligenes Escherichia coli*	*Corynebacterium* spp.	*Pseudomonas aeruginosa Pasteurella multocida Escherichia coli*

Table 6 Antibiogram and the bacterial profile in dogs.

Bacteria Profile																								
Antibiotics	***Actinomyces* spp. (n=2)**	***Bacillus* spp. (n=3)**	***Bacillus cereus* (n=1)**	***Corynebacterium* spp. (n=5)**	***Corynebacterium* spp. No 1 (n=1)**	***Corynebacterium* spp. No 2 (n=1)**	***Enterococcus* spp. (n=2)**	***Lactobacillus* spp. (n=1)**	***Staphylococcus* spp. (n=1)**	***Staphylococcus aureus* (n=1)**	***Staphylococcus intermedius* (n=1)**	***Aeromonas salmonicida* (n=3)**	**CDC group VE-2 (n=1)**	**Enteric group 8 (n=1)**	***Enterobacter cloacae* (n=1)**	***Escherichia coli* (n=3)**	***Moraxella* spp. (n=4)**	***Pasteurella* spp. (n=2)**	***Pasteurella canis* (n=1)**	***Pasteurella multocida* (n=5)**	***Pasteurella pneumotropica (n=1***	***Proteus mirabilis* (n=1)**	***Pseudomonas aeruginosa (n=3)***	***Pseudomonas alcaligenes* (n=1)**	***Weeksella virosa* (n=1)**
Amikacin	50	100	100	80	100	100	100		100	50	100	66.66	50	50	100	33.33	87.5	100	100	70	100	0	0	100	100
Amoxycillin / Ampicillin	50	66.66	0	20	100	0	50		100	100	0	0	0	100	0	0	50	100	0	60	100	0	0	0	100
Doxycycline / Oxitetracycline	100	100	100	0	50	0	0		100	100	100	66.66	0	100	100	100	75	50	100	90	100	100	100	100	0
Enrofloxacin	100	50	0	60	50	50	100		0	0	0	66.66	0	50	0	33.33	100	25	100	80	100	100	0	100	0
Gentamicin	100	100	100	100	100	50	25		100	100	100	83.33	100	100	100	100	75	75	100	70	100	100	50	100	100
Penicilin G	100	83.33	100	100	100	50	75		100	100	100	83.33	50	100	100	100	87.5	100	100	90	100	100	100	100	100
Sulpha / Trimethropim	100	100	0	20	100	0	100		100	0	100	66.66	0	100	100	66.66	75	75	100	90	100	100	0	100	0
Cephalothin / Lexin	100	100	0	100	100	100	100		100	100	0	16.66	0	0	0	0	62.5	100	100	60	100	100	0	100	0
Kanamycin	100	66.66	50	25	100	50	25		100	100	100	66.66	50	100	100	100	83.33	75	100	90	100	100	0	100	50
Lyncomycin	100	33.33	0	20	0	50	25		0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0
Lincospectin	100	100	100	40	100	50	50		50	0	100	100	0	100	100	100	87.5	75	100	100	100	100	0	100	100
Orbifloxacin	100	100	50	100	100		100		100	100	0	66.66		50	0	83.33	100	75	100	80	100	100	0		100
Amoxycillin-Clavulanic Acid	100	83.33	100	100	100	100	100		100	100	0	50	0	0	0	0	75	75	100	60	100	0	0	0	100
Tylosin Tartrate	100	100	100	100	100	100	100		100	100	0	100	0	100	100	100	100	100	100	100	100	100	0	100	
Chloramphenicol	37.5	100	0	70	50	50	100		0	0	0	33.33	0	0	0	0	50	25	100	80	100	50	0	50	50

Table 7 Efficacy of the different antibiotics tested against all the aerobic and facultative anaerobic bacteria isolated in dogs.

Antibiotics	Efficacy
Gentamicin	92.39 %
Chloramphenicol	89.13 %
Enrofloxacin	85.21 %
Orbifloxacin	76.08 %
Amoxycillin-Clavulanic Acid	73.91 %
Doxycycline / Oxitetracycline	72.82 %
Kanamycin	69.74 %
Amikacin	69.56 %
Sulpha / Trimethropim	65.21 %
Cephalothin / Lexin	60.86 %
Tylosin tartrate	60.13 %
Amoxicillin / Ampicillin	55.43 %
Penicilin G	47.28 %
Lincospectin	39.13 %
Lincomycin	13.04 %

Table 8 Bacterial isolates from 36 pulps exposed due to CCF in the canine teeth of cheetahs (N = 59).

	Isolates	Total isolates (%)
Gram-positive		
Facultative anaerobic		
Actinomyces spp.	1	1.69 %
Actinomyces hordeovulnaris	1	1.69 %
Bacillus spp.	13	22.13 %
Corynebacterium spp.	5	8.47 %
Enterococcus spp.	5	8.47 %
Lactobacillus spp.	1	1.69 %
Streptococcus anginosus	1	1.69 %
Anaerobic		
Clostridium septicum	2	3.38 %
Clostridium sordelli	3	5.08 %
	32	**54.29 %**
Gram-negative		
Aerobic		
Acinetobacter calco var. Anitratus	1	1.69 %
Moraxella spp.	5	8.47 %
Moraxella lacunata	1	1.69 %
Pseudomonas aeuroginosa	9	15.25 %
Stenotrophomonas maltophila	1	1.69 %
Facultative Anaerobic		
Aeromonas salmonicida	1	1.69 %
Pasteurella spp.	1	1.69 %
Pasteurella multocida	6	10.16 %
Vibrio spp.	1	1.69 %
Vibrio parahaemolyticus	1	1.69 %
	27	**45.71 %**
Total isolates	**59**	**100 %**

Table 9 Representation of the bacteria isolated from 36 pulps exposed due to CCF in the canine teeth of cheetahs (N = 59), according to the diet cheetahs were fed during the period of the study.

	Meat & IAMS® **Total isolates (%)**	**Meat** **Total isolates (%)**
Gram-positive		
Facultative anaerobic		
Actinomyces spp.		1 (5.88)
Actinomyces hordeovulnaris		1 (5.38)
Bacillus spp.	9 (21.4)	4 (23.52)
Corynebacterium spp.	3 (7.14)	2 (11.76)
Enterococcus spp.	5 (11.9)	
Lactobacillus spp.		1 (5.88)
Streptococcus anginosus		1 (5.88)
Anaerobic		
Clostridium septicum	1 (2.38)	1 (5.88)
Clostridium sordelli	2 (4.76)	1 (5.88)
	20 (47.64)	12 (70.56)
Gram-negative		
Aerobic		
Acinetobacter calco var. Anitratus	1 (2.38)	
Moraxella spp.	4 (9.52)	1 (5.88)
Moraxella lacunata	1 (2.38)	
Pseudomonas aeuroginosa	9 (21.4)	
Stenotrophomonas maltophila	1 (2.38)	
Facultative Anaerobic		
Aeromonas salmonicida		1 (5.88)
Pasteurella spp.	1 (2.38)	
Pasteurella multocida	4 (9.52)	2 (11.76)
Vibrio spp.		1 (5.88)
Vibrio parahaemolyticus	1 (2.38)	
	22 (52.36)	5 (29.4)
Total isolates	**42**	**17**

Table 10 Bacteria isolated from those dental pulps of cheetahs in which more than one CCF was sampled.

	Canine teeth sampled			
Animal	**104**	**204**	**304**	**404**
1		*Lactobacillus* spp.	*Pasteurella multocida* *Aeromonas salmonicida*	*Bacillus* spp.
2	*Acinetobacter calco var. Anitratus* *Bacillus* spp. *Pasteurella* spp.	*Bacillus* spp. *Corynebacterium* spp.		
3		*Bacillus* spp.	*Bacilllus* spp. *Vibrio parahaemolyticus* *Pasteurella multocida* *Moraxella* spp. *Clostridium septicum.*	*Bacillus* spp. *Pseudomonas aeruginosa.*
4	*Pseudomonas aeruginosa* *Enterococcus* spp.	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	*Bacillus* spp.
5	*Stenotrophomonas maltophila*	*Pseudomonas aeruginosa* *Moraxella* spp.		
6	*Enterococcus* spp.	*Enterococcus* spp.	*Enterococcus* spp. *Pasteurella multocida* *Clostridium sordelli*	*Pseudomonas aeruginosa*
7	No growth	*Pseudomonas aeruginosa*	No growth	*Clostridium sordelli*
8	*Bacillus* spp. *Corynebacterium* spp.	*Bacillus* spp.		
9		*Corynebacterium* spp.		No growth.

Table 11 Antibiogram and the bacterial profile in cheetahs.

Bacteria Profile																	
Antibiotics	***Actinomyces* spp. (n=1)**	***Actinomyces hordeovulnaris* (n=1)**	***Bacillus* spp. (n=13)**	***Corynebacterium* spp. (n=5)**	***Enterococcus* spp. (n=5)**	***Lactobacillus* spp. (n=1)**	***Streptococcus anginosus* (n=1)**	***Acinetobacter calco var. Anitratus* (n=1)**	***Aeromonas salmonicida* (n=1)**	***Moraxella* spp. (n=5)**	***Moraxella lacunata* (n=1)**	***Pasteurella* spp. (n=1)**	***Pasteurella multocida* (n=6)**	***Pseudomonas aeuroginosa* (n=9)**	***Stenotrophomonas maltophila* (n=1)**	***Vibrio* spp. (n=1)**	***Vibrio parahaemolyticus* (n=1)**
Amikacin	100	100	96.15	100	20	0	100	100	100	50	0	100	41.66	100	100	100	50
Amoxycillin / Ampicillin	50	100	76.92	60	100	100	100	0	100	100	50	100	66.66	0	100	100	0
Doxycycline / Oxitetracycline	100	0	96.1	100	100	100	100	100	100	100	50	100	91.66	0	100	100	100
Enrofloxacin	100	100	100	100	80	100	100	100	100	100	50	100	100	33.33	100	100	100
Gentamicin	100	100	100	100	30	50	100	100	100	80	50	100	58.33	100	100	100	100
Penicillin G	50	100	92.30	60	100	50	100	0	100	70	50	100	58.33	0	100	50	0
Sulpha / Trimethropim	100	100	88.46	80	80	100	100	100	100	100	0	100	100	0	100	50	100
Cephalothin / Lexin	100	100	100	100	90	100	100	0	100	100	50	100	83.33	0	100	100	0
Kanamycin	100	100	100	90	20	0	100	100	100	60	0	100	41.66	0	100	100	50
Lyncomycin	50	100	38.46	20	20	0	100	0	100	0	0	100	8.33	0	0	0	0
Lincospectin	100	100	38.46	40	60	100	100	100	100	60	0	100	25	0	100	100	0
Orbifloxacin	100	100	76.92	90	50	100	100	100	100	100	50	100	100	0	100	100	100
Amoxycillin-Clavulanic Acid	100	100	96.15	100	100	100	100	0	100	100	100	100	66.66	0	100	100	100
Tylosin Tartrate	100	100	96.15	90	90	100	100	0	100	80	50	100	41.66	0	100	0	0
Chloramphenicol	100	100	100	100	80	100	100	0	100	100	100	100	100	0	0	100	100

Table 12 Efficacy of the different antibiotics tested against all the aerobic and facultative anaerobic bacteria isolated from the cheetahs.

Antibiotics	Efficacy
Enrofloxacin	91.96 %
Gentamicin	86.37 %
Orbifloxacin	86.28 %
Amoxycillin-Clavulanic Acid.	86.04 %
Doxycycline / Oxitetracycline	84.57 %
Sulpha / Trimethropim	82.26 %
Chloramphenicol	81.17 %
Cephalothin / Lexin	77.84 %
Amikacin	73.98 %
Amoxycillin / Ampicillin	70.79 %
Kanamycin	68.33 %
Tylosin Tartrate	67.51 %
Lincospectin	66.08 %
Penicilin G	63.56 %
Lincomycin	31.57 %

Table 13 Results of the measurements of the clinical crown length of the canine teeth, and the distance between the occlusal part of both maxillary canine teeth (104-204) and mandibular canine teeth (304-404) performed in twenty cheetah skulls (all measurements in millimetres).

Skull label	104	204	304	404	Distance between 104-204	Distance between 304-404
G00364	22	21	16	16	42	33
248/10	21	21	16	17	33	21
F278	18	19	14	14	35	30
No ID	19	19	15	15	40	28
M264	21	20	16	16	38	30
PM10/146	19	20	14	15	42	32
5	20	20	15	15	36	31
M403	22	22	16	16	34	28
No ID	21	21	17	17	33	27
M184	18	18	15	15	37	30
No ID	22	22	18	17	36	26
M382	19	19	16	16	35	23
No ID	19	20	17	17	43	30
M 2004 wild caught	23	22	16	17	40	32
F183	16	16	12	12	37	29
F303	17	18	14	15	33	28
04/156	19	19	15	15	35	29
No ID	18	17	14	14	35	28
No ID	20	20	15	15	37	26
F 4yrs	19	18	15	15	28	26
Mean	**19.65**	**19.6**	**15.3**	**15.45**	**36.45**	**28.35**
Length Range	**16-23**	**16-22**	**12-18**	**12-17**	**28-43**	**21-33**

Table 14 Representation of the patient data and nucleic acid-base detection results.

Sample	Transfer No	Patient	Tooth	Species	PCR		
					Clone	Genus or species match	Acc. No
1	28237	Paws	104	Cheetah	F3PJET	*Clostridiale bacteruim*	EU289058
					R6B2275F	*Cardiobacteruim* spp.	Y827877
					6C2275F	*Bacteruim enrichment*	HQ122965
					6G227F	*Clostridiales bacteruim*	EU289058
					A2PJETR	*Tissierella praeacuta*	GQ461814
					BOSA2	*Clostridiales bacteruim*	EU289058
					BOSC2	*Uncultured Synergistetes*	AB522155
					BOSG2	*Clostridiales bacteruim*	EU289058
					D2PJETR	*Caloranaerobacter azorensis*	NR028919
					E2PJETR	*Bacteroidetes bacteruim*	CU922596
					F2PHETR	*Uncultured Clostridiales*	EU289058
2	28234	Selkie	404	Cheetah	BOSH3	*Uncultured bacteruim*	HQ400334
					5E3F0618	*Ehrlichia coli*	AP012030
					6C327F	*Uncultured Eubacteruim*	AM419990
					6E327F	*Uncultured bacteruim*	GQ016861
					B3PJETRE	*Uncultured Eubacteruim*	AM419990
					BOSB3PJET	*Pseudoramibacteruim alactolyticus*	BO36759
					BOSC3PJET	*Delfti tsuruhatensis str*	EF440614
					BOSD3PJET	*Uncultured bacteruim*	EU775855
					BOSE3PJET	*Propionibacteruim* sp. *aura*	GQ4226728
					BOSF3PJET	*Leuconostoc mesenteroides str*	FJ65776
3	28234	Selkie	204	Cheetah	C6PJETR	*Syntrophomonas curvata*	MR025752
					3F627FG	*Pseudoramibacteruim alactolyticus*	B036759
					3G627FH	*Uncultured bacteria camel*	HQ008629
					3D6B0205	*Uncultured rumen bacteruim*	HQ400334
					3E6C0208	*Uncultured bacteruin camel*	HQ008603
					4H6F0217	*Uncultured bacteruim camel*	HQ008629
					5E6PJET	*Uncultured rumen bacteria*	HQ400334
					5F6PJET	*Uncultured bacteruim*	HM248358
					5H6PJETR	*Uncultured bacteruim*	HM248358
					A6PJETR	*Streptococcus gallolycticus*	EU163484
					BOSA6PJET	*Uncultured bacteruim*	GQ016861
					BOSB6PJET	*Uncultured bacteruim*	FJ032552
					BOSD6PJET	*Uncultured Bacteruim*	EU458979
4	28235	Charley	104	Cheetah	F7PJET	*Paenibacillus barcinonensis*	DQ870733
					B11PJET	*Uncultured bacteruim*	HQ728208
					B12PJETR	*Bacillus* sp.	AB425363
					BOSF6PJET	*Uncultured Delftia*	GU563748
					BOSH6PJET	*Uncultured bacteruim*	HQ008619
					C7PJET	*Uncultured bacteruim*	HM272655
					D75PJET	*Uncultured rumen bacteria*	GQ327262
					D7PJETR	*Uncultured bacteruim*	FN985404
					E75PJET	*Uncultured bacteruim*	HM366499
					E11PJETR	*Uncultured bacteruim*	EU748123
					F75PJETR	*Uncultured bacteruim*	HM272655

Table 14 continued

Sample	Transfer No	Patient	Tooth	Species	PCR		
					Clone	Genus or species match	Acc. No
5	28235	Charley	204	Cheetah	H9PJET	*Uncultured rumen bacteruim*	HQ400334
					3H427FF	*Uncultured Bacillus* sp.	EF636830
					5C427FD	*Tissierella praeacuta*	GQ461814
					5F227F	*Tissierella praeacuta*	GQ461814
					A5PETRD	*Tissierella praeacuta*	GQ461814
					B4PJETRF0217	*Bacteroides*	EU136689
					B6PJETRB0606	*Clostriduim hastiforme*	X80841
					C4PJETRG0202	*Fusobacterium russi*	M58681
					C5PJETR	*Uncultured bacteruim camel*	HQ008619
					D10PJET	*Uncultured bacteruim*	EU458979
					E4PJETRH	*Bacteroides suis*	AB542771
					F10PJETRF	*Uncultured Peptostreptococcus acaea*	EU289040
6	28236	Tongs	204	Cheetah	E7PJET	*Porphyromonas* sp.	EU012331
					1G115E1	*Lactobacillus curvateos*	AB494734
					711B27	*Uncultured bacteria*	FJ959685
					711B31	*Uncultured Eubacteriaceae*	AM419965
					712A27	*Uncultured bacterium*	FM873231
					712B27	*Delftia tsuruhatensis*	HM003215
					715F27	*Uncultured bacteria*	FJ959686
					712H27	*Fusobacterium russi*	M58681
					BOSA5PJ	*Uncultured bacteruim*	EU844467
					BOSC5PJET	*Uncultured bacteruim*	FJ55776
					BOSE5PJET	*Uncultured bacteruim*	HM336345
					BOSF5PJET	*Uncultured bacteruim*	EU844467
					C118	*Fusobacterium necrophorum*	AB525413
7	28232	Tosca	304	Canine	E118	*Uncultured bacteruim*	Q308572
					5B8	*Uncultured bacteruim*	EU681991
					5F8	*Uncultured bacteria*	FJ959656
					EH8	*Uncultured*	HM272655
					B118	*Fusobacterium necrophorum*	AB525413
					BOSD7	*Uncultured Bacteruim*	HM272655
					BOSE7	*Uncultured Bacteruim*	HM272655
					BOSF7	*Uncultured Bacteruim*	HM272655
					3G8G0220	*Uncultured bacteruim*	HM272655
					C118	*Fusobacterium necrophorum*	AB525413
8	28233	Jabu	204	Canine	F1PJET	*Uncultured bacteruim*	HM341046
					5E1C0609	*Clostridium sp*	FJ384368
					5G1D0612	*Uncultured bacteria*	HM272655
					6B127F	*Clostridium* sp.	FJ159526
					6C127F	*Uncultured bacterium*	HM336453
					BOSA1	*Uncultured bacteruim*	CU915048
					BOSB1	*Uncultured Delftia* sp.	GU563745
					BOSD1	*Uncultured bacteruim*	FJ024720
					BOSF1	*Achromobacter* sp.	HQ619222
					BOSG1	*Filifactor villosus*	F537211
					BOSH1	*Clostridium bifermentans*	AB538434

Table 15 Comparative results of standard culture and 16S rRNA PCR for detection of bacteria in RC with necrotic pulps of canine teeth in those dogs and cheetahs where both methods were applied.

Cheetahs	PCR	Culture
Paws 104	*Clostridiales bacteruim* (Freq. 4) G+ Anaerobic *Cardiobacterium* spp. G- Fac. Anaerobic *Bacteruim enrichment* *Tisierella Praeacuta* G- Anaerobic *Uncultured Synergistetes* G- Anaerobic *Caloranaerobacter azorensis* G- Anaerobic *Bacteroridetes bacteruim* G- Anaerobic *Uncultured Clostridiales* G+ Anaerobic	*Moraxella* spp. G- Aerobic *Moraxella lacunata* G- Aerobic
Selkie 404	*Uncultured Bacteruim* (Freq. 3) *Ehrlichia coli* GI disorders in foal *Uncultured Eubacterium* (Freq. 2) G- Anaerobic *Pseudoramibacterium alactolyticus* G+ Anaerobic *Delftia tsuruhatensis str* G- Aerobic *Propionibacterium* sp. *aura* G+ Anaerobic *Leuconostoc mesenteroides str* G+ Fac. Anaerobic	No growth after 72 h of incubation
Selkie 204	*Syntrophomonas curvata* G+ Anaerobic *Pseudoramibacterium alactolyticus* G+ Anaerobic *Uncultured bacteruim camel* (Freq. 3) *Uncultured rumen bacteria* (Freq. 2) *Uncultured bacteruim* (Freq. 5) *Streptococcus gallolycticus (S. Bovis* type I) G+ Fac. Anaerobic	*Corynebacterium* spp. G+ Fac. Anaerobic
Charley 104	*Paenibacillus barcinonensis* G+ Fac. Anaerobic *Uncultured Bacteruim* (Freq. 7) *Bacillus* spp. G+ Fac. Anaerobic *Uncultured Delftia* G- Aerobic *Uncultured rumen bacteria*	*Bacillus* spp. G+ Fac. Anaerobic *Corynebacterium* spp. G+ Fac. Anaerobic
Charley 204	*Uncultured rumen bacteria* *Uncultured Bacillus* spp. G+ Fac. Anaerobic *Tisierella praeacuta* G- Anaerobic (Freq. 3) *Bacteroides* G- Anaerobic *Clostriduim hastiforme (*Synonym *Tisierella praeacuta)* G- Anaerobic *Fusobacterium russi* G- Anaerobic *Uncultured bacteruim camel* *Uncultured bacteruim* *Bacteroides suis* G- Anaerobic *Uncultured Peptostreptococcus acaea* G+ Anaerobic	*Bacillus* spp. G+ Fac. Anaerobic
Tongs 204	*Porphyromonas* spp. G- Anaerobic *Lactobacillus curvateos* G+ Fac. Anaerobic *Uncultured bacteruim* (Freq. 7) *Uncultured Eubacteriaceae* *Delftia tsuruhatensis* G- Aerobic *Fusobacterium russi* G- Anaerobic *Fusobacterium necrophorum* G- Anaerobic	*Bacillus* spp. G+ Fac. Anaerobic *Pasteurella multocida* G- Fac. Anaerobic
Dogs	**PCR**	**Culture**
Tosca 304	*Uncultured bacteruim* (Freq. 8) *Fusobacterium necrophorum* (Freq. 2) G- Anaerobic	*Actinomyces* spp. G+ Fac. Anaerobic
Jabu 204	*Uncultured bacteruim* (Freq. 5) *Clostridium* spp. (Freq. 2) G+ Anaerobic *Uncultured Delftia* G- Aerobic *Achromobacter* spp. G- Anaerobic *Filifactor villosus synonym Clostridium villosum* G+ Anaerobic *Clostridium bifermentans* G+ Anaerobic	*Enterobacter cloacae* G+ Fac. Anaerobic *Pasteurella multocida* G- Fac. Anaerobic *Aeromonas salmonicida* G- Fac. Anaerobic *Clostridium acetobulyticum* G+ Anaerobic

Chapter 6
Conclusion

Feeding habits and behaviour problems can have negative effects on the dentition as has been shown in this study. Examples of this are the cheetahs eating meat covered in sand and those animals with cage biter syndrome.

This study revealed a diverse microbiota in the dogs and cheetahs examined by conventional culture mechanisms. However it did differ between dogs and cheetahs. Gram negative facultative anaerobic bacteria were predominant in the RC of the dogs, while in the cheetahs, Gram positive facultative anaerobic bacteria showed a higher prevalence. *Corynebacterium* spp. and *Pasteurella multocida* were the bacterial species with a higher prevalence in the dogs; while, *Pseudomonas aeruginosa*, *Bacillus* spp. and *Pasteurella multocida* were more prevalent in the cheetahs.

The susceptibility of the bacteria isolated from both dogs and cheetahs was determined using various antimicrobial agents; Enrofloxacin and Gentamicin were indicated as the antimicrobial agents with the highest efficacy. Systemic antibiotics may be used pre-operatively in dogs or cheetahs with necrotic pulps in order to improve the success rate of the RCT. The duration and success of this treatment should be evaluated in follow-up studies.

Using Nucleic acid-base detection methods, this study has indicated that the microbial flora in any single infected RC is much more diverse than has been shown using conventional culturing techniques alone and can contain potentially uncultivable bacteria. Some of these bacteria may represent potentially new phylotypes, which may be involved in endodontic infections and, ultimately, the disease process of periradicular periodontitis and should therefore be considered in any future studies involved in defining endodontic pathogens.

Further investigations (i.e. on teeth with periapical abcceses; on teeth with draining tracts; on the relation of radiographic findings with bacteria profiles; study outcomes of cases where antibiotics have been used peri- or post-operatively; biomechanical forces that explain the higher incidence of CCF in the maxillary canine teeth), based on the results of this study should be performed. Changes should be made in the sampling techniques and culture media used according to the nucleic acid-base detection results obtained in this study. Furthermore, examination of larger number of teeth will be necessary in order to give more reliable results.

Chapter 7
References

1. Fagan DA, Oosterhuis JE. A review of the expanding field of exotic animal oral health care - Veterinary Dentisty. *Journal of Veterinary Dentistry*. 1998; 15(3):117-28.

2. Yoshida M, Fukushima H, Yamamoto K, Ogawa K, Toda T, Sagawa H, Correlation between clinical symptoms and microorganisms isolated from root canals of teeth with periapical pathosis. *J Endod*. 1987 Jan; 13(1):24-8.

3. Gomes BPFA. An investigation into the root canal microflora (Dissertation). Manchester: University Dental Hospital of Manchester; 1995.

4. Jacinto RC, Gomes BP, Ferraz CC, Zaia AA, Filho FJ. Microbiological analysis of infected root canals from symptomatic and asymptomatic teeth with periapical periodontitis and the antimicrobial susceptibility of some isolated anaerobic bacteria. *Oral Microbiol Immunol*. 2003 Oct; 18(5):285-92.

5. Lana MA, Ribeiro-Sobrinho AP, Stehling R, Garcia GD, Silva BK, Hamdan JS, *et al.* Microorganisms isolated from root canals presenting necrotic pulp and their drug susceptibility in vitro. *Oral Microbiol Immunol*. 2001 Apr; 16(2):100-5.

6. Siqueira JFJr, Rocas IN. Clinical implications and microbiology of bacterial persistence after treatment procedures. *J Endod*. 2008 Nov; 34(11):1291-301.

7. Sundqvist G, Figdor D, Persson S, Sjogren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 1998 Jan; 85(1):86-93.

8. Ferreira FB, Campos Rabang HR, Pinheiro ET, Gade-Neto CR, Zaia AA, Ferraz CC, *et al.* Root canal microbiota of dogs' teeth with periapical lesions induced by two different methods. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2006 Oct; 102(4):564-70.

9. Molven O, Halse A. Success rates for gutta-percha and kloroperka N-Ø root fillings made by undergraduate students: radiographic findings after 10-17 years. *Int Endod J*. 1988; 21:243-50.

10. Sjögren U, Hägglund B, Sundquist G, Wing K. Factors affection the long-term results of endodontic treament. *J Endod*. 1990; 16:498-504.

11. Kuntsi-Vaattovaara H, Verstraete FJ, Kass PH. Results of root canal treatment in dogs: 127 cases (1995-2000). *J Am Vet Med Assoc*. 2002 Mar 15; 220(6):775-80.

12. Nair PNR, Sjögren U, Schumacher E, Sundquist G. Radicular cyst affectiong a root-filled human tooth: a long-term post-treament follow-up. *Int Endod J*. 1993; 26:225-33.

13. Nair PNR, Sjögren U, Krey G, Sundquist G. Therapy-resistant foreign body giant cell granuloma at the periapex of a root-filled human tooth. *J Endod*. 1990; 16:589-95.

14. Luskin IR, Kressin DJ. Endodontic decisions based on radiographic appearance. *Clin Tech Small Anim Pract.* 2001 Aug;16(3):173-81.

15. Lobprise HB, Bloom BC. Endodontic decisions based on clinical appearance. *Clin Tech Small Anim Pract.* 2001 Aug; 16(3):133-8.

16. Nair PN, Sjogren U, Krey G, Kahnberg KE, Sundqvist G. Intraradicular bacteria and fungi in root-filled, asymptomatic human teeth with therapy-resistant periapical lesions: a long-term light and electron microscopic follow-up study. *J Endod.* 1990 Dec; 16(12):580-8.

17. Steenkamp G. "Cheetahs in captivity suffer from exposed pulp due to the attrition of the teeth". In: Day F, editor. Pretoria, South Africa. 2009.

18. Girard N, Southerden P, Hennet P. Root canal treatment in dogs and cats. *J Vet Dent.* 2006 Sep; 23(3):148-60.

19. Crossley DA, Penman S. Endodontics in small carnivores. In: Association BSAV, editor. Manual of small animal dentistry. 2nd ed. Gloucestershire, UK: British Small Animal Veterinary Association; 1995. p. 168-81.

20. Giuliana G, Ammatuna P, Pizzo G, Capone F, D'Angelo M. Occurrence of invading bacteria in radicular dentin of periodontally diseased teeth: microbiological findings. *J Clin Periodontol.* 1997 Jul; 24(7):478-85.

21. Pinheiro ET, Gomes BP, Ferraz CC, Sousa EL, Teixeira FB, Souza-Filho FJ. Microorganisms from canals of root-filled teeth with periapical lesions. *Int Endod J.* 2003 Jan; 36(1):1-11.

22. Nair PNR. Light and electron microscopic studies of root canal flora and periapical lesions. *J Endod.* 1987; (13):29-39.

23. Suchitra U, Kundabala M, Shenoy MM. In search of endodontic pathogens. Kathmandu Univ Med J (KUMJ). 2006 Oct-Dec; 4(4):525-9.

24. Peters LB, Wesselink PR, Buijs JF, Van Winkelhoff AJ. Viable bacteria in root dentinal tubules of teeth with apical periodontitis. *J Endod.* 2001 Feb; 27(2):76-81.

25. McCormick JE, Weine FS, Maggio JD. Tissue pH of developing periapical lesions in dogs. *J Endod.* 1983 Feb; 9(2):47-51.

26. Lomcali G, Sen BH, Cankaya H. Scanning electron microscopic observations of apical root surfaces of teeth with apical periodontitis. *Endod Dent Traumatol.* 1996 Apr; 12(2):70-6.

27. Melville TH, Birch RH. Root canal and periapical floras of infected teeth. *Oral Surg Oral Med Oral Pathol.* 1967 Jan; 23(1):93-8.

28. Fouad AF, Rivera EM, Walton RE. Penicillin as a supplement in resolving the localized acute apical abscess. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 1996 May; 81(5):590-5.

29. Nair PN. Pathogenesis of apical periodontitis and the causes of endodontic failures. *Crit Rev Oral Biol Med.* 2004; 15(6):348-81.

30. Tronstad L, Barnett F, Riso K, Slots J. Extraradicular endodontic infections. *Endod Dent Traumatol.* 1987 Apr; 3(2):86-90.

31. Wang CY, Stashenko P. Characterization of bone-resorbing activity in human periapical lesions. *J Endod.* 1993 Mar; 19(3):107-11.

32. Sundqvist GK, Eckerbom MI, Larsson AP, Sjogren UT. Capacity of anaerobic bacteria from necrotic dental pulps to induce purulent infections. *Infect Immun.* 1979 Aug; 25(2):685-93.

33. De Sousa EL, Ferraz CC, Gomes BP, Pinheiro ET, Teixeira FB, De Souza-Filho FJ. Bacteriological study of root canals associated with periapical abscesses. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2003 Sep; 96(3):332-9.

34. Assed S, Ito IY, Leonardo MR, Silva LA, Lopatin DE. Anaerobic microorganisms in root canals of human teeth with chronic apical periodontitis detected by indirect immunofluorescence. *Endod Dent Traumatol.* 1996 Apr; 12(2):66-9.

35. Rolph HJ, Lennon A, Riggio MP, Saunders WP, MacKenzie D, Coldero L, *et al.* Molecular identification of microorganisms from endodontic infections. *J Clin Microbiol.* 2001 Sep; 39(9):3282-9.

36. Siqueira JFJr, Rocas IN, Cunha CD, Rosado AS. Novel bacterial phylotypes in endodontic infections. *J Dent Res.* 2005 Jun; 84(6):565-9.

37. Siqueira JFJr. Aetiology of root canal treatment failure: why well-treated teeth can fail. *Int Endod J.* 2001 Jan; 34(1):1-10.

38. Figdor D, Sundqvist G. A big role for the very small--understanding the endodontic microbial flora. *Aust Dent J.* 2007 Mar; 52(1 Suppl):S38-51.

39. Rietschel ET BH. Bacterial endotoxins. *Sci Am.* 1992; (267):54-61.

40. Costerton JW, Cheng KJ, Geesey GG, Ladd TI, Nickel JC, Dasgupta M, *et al.* Bacterial biofilms in nature and disease. *Annu Rev Microbiol.* 1987; 41:435-64.

41. Sundqvist G. Associations between microbial species in dental root canal infections. *Oral Microbiol Immunol.* 1992 Oct; 7(5):257-62.

42. Baumgartner JC, Watkins BJ, Bae KS, Xia T. Association of black-pigmented bacteria with endodontic infections. *J Endod.* 1999 Jun; 25(6):413-5.

43. Marsh PD. Are dental diseases examples of ecological catastrophes? *Microbiology.* 2003; (149):279-94.

44. Costerton W, Veeh R, Shirtliff M, Pasmore M, Post C, Ehrlich G. The application of biofilm science to the study and control of chronic bacterial infections. *J Clin Invest.* 2003 Nov; 112(10):1466-77.

45. Distel JW, Hatton JF, Gillespie MJ. Biofilm formation in medicated root canals. *J Endod.* 2002 Oct; 28(10):689-93.

46. Svensäter G, Bergenholtz G. Biofilms in endodontics infections. *Endod Top.* 2004; (9):27-36.

47. Paster BJ, Olsen I, Aas JA, Dewhirst FE. The breadth of bacterial diversity in the human periodontal pocket and other oral sites. *Periodontol 2000.* 2006; 42:80-7.

48. Gutmann JL. Clinical, radiographic, and histologic perspectives on success and failure in endodontics. *Dent Clin North Am.* 1992 Apr; 36(2):379-92.

49. Lin LM, Pascon EA, Skribner J, Gangler P, Langeland K. Clinical, radiographic, and histologic study of endodontic treatment failures. *Oral Surg Oral Med Oral Pathol.* 1991 May; 71(5):603-11.

50. Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the normal bacterial flora of the oral cavity. *J Clin Microbiol.* 2005 Nov; 43(11):5721-32.

51. Roças IN, Siqueira JFJr, Aboim MC, Rosado AS. Denaturing gradient gel electrophoresis analysis of bacterial communities associated with failed endodontic treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2004 Dec; 98(6):741-9.

52. Sundqvist G. Ecology of the root canal flora. *J Endod.* 1992 Sep; 18(9):427-30.

53. Siqueira JF, Jr. Endodontic infections: concepts, paradigms, and perspectives. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2002 Sep; 94(3):281-93.

54. Lin LM, Skribner JE, Gaengler P. Factors associated with endodontic treatment failures. *J Endod.* 1992 Dec; 18(12):625-7.

55. Sathorn CP, P. Messer HH. How usefull is root canal culturing in predicting the treatment outcome? *JOE.* 2007; 33(3):220-5.

56. Sjogren U, Figdor D, Persson S, Sundqvist G. Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. *Int Endod J.* 1997 Sep; 30(5):297-306.

57. Fabricius L, Dahlen G, Sundqvist G, Happonen RP, Moller AJ. Influence of residual bacteria on periapical tissue healing after chemomechanical treatment and root filling of experimentally infected monkey teeth. *Eur J Oral Sci.* 2006 Aug; 114(4):278-85.

58. Kantz WE, Henry CA. Isolation and classification of anaerobic bacteria from intact pulp chambers of non-vital teeth in man. *Arch Oral Biol.* 1974 Jan; 19(1):91-6.

59. Sundquist G. Life as an endodontic pathogen. Ecological differences between thee untreated and the root-filled root canals. *Endodon Topics.* 2003; 6:3-28.

60. Ito HO, Shuto T, Takada H, Koga T, Aida Y, Hirata M. Lipopolysaccharides from Porphyromonas gingivalis, Prevotella intermedia and Actinobacillus actinomycetemcomitans promote osteoclastic differentiation in vitro. *Arch Oral Biol.* 1996 May; 41(5):439-44.

61. Tronstad L, Kreshtool D, Barnett F. Microbiological monitoring and results of treatment of extraradicular endodontic infection. *Endod Dent Traumatol.* 1990 Jun; 6(3):129-36.

62. Wittgow WCJr, Sabiston CBJr. Microorganisms from pulpal chambers of intact teeth with necrotic pulps. *J Endod.* 1975 May; 1(5):168-71.

63. Roças IN, Hulsmann M, Siqueira JF, Jr. Microorganisms in root canal-treated teeth from a German population. *J Endod.* 2008 Aug; 34(8):926-31.

64. Keudell K, Conte M, Fujimoto L, Ernest M, Berry HG. Microorganisms isolated from pulp chambers. *J Endod.* 1976 May; 2(5):146-8.

65. Grenier D, Mayrand D. Nutritional relationships between oral bacteria. *Infect Immun.* 1986 Sep; 53(3):616-20.

66. Sen BH, Piskin B, Demirci T. Observation of bacteria and fungi in infected root canals and dentinal tubules by SEM. *Endod Dent Traumatol.* 1995 Feb; 11(1):6-9.

67. Grossman LI. Origin of microorganisms in traumatized, pulpless, sound teeth. *J Dent Res.* 1967 May-Jun; 46(3):551-3.

68. Roças IN, Jung IY, Lee CY, Siqueira JF, Jr. Polymerase chain reaction identification of microorganisms in previously root-filled teeth in a South Korean population. *J Endod.* 2004 Jul; 30(7):504-8.

69. Siqueira JFJr, Roças IN. Polymerase chain reaction-based analysis of microorganisms associated with failed endodontic treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2004 Jan; 97(1):85-94.

70. Ando N, Hoshino E. Predominant obligate anaerobes invading the deep layers of root canal dentin. *Int Endod J.* 1990 Jan; 23(1):20-7.

71. Sundqvist G, Johansson E, Sjogren U. Prevalence of black-pigmented bacteroides species in root canal infections. *J Endod.* 1989 Jan; 15(1):13-9.

72. Olsen I, Solberg CO, Finegold SM. A primer on anaerobic bacteria and anaerobic infections for the uninitiated. *Infection.* 1999 May-Jun; 27(3):159-65.

73. Zavistosky J, Dzink J, Orderdonk A, Barlett J. Quantitative bacteriology of endodontic infections. *Oral Surg.* 1980; (49):171-4.

74. Saito D, Coutinho LL, Borges Saito CP, Tsai SM, Hofling JF, Goncalves RB. Real-time polymerase chain reaction quantification of Porphyromonas gingivalis and Tannerella forsythia in primary endodontic infections. *J Endod.* 2009 Nov; 35(11):1518-24.

75. Sundqvist G. Taxonomy, ecology, and pathogenicity of the root canal flora. *Oral Surg Oral Med Oral Pathol.* 1994 Oct; 78(4):522-30.

76. Gomes BP, Lilley JD, Drucker DB. Variations in the susceptibilities of components of the endodontic microflora to biomechanical procedures. *Int Endod J.* 1996 Jul; 29(4):235-41.

77. Estrela C, Holland R, Bernabe PF, De Souza V, Estrela CR. Antimicrobial potential of medicaments used in healing process in dogs' teeth with apical periodontitis. *Braz Dent J.* 2004; 15(3):181-5.

78. Westphal O. Bacterial endotoxins. The second Carl Prausnitz Memorial lecture. *Int Arch Allergy and Appl Immunol.* 1975; (49):1-43.

79. Marsh P. Are dental diseases examples of ecological catastrophes? *Microbiology.* 2003; (149):279-94.

80. Siqueira JFJr, Araujo MC, Garcia PF, Fraga RC, Dantas CJ. Histological evaluation of the effectiveness of five instrumentation techniques for cleaning the apical third of root canals. *J Endod.* 1997 Aug; 23(8):499-502.

81. Walton RE. Histologic evaluation of different methods of enlarging the pulp canal space. *J Endod.* 1976 Oct; 2(10):304-11.

82. Wu MK, Van der Sluis LW, Wesselink PR. The capability of two hand instrumentation techniques to remove the inner layer of dentine in oval canals. *Int Endod J.* 2003 Mar; 36(3):218-24.

83. Akpata ES, Blechaman H. Bacterial invasion of pulpal dentin wall in vitro. *J Dent Res.* 1982; (61):435-8.

84. Shovelton DS. The presence and distribution of microorganisms with in non vital teeth. *Braz Dent J.* 1964; (117):101-7.

85. Orstavik D, Haapasalo M. Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules. *Endod Dent Traumatol.* 1990 Aug; 6(4):142-9.

86. Haapasalo M, Orstavik D. In vitro infection and disinfection of dentinal tubules. *J Dent Res.* 1987 Aug; 66(8):1375-9.

87. Perez F, Rochd T, Lodter JP, Calas P, Michel G. In vitro study of the penetration of three bacterial strains into root dentine. *Oral Surg Oral Med Oral Pathol.* 1993 Jul; 76(1):97-103.

88. Loesche WJ, Gusberti F, Mettraux G, Higgins T, Syed S. Relationship between oxygen tension and subgingival bacterial flora in untreated human periodontal pockets. *Infect Immun.* 1983 Nov; 42(2):659-67.

89. Andreasen J. Relationship between surface and inflamatory resorption and changes in the pulp after replantation of permanent incisors in monkeys. *J Endodon.* 1981; (7):294-301.

90. Harvey CE. Periodontal disease in dogs. Etiopathogenesis, prevalence, and significance. *Vet Clin North Am Small Anim Pract.* 1998 Sep; 28(5):1111-28, vi.

91. Le Brench C HL, Le Nihouannen JC, Daculsi G. Epidemiological study of canine teeth fractures in military dogs. *Journal of Veterinary Dentistry.* 1997; 14(2 June):51-5.

92. Stewart RE, Hale KJ. The paradigm shift in the etiology, prevention, and management of dental caries: its effect on the practice of clinical dentistry. *J Calif Dent Assoc.* 2003 Mar; 31(3):247-51.

93. Gomes BP, Ferraz CC, Vianna ME, Berber VB, Teixeira FB, Souza-Filho FJ. In vitro antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of Enterococcus faecalis. *Int Endod J.* 2001 Sep; 34(6):424-8.

94. Sundqvist G, Figdor D. Life as an endodontic pathogen. *Endodon Topics.* 2003; 6:3-28.

95. Griffee MB, Patterson SS, Miller CH, Kafrawy AH, Newton CW. The relationship of Bacteroides melaninogenicus to symptoms associated with pulpal necrosis. *Oral Surg Oral Med Oral Pathol.* 1980 Nov; 50(5):457-61.

96. Siqueira JFJr, Roças IN, Souto R, Uzeda M, Colombo AP. Microbiological evaluation of acute periradicular abscesses by DNA-DNA hybridization. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2001 Oct; 92(4):451-7.

97. Williams BL, McCann GF, Schoenknecht FD. Bacteriology of dental abscesses of endodontic origin. *J Clin Microbiol.* 1983 Oct; 18(4):770-4.

98. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germfree and conventional laboratory rats. *J South Calif Dent Assoc.* 1966 Sep; 34(9):449-51.

99. Yamasaki M, Kumazawa M, Kohsaka T, Nakamura H, Kameyama Y. Pulpal and periapical tissue reactions after experimental pulpal exposure in rats. *J Endod.* 1994 Jan; 20(1):13-7.

100. Stern MH, Dreizen S, Mackler BF, Levy BM. Antibody-producing cells in human periapical granulomas and cysts. *J Endod.* 1981 Oct; 7(10):447-52.

101. Aqrabawi J, Schilder H, Toselli P, Franzblau C. Biochemical and histochemical analysis of the enzyme arylsulfatase in human lesions of endodontic origin. *J Endod.* 1993 Jul; 19(7):335-8.

102. Silva LA, Leonardo MR, Assed S, Tanomaru Filho M. Histological study of the effect of some irrigating solutions on bacterial endotoxin in dogs. *Braz Dent J.* 2004; 15(2):109-14.

103. Soares JA, Leonardo MR, Da Silva LA, Tanomaru Filho M, Ito IY. Effect of rotary instrumentation and of the association of calcium hydroxide and chlorhexidine on the antisepsis of the root canal system in dogs. *Braz Oral Res.* 2006 Apr-Jun; 20(2):120-6.

104. Carlsson J, Frolander F, Sundquist G. Oxygen tolerance of anaerobic bacteria isolated from necrotic dental pulps. *Acta Odontol Scand.* 1977; 35(3):139-45.

105. Dunavant TR, Regan JD, Glickman GN, Solomon ES, Honeyman AL. Comparative evaluation of endodontic irrigants against Enterococcus Faecalis Biofilms. *JOE.* 2006 June; 32(6).

106. Estrela C, Sydeney GB. Antibacterial efficacy of intracanal medicaments on bacterial biofilm: A critical review. *J Appll Oral Sci.* 2009; 17(1):1-7.

107. Chavez de Paz LE, Bergenholtz G, Dahlen G, Svensater G. Response to alkaline stress by root canal bacteria in biofilms. *Int Endod J.* 2007 May; 40(5):344-55.

108. Guardabassi L, Schwarz S, Lloyd DH. Pet animals as reservoirs of antimicrobial-resistant bacteria. *J Antimicrob Chemother.* 2004 Aug; 54(2):321-32.

109. Relman DA. Emerging infections and newly-recognised pathogens. *Neth J Med.* 1997; 50:216-20.

110. Roças IN, Hülsman M, Siqueira JFJr. Microorganisms in root canal-treated teeth from a german population. *JOE.* 2008; 34(8):926-31.

111. Pace NR. A molecular view of microbial diversity and the biosphere. *Science.* 1997; 276:734-40.

112. Siqueira JFJr, Rôças IN. Exploiting molecular methods to explore endodontic infections: Part 1-Current molecular technologies for microbiological diagnosis. *JOE.* 2005; 31(6):411-23.

113. Paster BJ, Boches SK, Galvin JL, *et al.* Bacterial diversity in human subgingival plaque. *J Bacteriol.* 2001; 183:3770-83.

114. Paster BJ, Falkler WAJ, Enwonwu CO, *et al.* Prevalent bacterial species and novel phylotypes in advance noma lesions. *J Clin Microbiol.* 2002; 40:2187-91.

115. Lepp PW, Brinig MM, Ouverney CC, Palm K, Armitage GC, Reldman DA. Methanogenic Archaea and human periodontal disease. *Proc Natl Acad Sci USA.* 2004; 101:6176-81.

116. Kumar PS, Griffen AL, Barton JA, Paster BJ, Moeschberger ML, Leys EJ. New bacterial species associated with chronic periodontitis. *J Dent Res.* 2003; 82:338-40.

117. Sakamoto M, Huang Y, Umeda M, Ishikawa I, Benno Y. Detection of novel oral phylotypes associated with periodontitis. *FEMS Microbial Lett.* 2002; 217:65-9.

118. Griffen AL, Kumar PS, Leys EJ, editors. A quantitative, molecular view of oral biofilms communities in health and disease suggest s a role for uncultivated species. Polymicrobial diseases, Am Society for Microbiology conferences; 2003; Lake Tahoe, Nevada.

119. Felsenstein J. PHYLIP (Phylogeny Inference Package). 3.5c. ed. Seatle: Department of Genetics, University of Wahsington; 1993.

120. Jung IY, Choi BK, Kum KY, Roh BD, Lee SJ, Lee CY, *et al.* Molecular epidemiology and association of putative pathogens in root canal infection. *J Endod.* 2000 Oct; 26(10):599-604.

121. Siqueira JF, Rôças IN, Souto R, Uzeda M, Colombo AP. Checkerboard DNA-DNA hybridation analysis of endodontic infections. *Oral Surg Oral Med Oral Pathol.* 2000; 89:744-48.

122. Giovannoni SJ, Britschgi TB, Moyer CL, Field KG. Genetic diversity in Sargasso Sea Bacterioplankton. *Nature.* 1990; 345:60-3.

123. Hugenholtz P, Goebel BM, Pace NR. Impact of culture independent studies on the emerging phylogenetic view of bacterial diversity. *J Bacteriol.* 1998; 180:4765-74.

124. Ward DM, Weller R, Bateson MM. 16S rRNA sequences reveal numerous uncultured microorganisms in a natural community. *Nature.* 1990; 345:63-5.

125. Relman DA, Loutit JS, Schmidt TM, Falkow S, Tompkins LS. The agent of bacilliary angiomatosis-an approach to the identification of uncultured pathogens. *N Engl J Med.* 1990; 323:1573-80.

126. Dymock D, Weightman AJ, Scully C, Wade WG. Molecular analysis of microflora associated with dentoalveolar abscesses. *J Clin Microbiol.* 1996; 34:537-42.

127. Kroes I, Lepp PW, Relman DA. Bacterial diversity within the human subgingival crevice. *Proc Natl Acad Sci USA.* 1999; 96:14547-52.

128. Sakamoto M, Umeda M, Ishikawa I, Benno Y. Comparisson of the oral bacterial flora in saliva from a healthy subject and two periodontitis patientsby sequence analysis of 16S rDNA libraries. *Microbiol Immunol.* 2000; 44:643-52.

129. Choi BK, Paster BJ, Dewhirst FE, Göbel UB. Diversity of cultivable and uncultivable oral spirochetes from a patient with destructive severe periodontitis. *Infect Immun.* 1994; 62:1889-95.

130. Spratt DA, Weightman A, Wade WG. Diversity of oral asaccharolytic *Eubacterium* species in periodontitis - identification of novel phylotypes representing uncultivated taxa. *Oral Microbiol Immunol.* 1999; 14:56-9.

131. Sakamoto M, Rôças IN, Siqueira JFJr, Benno Y. Molecular analysis of bacteria in asymptomatic and symptomatic endodontic infections. *Oral Microbiol Immunol.* 2006; 21:112-22.

132. Wingfield JC, Lynn S, Soma KK. Avoiding the 'costs' of testosterone: ecological bases of hormone-behavior interactions. *Brain Behav Evol.* 2001 May; 57(5):239-51.

133. Baum M. Neuroendocrinology and sexual behavior in the male. In: Becker J, Breedlove S, Crews D, McCarthy M, editors. Behavioral Endocrinology. Cambridge: MTI Press; 2002. p. 153-204.

134. Fusani L, Day LB, Canoine V, Reinemann D, Hernandez E, Schlinger BA. Androgen and the elaborate courtship behavior of a tropical lekking bird. *Horm Behav.* 2007 Jan; 51(1):62-8.

135. Wickings EJ, Dixson AF. Testicular function, secondary sexual development, and social status in male mandrills (Mandrillus sphinx). *Physiol Behav.* 1992 Nov; 52(5):909-16.

136. Allaker RP, Lloyd DH, Bailey RM. Population sizes and frequency of staphylococci at mucocutaneous sites on healthy dogs. *Vet Rec.* 1992 Apr 4; 130(14):303-4.

137. Harvey CE, Thornsberry C, Miller BR. Subgingival bacteria--comparison of culture results in dogs and cats with gingivitis. *J Vet Dent.* 1995 Dec; 12(4):147-50.

138. Haapasalo M, Ranta H, Ranta K, Shah H. Black-pigmented Bacteroides spp. in human apical periodontitis. *Infect Immun.* 1986 Jul; 53(1):149-53.

139. Gomes BP, Drucker DB, Lilley JD. Endodontic microflora of different teeth in the shame mouth. *Anaerobe.* 1999; (5):241-5.

140. Brook I. Human and animal bite infections. *J Fam Pract.* 1989 Jun; 28(6):713-8.

141. Goldstein EJ. Bite wounds and infection. *Clin Infect Dis.* 1992 Mar; 14(3):633-8.

142. Samaranayake LP. Normal oral flora, the oral ecosystem and dental plaque. In: Samaranayake LP, editor. Essential microbiology for dentistry. London: Harcourt Publishers Limited; 2002. p. 207-13.

143. Kasempimolporn S, Benjavongkulchai M, Saengseesom W, Sitprija V. Oral bacterial flora of dogs with and without rabies: a preliminary study in Thailand. *J Med Assoc Thai.* 2003 Dec; 86(12):1162-6.

144. Goldstein EJ, Citron DM, Finegold SM. Role of anaerobic bacteria in bite-wound infections. *Rev Infect Dis.* 1984 Mar-Apr; 6 Suppl 1:S177-83.

145. Al-Haroni M. Bacterial resistance and the dental professionals' role to halt the problem. *J Dent.* 2008 Feb; 36(2):95-103.

146. Sassone LM, Fidel R, Faveri M, Fidel S, Figueiredo L, Feres M. Microbiological evaluation of primary endodontic infections in teeth with and without sinus tract. *Int Endod J.* 2008 Jun; 41(6):508-15.

147. Kuriyama T, Karasawa T, Nakagawa K, Yamamoto E, Nakamura S. Incidence of beta-lactamase production and antimicrobial susceptibility of anaerobic gram-negative rods isolated from pus specimens of orofacial odontogenic infections. *Oral Microbiol Immunol.* 2001 Feb; 16(1):10-5.

148. Skucaite N, Peciuliene V, Vitkauskiene A, Machiulskiene V. Susceptibility of endodontic pathogens to antibiotics in patients with symptomatic apical periodontitis. *J Endod.* 2010 Oct; 36(10):1611-6.

149. Baumgartner JC, Xia T. Antibiotic susceptibility of bacteria associated with endodontic abscesses. *J Endod.* 2003 Jan; 29(1):44-7.

150. Watson ADJ, Rosin E. Antimicrobial drug use in dogs and cats. In: Prescott JF, Baggot JD, Walker RD, Eds, editors. Antimicrobial therapy in Veterinary medicine. Iowa: Iowa State University Press, USA; 2000. p. 537-75.

151. Cohen FL, Tartasky D. Microbial resistance to drug therapy: a review. *Am J Infect Control.* 1997 Feb; 25(1):51-64.

152. Muder RR, Brennen C, Drenning SD, Stout JE, Wagener MM. Multiply antibiotic-resistant gram-negative bacilli in a long-term-care facility: a case-control study of patient risk factors and prior antibiotic use. *Infect Control Hosp Epidemiol.* 1997 Dec; 18(12):809-13.

153. Peciuliene V, Reynaud AH, Balciuniene I, *et al.* Isolation of yeast and enteric bacteria in root-filled teeth with chronic apical periodontitis. *Int Endod J.* 2001; (34):429-34.

154. Foschi F, Cavrini F, Montebugnoli L, *et al.* Detection of bacteria in endodontic samples by polymerase chain reaction assays and association with defined clinical signs in Italian patients. *Oral Microbiol Immunol.* 2005; (20):289-95.

155. Plumb DC. Gentamicin. In: Plumb DC, editor. Plumb´s Veterinary Drug Handbook Fifth Edition. Ames, Iowa: Blackwell Publishing; 2008.

156. Plumb DC. Enrofloxacin. In: Plumb DC, editor. Plumb´s Veterinary Drug Handbook Fifth Edition. Ames, Iowa: Blackwell Publishing; 2008.

157. Debelian GJ, Olsen I, Tronstad L. Electrophoresis of whole-cell soluble proteins of microorganisms isolated from bacteremias in endodontic therapy. *Eur J Oral Sci.* 1996 Oct-Dec; 104(5-6):540-6.

158. Debelian GJ, Eribe ER, Olsen I, Tronstad L. Ribotyping of bacteria from root canal and blood of patients receiving endodontic therapy. *Anaerobe.* 1997 Aug; 3(4):237-43.

159. Debelian GJ, Olsen I, Tronstad L. Bacteremia in conjunction with endodontic therapy. *Endod Dent Traumatol.* 1995 Jun; 11(3):142-9.

160. Savarrio L, Mackenzie D, Riggio M, Saunders WP, Bagg J. Detection of bacteraemias during non-surgical root canal treatment. *J Dent.* 2005 Apr; 33(4):293-303.

161. Baumgartner JC. Antibiotics in endodontic therapy. In: Newman MG, Van Winkelhoff AJ, editors. Antibiotics and antimicrobial use in dental practice. Carol Stream, IL: Quistence publishing Co, Inc; 2001. p. 143-57.

162. Muson MA, Pitt-Ford T, Chong B, Weightman A, Wade WG. Molecular and cultural analysis of the microflora associated with endodontic infections. *J Dent Res.* 2002; 11(81):761-6.

163. Boissinot M, Bergeron MG. Toward rapid real-time molecular diagnostic to guide smart use of antimicrobials. *Curr Opin Microbiol.* 2002; (5):478-82.

164. Kempsell KE, Cox CJ, Hurle M, Wong A, Wilkie S, Zanders ED, *et al.* Reverse transcriptase-PCR analysis of bacterial rRNA for detection and characterization of bacterial species in arthritis synovial tissue. *Infect Immun.* 2000 Oct; 68(10):6012-26.

165. Rantakokko-Jalava K, Nikkari S, Jalava J, *et al.* Direct amplification of rRNA genes in diagnosis of bacteria infections. *J Clin Microbiol.* 2000; (38):32-9.

166. Siqueira JF, Rôças IN. PCR methodology as a valuable tool for identification of endodontic pathogens. *J Dent.* 2003; (31):333-9.

167. Wang R-F, Cao W-W, Cerniglia CE. PCR detection and quantitation of predominant anaerobic bacteria in human and animal faecal samples. *Appl Environ Microbiol.* 1996; (62):1242-7.

168. Bernardi G. Chromatography of nucleic acids on hydroxyapatite. *Nature.* 1965; (206):778-83.

169. Marchesi JR, Sato T, Weightman AJ, Martin TA, Fry JC, Hiom SJ, *et al.* Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. *Appl Environ Microbiol.* 1998 Feb; 64(2):795-9.