Streptococci in an Aboriginal Australian community: is there a link between dogs and humans?

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Abstract

Dogs are an important part of modern Aboriginal Australian life in their role as hunters, companions and spiritual protectors. People in Aboriginal communities want their dogs to be healthy and therefore dog health programs that treat dogs and assist people to care for their dogs are popular. In the Yarrabah Aboriginal community, dogs are known to carry a number of zoonotic micro-organisms with the potential to cause disease in humans. A combination of the unhealthy appearance of free roaming dogs and lack of veterinary services has resulted in community concerns about the transmission of zoonoses. The community needs accurate information relevant to their local situation in order to develop strategies to manage canine zoonoses. This thesis is a small attempt to provide evidence about one group of bacteria with zoonotic potential, streptococci.

This study was conducted in the Yarrabah Aboriginal community of far north Queensland. In other Aboriginal Australian communities dogs have been previously found to carry streptococci. In Aboriginal populations, streptococcal disease causes significant morbidity and mortality associated with invasive infections, post-streptococcal glomerulonephritis and rheumatic fever, all of which are over-represented in the Aboriginal population. The shared environment of dogs and people in many Aboriginal communities has led to the hypothesis that dogs in these communities are reservoirs for some species of streptococci capable of causing disease in humans.

This thesis had four aims: (1) Isolate streptococci from dogs and characterise the strains; (2) Investigate associations between health and social parameters of dogs and isolation of streptococci; (3) Isolate streptococci from children with skin sores and determine if there was any indication of the strains being shared with dogs; (4) Translate scientific knowledge, including the new information from this study into community action to improve the health of dogs.

The methods used to achieve these aims are as follows:

Aim 1: Dog samples were collected by swabbing the pharynx of dogs and transporting these to the laboratory in transport medium. Samples were collected from 61 dogs consisting of 57 community dogs and 4 wild dogs. Streptococci were cultured and purified on selective and blood agar plates incubated in 5% CO_2 at 35°C. Species of streptococci were identified using morphological and phenotypic techniques and 16S rRNA sequencing. All isolates were investigated for the presence of genes encoding known *S. pyogenes* virulence genes. *S. dysgalactiae* strains (mainly subsp. *equisimilis* (SDSE)) were further characterised using *emm* typing and multilocus sequence typing (MLST) of 7 housekeeping genes and the *S. canis* isolates were also characterised using MLST.

Aim 2: Associations between dog health and social parameters and carriage of streptococci were assessed through further data collection. Dogs sampled in the study were assessed for body condition (using Purina body condition score) and skin condition. Data related to sex, age and the number of dogs belonging to the household were also collected at the time of sampling. A Chi-squared analysis assessing each of these parameters and presence or absence of β-haemolytic streptococci was performed on this data.

Aim 3: Six humans were sampled for the presence of streptococci. Two children were sampled by a medical practitioner who took skin sore and pharyngeal samples of children attending the community controlled health service with skin sores and living with a dog. Two children had skin swabs taken during house visits to swab dogs and two adults self-swabbed their nasal cavities. Swabs were transported to the laboratory in transport medium where they were cultured, identified and characterised using the same techniques as for the dog samples.

Aim 4: Knowledge translation (KT) strategies were developed by documenting conversations with community members using a 'yarning' protocol in which the subject matter of dog issues in the community and strategies for improvement were

deliberately introduced in an open manner. Participants were associated with the study community and included; 4 Aboriginal dog owners, 3 Aboriginal community members who did not own dogs and 3 non-Indigenous people who worked in the community. Conversations were recorded in writing, validated by participants and thematically analysed using NVIVO qualitative data analysis tool. The resulting suggestions and knowledge translation strategies were discussed, planned, implemented and re-assessed by the researcher, environmental health officer (EHO) and animal management worker (AMW) collaboratively using a participatory action research methodology.

Results

Approximately fifty percent of dogs carried at least one species of β-haemolytic streptococci. Twenty three isolates of *S. canis*, 9 isolates of *S. dysgalactiae* (SD) with 6 of these isolates further identified to subsp. *equisimilis* (SDSE), 2 isolates of *S. agalactiae* and 1 isolate of *S. equi* subsp. *zooepidemicus* (SESZ) were collected from a total of 61 dogs sampled. Streptococci recovered from the children sampled were identified as *S. pyogenes* (4 isolates) and SDSE (2 isolates). Therefore, a total of 41 streptococcal isolates were retrieved from dogs and humans in this study. There were no significant associations with dog health or social parameters in dogs and carriage of β-haemolytic streptococci in the dog.

The *S. canis* and SDSE isolates displayed biochemical heterogeneity; three isolates of *S. canis* were found to possess the Lancefield group C antigen and one isolate of SDSE agglutinated both A and G antigens.

Genetic characterisation of the 11 SD/SDSE isolates retrieved from both dogs and children belonged to 7 *emm* types and multilocus sequence types (ST). Isolates collected from a child and dog within the same household had identical *emm* type and ST. The majority of isolates retrieved from dogs belonged to new STs that were phylogenetically diverged from the human STs available in the MLST database. All *S. pyogenes* and SD/SDSE isolates were positive for genes encoding at least one *S.*

pyogenes virulence gene. The characterisation of the 23 isolates of *S. canis* separated them into 4 new STs. No *S. canis*, *S. agalactiae* or SESZ isolates tested positive to any of the *S. pyogenes* virulence genes investigated in this study.

The results of the community 'yarns' revealed that community members were concerned about; their (and their families) safety due to dog aggression and diseases, the health and welfare of dogs, the capacity of dog owners to care for dogs, and the high numbers of stray or unwanted dogs. Suggested strategies for improvements made by community members included; dog registrations, limiting numbers of dogs per household, dog desexing, improved fencing, regular veterinary services and a mobile dog wash. Community engagement was perceived as extremely important in the successful implementation of any strategies. All community respondents suggested a community day in which dog owners could bring their dogs for anti-parasitic treatments, information about basic dog care and zoonoses and an opportunity to talk to a veterinarian. The EHO and AMW decided that the implementation of a Yarrabah dog care day would be beneficial in delivering further KT strategies to build capacity of dog owners to care for their dogs. KT resources were developed by the AMW and researcher in community context and included posters, microscope sessions and specimens of dog parasites. KT strategies used to feedback results of the streptococcal research included letters to participants, emails to relevant community organisations and a poster displayed at the community controlled health service, council office and at the dog care days implemented during this study.

Discussion

The characterisation of SD/SDSE has revealed that strains isolated from humans and animals are genetically diverged. However, it is also evident that recombination and genetic transfer between strains from the same host, strains isolated from different hosts and other species of streptococci has occurred. Furthermore, this study has found evidence of cross-species transfer in which SDSE strains that are typically human in origin have been isolated from dogs and vice versa. The finding of a

homologous strain of SDSE in both a child and dog from the same household illustrated that cross-species transfer can occur at the household level. Therefore, the shared environment (and host) of some species of streptococci may be facilitating genetic and biochemical variability among species of streptococci.

The contribution that SDSE makes to disease is largely unknown within this study community. This study found no association between dog health and social parameters and presence or absence of β-haemolytic streptococci in dogs. However, these methods did not allow for any comment on bacterial load which may be expected to be higher in dogs with poor body condition. Therefore it is unknown whether dog health improvements will reduce carriage of SDSE or other streptococci. However, dog health improvements may reduce the carriage of other canine zoonoses that represent risks to human health in this community.

The results of the qualitative component revealed that many community members believed that building the capacity of dog owners through knowledge translation, access to veterinary services or basic veterinary care and improved infrastructure would facilitate empowering dog owners to care for their own animals, ultimately resulting in improvements in dog health. Yarrabah dog care days were successful in providing a place for community dog owners, community animal workers and veterinarians to discuss dog health issues, collect information on basic dog care, improve zoonoses awareness, and apply worming, flea and mange treatments to their dogs. These days also provided a platform to feedback results of the streptococcal component to dog owners.

Further studies investigating possible zoonoses in populations where animals and humans share a close environment should be based on longitudinal studies of both human and animal populations in the same area. The methods should include genetic identification of bacteria to the strain level and methods to quantify bacterial load. Resources focussed on building early collaborations with the medical services and other relevant community members are extremely beneficial. The ability to include study components that are beneficial and relevant to the

community are also important in providing tangible outcomes to the study community in which the results of research can be put into practice.

Declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any University or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis. This thesis is less than 40,000 words in length, exclusive of table, maps, figures, reference list and appendices.

Layla Schrieber (21/05/2012)

Preface

Wild dogs were trapped and sampled by Dr. Felicity Smout of James Cook University, Townsville.

Genotypic methods for the identification of strains and virulence genes were carried out at Menzies School of Health Research in Darwin under the supervision of Dr. Rebecca Towers.

Part of this work has been presented as a poster presentation at the XVIII Lancefield International Symposium (2011), Palermo, Italy:

> Towers, R. J. & Schrieber, L. J. "Development of a 16S-based HRM assay to differentiate *S. dysgalactiae* subsp. *equisimilis* from other large colony forming beta-haemolytic streptococci of clinical and veterinary significance."

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To my late grandmother, Lealon (Lorna) Reece Wenitong who encouraged me to take every opportunity including this Master degree; even after death her life continues to inspire me...and to her best friend (her red cattle dog)... "Nyarla"... this thesis is for you...

Abbreviations

The following abbreviations are used throughout this thesis:

ABS	Australian Bureau of Statistics
AMW	animal management worker
ARF	acute rheumatic fever
atoB	acetoacetyl coathioloase
BHS	beta-haemolytic streptococci
bp	base pair
°C	degrees Celsius
CC	Clonal Complexes
CDC	Centers for Disease Control and Prevention
CDEP	Community Development and Employment Program
CO_2	carbon dioxide
cpa	collagen type I binding protein
C_T	amplification curve threshold cycle
DNA	deoxyribonucleic acid
EHO	Environmental Health Officer
ESRD	end-stage renal disease

gki	glucose kinase
gtr	glutamine transport protein
HDHC	Healthy dogs, healthy communities project
HGT	horizontal gene transfer
HRM	high resolution melt
km	kilometre
KT	knowledge translation
MLST	multilocus sequence typing
mL	millilitre
mM	milliMoles
murI	glutamate racemase
mutS	DNA mismatch repair protein
µg	microgram
µL	microlitre
µM	microMoles
PAR	participatory action research
PCR	polymerase chain reaction
prtF2	fibronectin binding protein F2

PSGN	post-streptococcal glomerulonephritis
qPCR	Real-time PCR
recP	transketolase
RHD	rheumatic heart disease
16S rRNA	16S ribosomal ribonucleic acid gene
sdaB	mitogenic factor
sdn	streptodornase
SDSE	*Streptococcus dysgalactiae* subsp. *equisimilis*
SDSD	*Streptococcus dysgalactiae* subsp. *dysgalactiae*
SD	*Streptococcus dysgalactiae*
SESZ	*Streptococcus equi* subsp. *zooepidemicus*
sfbI	fibronectin binding protein
sfbX	fibronectin binding protein X
SLV	single locus variant
speG	streptococcal pyrogenic exotoxin G
ST	sequence type
xpt	xanthinine phosphoribosyl transferase

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Chapter 1: Introduction

In 2007 Virginia Boon found that dogs in two Aboriginal communities were infected with species of streptococci that could potentially cause disease in humans (Boon 2007). In this thesis the term 'Aboriginal' refers to Aboriginal Australian populations. In Aboriginal populations, streptococci are major causes of mortality and morbidity mainly because of the role they play in the development of acute rheumatic fever (ARF) and post-streptococcal glomerulonephritis (PSGN) (Gogna, Nossar & Walker 1983; Currie & Brewster 2002). The notion that dogs could be reservoirs of streptococci is rational because many Aboriginal communities have large numbers of dogs that appear unhealthy and are living in close proximity to humans. Many dogs in Aboriginal communities (referred to as 'community dogs' in this thesis) although owned by people are allowed to roam freely, are in large numbers and their appearance often reflects those of stray or unwanted dogs. Regardless of their appearance, the bond between people and their dogs is still very strong. Constable et al. (2008) found that many Aboriginal people, regardless of the condition of their dogs, still have a strong bond with their dogs that goes beyond companionship. In some Aboriginal communities dogs are accepted as family which is reflected in the bestowment of skin names and ceremonial burials (Constable et al. 2008; Smith & Litchfield 2009). This strong connection extends even further into spiritual ties evident by the belief that harm or sickness in a dog could result in physical harm or sickness to the community or to individuals within. Further evidence of this strong connection is the inclusion of the dingo in Dreaming stories (Constable et al. 2008; Smith & Litchfield 2009).

The Healthy Dogs, Healthy Communities project began in 2007 and over the three years it was operating engaged with several Aboriginal communities aimed at improving dog health and welfare through education programs (Dixon, Dixon & Constable 2007). The Healthy Dogs, Healthy Communities project was keen to engage with an Indigenous student and to build their capacity for research through a research project comparing streptococci carried by both dogs and humans. I

applied for this project and, in 2009, enrolled as a Master of Science in Veterinary Science student aiming to complete the streptococcal research project by conducting a comparative study in the Yarrabah Aboriginal community.

I am an Aboriginal woman, descendant from the Kabi Kabi tribe of south-east Queensland, Glass House Mountains area. I hold a Bachelor's degree in Science, majoring in Zoology and have, since childhood, held a keen interest in animals. My father was one of the first Aboriginal people in Australia to gain a Medical degree and therefore the passion to improve health outcomes of our people (Aboriginal people) has been inherited. The opportunity of doing this Masters degree allowed me to bring both of these together.

My husband is a Gungganyji man whose tribal area encompasses the Yarrabah region. He and his family are traditional owners of the land in which Yarrabah is located and they have connections in many facets of the community. He and his brothers are one of the few families who are familiar with and continue to practice Gungganyji dance and song. This has meant that he is an extremely proud Gungganyji man who follows the traditions and beliefs of his people and is consequently well respected in the community. My marital ties to the community (and my Aboriginality) have imposed on me a level of responsibility to the people of the Yarrabah community that goes beyond the research project.

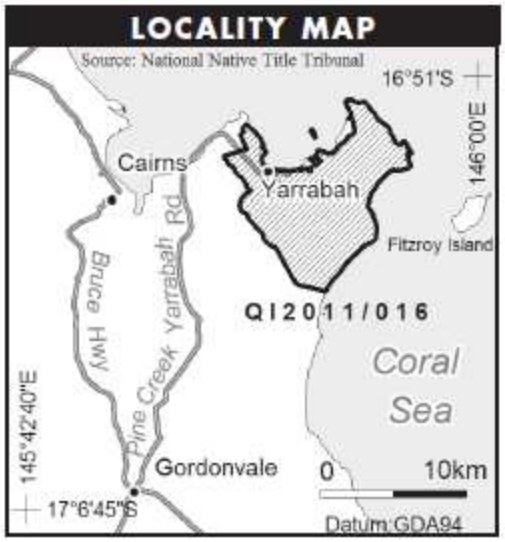

Figure 1.1. Location of Yarrabah (National Native Title Tribunal 2011).

The Yarrabah Aboriginal Shire is shown in Figure 1.1. It is a coastal community situated approximately 40 km from Cairns separated by a mountain range. The Yarrabah community was established in 1892 as an Anglican mission where up to forty tribal groups from surrounding areas were forcibly re-located (Denigan 2008). Some of the children of that time are still alive today and have spoken about their experiences of being hand-cuffed and separated from their mothers. There are also accounts of abuse and starvation in which regardless of a lack of food in the mission, people were prohibited from hunting or picking fruit. Aboriginal people forced to live in Yarrabah were also forbidden to continue cultural practices of language and dance. Ultimately, it was a time where individuals' control over their own lives was revoked (Hume 1991; Denigan 2008). It was not until the late 1960 s that people living in the Yarrabah mission no longer had to seek permission to work, marry or leave the mission. Therefore, many of the people residing in Yarrabah have grown up in a dormitory separated from their parents, and have often been affected by the traumatic experiences they have endured. These past hurts and traumas are intergenerational and have affected the way that some Yarrabah people engage with others from outside their community. This history needs to be acknowledged and carefully considered in order to conduct respectful research in the community.

At present, Yarrabah has a population of approximately 2722 people according to the Australian Bureau of Statistics (ABS) (2010). However, ABS statistics of Aboriginal populations are often significant undercounts that are affected by the methods of data collection (Australian Human Rights Commission 2008). Discussions with locals suggest the population is currently closer to 4000. The Yarrabah population, like many Aboriginal communities, consists of individuals with familial links also to the Torres Strait Islands and the South Sea Islands. However, because the community is commonly recognised as an Aboriginal community, all participants are referred to as 'Aboriginal' in this thesis. Everyone in the community speaks English. However, some speak Kriol (Aboriginal English) in which common English words are pronounced differently and are combined with Gungganyji language.

Throughout the duration of the streptococcal research, the community was undergoing organisational change from a Deed of Grant in Trust (DOGIT) community to mainstream shire council status. Yarrabah has significant infrastructure; a main grocery shop, two corner stores, a primary school, high school, day care centre, community controlled health service, hospital, an employment agency, post office and Centrelink office. During the research project Yarrabah Council improved their dump facilities and implemented regular household garbage removal and recycling. However, the community, like many other Aboriginal communities, still suffers from health disadvantage. In 2006, Yarrabah had a reported 335 houses and up to 11 persons per dwelling (Calma 2006). Inappropriate housing design that does not factor the communal and extended family living conditions of many Aboriginal and Torres Strait Islander people has played a role in overcrowding. Furthermore, the community unemployment rate is high, increasing to 19.9% in 2010 from just 4.1% in 2008 (Australian Bureau of Statistics 2010). The government funded Community Development Employment Plan (CDEP) offered extensive employment for Yarrabah community members and its cessation in June 2009 has added to the current high rates of unemployment.

The Yarrabah community has a large number of free roaming dogs and currently employs an Environmental Health Officer (EHO), a ranger and an Animal Management Worker (AMW). The community has also experienced frequent outbreaks of PSGN and ARF, some of which are documented in the literature (Neilsen et al. 1993; Streeton et al. 1995; Minaur et al. 2004). Anecdotal reports from hospital staff suggested that a previous dog culling program was followed by a sharp decline in childhood impetigo (streptococcal skin sores) within the community. Therefore, the research project was supported by health organisations and many people within the community were keen to see the results.

As an Aboriginal researcher, conducting research in an Aboriginal community often comes with a large amount of death, family and community disruptions and other community priorities in which the research project was often understandably of lesser significance in comparison to other community priorities. Furthermore, like many Indigenous researchers, I had the added burden of shaping a methodology that could be both acceptable to the community and acceptable to supervisors, examiners and scientific peers.

Quantitative scientific research methods that may be acceptable in non-Aboriginal communities are often viewed with caution (and distrust) in Aboriginal communities. This view reflects a history of Aboriginal people whose perception and experience with scientific research has been negative, and this has included many residents of Yarrabah (Mayo, Tsey & Empowerment Research Team 2009). The Yarrabah community, like many Aboriginal communities, has been the subject of many research projects, some conducted without the community's permission or knowledge. Quantitative research methodologies are seen to be one of the most problematic in Indigenous Australian communities, and other Indigenous communities worldwide (Maddocks 1992; Tuhiwai Smith 1999; Humphery 2001; Rigney 2001; Smith 2001; Estey, Kmetic & Reading 2008; Saunders, West & Usher 2010). These methods have also been implicated as contributors to the health disadvantage of Indigenous peoples all over the world, because of their focus on scientific quality rather than health improvements. This is partly because

quantitative research methodologies focus on discovery rather than action for change. Indigenous populations on an international level have voiced their frustration with the time lag between research and action with many Indigenous communities subjected to research often seeing no impact at all (Wandersman 2003; World Health Organisation 2006; Estey, Kmetic & Reading 2008; Bammer, Michaux & Sanson 2010).

Whilst the research project was approved by the Yarrabah community, part of the research agreement required working closely with the EHO, AMW and Ranger. After discussion with the EHO, it became clear that they had strategies to improve dog health and ownership in the community. However, a lack of resources and time to manage the AMW with other environmental health issues in a community of 3000-4000 people was challenging the implementation of these strategies. Therefore, an underlying component of this study was aimed at developing and implementing strategies to improve dog health and ownership in the community. This was an extremely important part of the study because it facilitated the development of action, community engagement and capacity building in the research process. It also facilitated the ability to translate the research back to the community in context with community needs. To ignore the needs of the community by excluding this component would have been extremely unethical.

1.1 Thesis outline

This chapter introduces the study community and the researcher. It also provides a background of the project and the importance of both components of the research. Whilst the bulk of the thesis is concerned with the quantitative aspects of the research through investigating streptococci in dogs and community members, another important aspect is to document the development of knowledge translation strategies to improve dog health.

Chapter two reviews the literature pertaining to the role of dogs in Aboriginal communities and their association with zoonotic disease. The pathogenesis of streptococcal disease in Aboriginal communities is also described with particular reference to dogs as a source of infection. The review discusses the most recent identification and classification scheme for the streptococci and factors associated with increasing virulence and pathogenicity, with a focus on species and strains evident in Aboriginal Australian communities. This chapter concludes by highlighting the aims of the studies that make up this thesis.

Chapter three describes the materials and methods used to collect samples including the recovery and isolation of streptococci and biochemical and molecular identification techniques. This chapter also describes the methodology adopted to collect and implement strategies to improve dog health and ownership in the community.

Chapter four explores the streptococcal species recovered from dogs and children in the Yarrabah community. Results describe the streptococci species recovered and the frequency as related to physiological and social parameters of dogs.

Chapter five explores the molecular genetics of the streptococci recovered from dogs and children in this study. Genetic characterisation using *emm* typing and multilocus sequence typing of isolates recovered from both dogs and humans are investigated with reference to the wider community. Isolates were also investigated for the presence of *S. pyogenes* virulence genes.

Chapter six discusses and documents the strategies developed by the community to improve dog health and ownership. It also describes how these discussions facilitated the development of knowledge translation strategies that were used to feedback research results but also as a tool to build capacity of dog owners to care for their pets.

The final chapter concludes the thesis and discusses the main findings in accordance with the aim of the research. It also offers recommendations for further studies.

Chapter 2: Review of the Literature

2.1 Introduction

As previously described, this study has two components; one aimed to investigate the streptococcal species carried by dogs and the possibility of streptococcal strains being shared in both dog and human populations in the study community. The other component aimed to document and implement strategies for dog health and ownership improvements as requested by the Yarrabah community.

This chapter aims to collate and review the role of dogs in Aboriginal communities. A brief history of dog health programs is also included to inform the reader of the current status of dogs and to emphasize the importance of capacity building in the success of dog health improvement programs within Aboriginal Australian communities.

Furthermore, the relevance of dogs in Aboriginal communities in transmitting zoonotic disease is described focussing on the potential of streptococci as zoonoses, especially those streptococci species producing the Lancefield group G and/or C antigen.

2.2 The role of dogs in Aboriginal Australian communities

The role of dogs in Aboriginal Australian tribes and communities differs between family, tribe and community. In traditional life, dingoes were used as companions, bed warmers, protectors, creatures of spiritual significance and hunting aides (Howe 1993; Smith & Litchfield 2009). Following European settlement, the dingo was replaced by European domestic dogs or dingo hybrids and the role of the dingo in traditional life was extended to the domestic dog in modern community life (Raw 2001).

Recent investigations suggest that domestic dogs were preferred over the dingo because they could be easily trained (Smith & Litchfield 2009). However, domestic dogs differ from dingoes in a number of very important ways that may have influenced the current condition of dogs in Aboriginal communities today. A recent review concluded that dingoes were not domesticated by Aboriginal people and were self surviving hunters that had the ability to forage for their own food (Smith & Litchfield 2009). Furthermore, dingoes did not commonly overpopulate tribal communities because they were encouraged to return to the wild once sexually mature. This contrasts with domestic dogs commonly found in Aboriginal communities in modern times which are dependent on their owners for food, care and attention and also breed twice a year (Raw 2001).

Today dogs in Aboriginal communities are often unhealthy and found in large numbers scavenging for food. Whilst it may appear that they are unwanted or stray dogs, the bond between people and dog is still very strong (Constable et al. 2008). In some Central and Northern Australian Aboriginal communities dogs are accepted as family reflected in the bestowment of skin names and ceremonial burials (Constable et al. 2008; Smith & Litchfield 2009). Prior to the introduction of culturally safe dog health programs, dogs were often shot by police and other outsiders without community consent, causing great distress to their owners (Donohoe, Garrawurri & Trudgen 2000).

In the Yarrabah community people believe that the bond between people and dogs in their community is cultural, even though dogs are not currently included in ceremony (Constable, Dixon & Dixon 2010). It is difficult to establish the spiritual connection of dogs in this area by investigating Dreaming stories and ceremony because the community consists of individuals from 40 tribal groups that were displaced from their tribal lands. Therefore, a lack of dog inclusion in ceremony is probably more reflective of the displacement experienced by many people that were forced to live in Yarrabah rather than a lack of spiritual connection with dogs.

Dogs are an important part of modern Yarrabah life as hunters and as physical and spiritual protectors (Constable, Dixon & Dixon 2010). Among many of my family members there is a belief that dogs have special senses that can detect spirits and therefore howl or bark when spirits are actively moving. This can also be described as howling to inform that someone has recently died or warning of an impending death (Constable et al. 2008). Elderly people and their dogs are also believed to have an extremely close spiritual bond. Therefore, if an owner dies the dog must be left alone for some time before removing it (Constable, Dixon & Dixon 2010). On the other hand, if the dog dies before the elderly person, it is often taken as a warning that the owner too may also die due to the loss of this strong connection. Furthermore, for many in the Yarrabah community, deliberate harm to their dog by an individual, warrants physical revenge or punishment (Constable, Dixon & Dixon 2010). To my knowledge, this is a reflection of the inclusion of a dog into a family network which results in protection of the dog (as with the rest of the family). Therefore, deliberate harm to a dog that belongs to a family network often results in physical revenge on the offender by the dog's human 'family'.

It is clear that dogs are important to the community and resources invested into dog health improvements have benefits for dogs, dog owners and other community members of Yarrabah.

2.3 History of dog health programs

Large numbers of dogs and poor dog health are a cause for concern in many Aboriginal communities and have resulted in the implementation of various dog health programs since the mid-1980s (English 2000). Much of the focus of these programs has been on lowering the dog population through either sterilisation or contraceptive treatments and mass treatment of dogs with anti-parasitic treatments such as avermectin or ivermectin (Currie 1995; English 2000). Literature searches of individuals involved in these programs prior to 1993 returned only one Northern Territory Government report (Palmer & Presson 1990). Around 1993, there were dog programs operating in both the Kimberley region and Cape York

region of Queensland (Speare & Mc Connell 1993; English 2000). However, to date there is only one peer reviewed publication regarding the operation of a dog health project (excluding the HDHC project), which was conducted in east Arnhem Land (Bradbury & Corlette 2006).The Numbulwar (a community in east Arnhem Land) dog health project did not include an educational component and concluded that the project needed to move beyond a reactive approach to include community and educational components in order to achieve a sustainable and functional disease prevention program. One of the most successful dog health programs is still effective in the Kimberley region and was supported and documented in a thesis by Kathryn Wilks (Wilks 1999). The success of this program was attributed to the collaboration between community members and the veterinary researcher. Furthermore, the continuation of the program was a result of capacity building, resulting in self-management of the project by the communities involved.

In 2006, Queensland Health distributed funding to 34 Aboriginal and Torres Strait Islander communities to support the employment and training of AMWs in these communities. The program was funded because of animal neglect, high animal populations and more importantly, dog attacks; one of which resulted in the death of a child (Abreu & D'Adonna 2009). The employment of AMWs in their own communities is obviously important in initiating self-management of dog programs.

In the Northern Territory, the Department of Health does not fund the employment of AMWs and until Animal Management in Rural and Remote Indigenous Communities (AMRRIC) provided funding, access to finances was often expected to be provided by each shire (Skunja 2011). The complacency of the Northern Territory Department of Health to provide AMWs for their Aboriginal communities is believed to be a result of prioritising funding for human health in which funding bodies believe that dogs in Aboriginal communities do not play a significant role in human health. This is mainly because of a lack of scientific evidence relating to the transmission of zoonotic diseases from dogs to humans in Aboriginal Australian communities (Currie 1995). However, since there are no studies that have assessed the impact of canine zoonoses in Aboriginal Australian

communities the arguments lack validity. Furthermore, showing that zoonotic pathogens exist in dogs highlights a potential risk to humans, but does not demonstrate a direct impact on human health. Therefore, the evidence is lacking on both sides of the debate, exacerbated by a lack of peer reviewed publications and comparative studies of zoonotic organisms from both human and animal populations within Aboriginal Australian communities.

2.4 Dogs and zoonoses in Aboriginal Australian communities

Published data of the prevalence of canine zoonoses in Aboriginal Australian communities are rare and the most extensive data refer to the Kimberley region of Western Australia (Meloni et al. 1993; Thompson et al. 1993). The most common zoonotic organisms found in dogs from the Aboriginal Australian communities that have been researched are the scabies mite *Sarcoptes scabiei*, dog hookworm *Ancylostoma caninum*, dog roundworm *Toxocara canis*, dog heartworm *Dirofilaria immitis*, dog tapeworm *Dyplidium caninum*, hydatid tapeworm *Echinococcus granulosus* and the dog whipworm *Trichuris vulpis.* However, since the methods of selection of dogs, collection techniques and source of dogs sampled differ between study and site, making meaningful comparisons of prevalence is difficult (Welch, Dobson & Freeman 1979; Jenkins & Andrew 1993; Meloni et al. 1993; Thompson et al. 1993) (Table 2.1).

Table 2.1. Prevalence of zoonotic organisms found in dogs from Aboriginal Australian communities published in peer reviewed journals.

Organism	Prevalence (%)	Sample size	Locations studied	Methods	Reference
Dog hookworm *Ancylostoma caninum*	51.1%	182	Kimberley region (north-west, Western Australia)	Faecal samples and autopsy. Direct stool microscopy and/or zinc flotation method.	Meloni et al (1993).
	52.6%	188			Thompson et al (1993).
	100%	15	South coast, New South Wales	Autopsy	Jenkins & Andrew (1993).
Dog roundworm *Toxocara canis*	70.5%	322	Mean of Various locations	Faecal samples	Welch et al (1979)
	1.1%	188	Kimberley region (north-west, Western Australia)	Faecal samples and autopsy. Direct stool microscopy and/or zinc flotation method.	Thompson et al (1993).
Dog heartworm *Dirofilaria immitis*	33.1%	444	Mean of Various locations	Wet blood mounts	Welch et al (1979)
Hydatid Tapeworm *Echinococcus granulosus*	6.7%	15	South-east, New South Wales	Autopsy	Jenkins & Andrew (1993).
Dog Tapeworm *Dipylidium caninum*	80%	15	South-east, New South Wales	Autopsy	Jenkins & Andrew (1993).
Dog whipworm *Trichuris vulpis*	80%	15	South-east, New South Wales	Autopsy	Jenkins & Andrew (1993).
	0%	188	Kimberley region (north-west, Western Australia)	Faecal samples and autopsy. Direct stool microscopy and/or zinc flotation method.	Thompson et al (1993).
Giardia duodenalis	17.0%	182	Kimberley region (north-west, Western Australia)	Faecal samples and autopsy. Direct stool microscopy and/or zinc flotation method.	Meloni et al (1993).
	15.9%	188			Thompson et al (1993).
Spirometra erinacei	6.7%	15	South-east, New South Wales	Autopsy	Jenkins & Andrew (1993).
	2.2%	182	Kimberley region (north-west, Western Australia)	Faecal samples and autopsy. Direct stool microscopy and/or zinc flotation method.	Meloni et al (1993).
	3.7%	188			Thompson et al (1993).
Entamoeba coli	1.1%	182	Kimberley region (north-west, Western Australia)	Faecal samples and autopsy. Direct stool microscopy and/or zinc flotation method.	Meloni et al (1993).
	1.1%	188			Thompson et al (1993).
Blastocystis	20%	10	Australian central desert	PCR genotyping	Parkar et al (2007).

Further studies have also identified cases where dogs in Aboriginal Australian communities have been infected with other zoonotic organisms including the dermatophyte, *Microsporum canis*; protozoans namely *Blastocystis hominis*, *Giardia duodenalis* and *Cryptosporidium* spp.; various gastrointestinal bacteria namely *Salmonella* spp., *Campylobacter* spp, *Yersinia* spp., *Escherichia coli;* and skin bacteria such as, *Streptococcus* spp. and *Staphylococcus aureus* (Kaminski & Green 1977; Palmer & Presson 1990; Wilks 1999; Boon 2007; Parkar et al. 2007; Speare 2011).

In dogs, these organisms can be carried with no clinical signs or be the cause of intense pruritus, anaemia, weight loss, enteritis, encystations, septicaemia, toxic shock syndrome and diarrhoea (Collins et al. 1987; Miller et al. 1996; Wilks 1999; Robertson & Thompson 2002; Weese 2011). Infections with these organisms, in humans, can produce clinical manifestations similar to those seen in dogs; ranging from self-limiting skin disease, gastroenteritis, eosinophilic enteritis to uni-ocular blindness, the development of cysts in major organs and bacteraemia (described further in Table 2.2) (Robertson & Thompson 2002; Gaskin et al. 2007).

In the Yarrabah community, a dog health program operating between 1993-1997 found approximately 80% of dogs to be infested with *Sarcoptes scabiei* and 83% with *Dirofilaria immitis* (Speare 2000). Preliminary data collected between May 2008 and August 2009 by the HDHC project in Yarrabah found that dogs in this community were infected with *Salmonella* spp., *Campylobacter* spp., *Giardia* spp., *Cryptosporidium* spp., *Dirofilaria immitis*, *Toxocara canis*, *Ancylostoma caninum* and *Sarcoptes scabiei* (G. K. Brown, Unpublished data).

Table 2.2. Zoonotic agents found in dogs in Aboriginal Australian communities and associated disease in humans.

Organism	**Associated symptoms/disease in humans**
Gastrointestinal pathogens	
Nematodes	
Ancylostoma caninum	Cutaneous larva migrans and eosinophilic enteritis.
Ancylostoma ceylanicum	anaemia
Toxocara canis	Visceral and ocular larva migrans (retinal granuloma).
Dirofilaria immitis	Dirofilariasis; pulmonary nodules.
Trichuris vulpis	Asymptomatic or mild infection; diarrhoea, weight loss, abdominal pain.
Cestodes	
Dipylidium caninum	Asymptomatic or mild infection; diarrhoea and abdominal pain.
Echinococcus granulosus	Hydatid disease; cysts develop in lung, liver or brain.
Spirometra erinacei	Sparganosis; larvae migrate through tissue and encyst in human tissue.
Protozoa	
Giardia duodenalis	Giardiasis; diarrhoea, weight loss, vomiting and abdominal pain.
Cryptosporidium spp.	Cryptosporidiosis; diarrhoea and abdominal pain.
Blastocystis hominis	Asymptomatic or mild infections; diarrhoea, abdominal pain, vomiting.
Sarcocystis spp.	none
Bacteria	
Salmonella spp.	Salmonellosis; diarrhoea, fever, vomiting and abdominal cramps.
Campylobacter spp.	Campylobacteriosis; diarrhoea (can be bloody), cramps and fever.
Yersinia spp	Mild entero-colitis; bloody diarrhoea and fever.
Escherichia coli	Can be commensal but some strains can cause diarrhoea, urinary tract infections, respiratory illness and pneumonia, and other illnesses.
Skin Irritations	
Sarcoptes scabiei var. *canis*	Itchiness potentially leading to skin trauma and secondary bacterial infection.
Microsporum canis	Ringworm
Ctenocephalides felis	Flea bite dermatitis; intermediate host of *D. caninum.*
Bacteria	
Streptococcus spp.	Depends on species; but potential for impetigo and invasive diseases ie. Bacteraemia and toxic shock syndrome (and perhaps PSGN and ARF).
Staphylococcus spp.	Impetigo, cellulitis, toxic shock syndrome.

Comparative data providing clear evidence of transmission of zoonoses between dogs and humans in Aboriginal Australian communities are almost non-existent, thereby neither proving nor disproving the role that dogs play in transmitting disease in these communities (Currie 1995; Gaskin et al. 2007). Many of the canine zoonoses commonly found in Aboriginal communities are not notifiable in Australia. Furthermore, many of these zoonoses have non-specific symptoms in humans and are not easily differentiated from their human parasitic counterparts, further exacerbating this lack of evidence. Since Aboriginal health services are already at full capacity and many zoonotic diseases are under recognised, infections may have been masked by other illness, undiagnosed and misdiagnosed. While many of the commonly prescribed human medications can cure disease associated with pathogens originating from dogs, prevention through avoiding transmission and reducing infectious sources is obviously still important and may also be beneficial in relation to suppressing the development of resistance in zoonotic pathogens to therapeutic agents.

2.5 The potential role of dogs and streptococcal disease in Aboriginal Australian communities

In Aboriginal Australian communities, skin trauma, such as that caused by scratching after an allergic reaction to the scabies mite has been a significant source of streptococcal pyoderma and impetigo (Currie & Carapetis 2000). Streptococcal pyoderma occurs in up to 50% of children and is believed to be responsible for the high rates of PSGN and possibly ARF with some Aboriginal communities of Northern Territory experiencing the highest rates in the world (Currie & Carapetis 2000; McDonald, Currie & Carapetis 2004). While the studies by Walton et al. (1999; 2004) showed that the scabies mite diverge into genetic strains according to host, it provided no evidence for or against the clinical effects of dog strains of scabies mites in humans. There are numerous documented cases of canine mites causing scabies in humans outside of Aboriginal Australian communities (Newton & Gerrie 1966; Smith & Claypoole 1967; Charlesworth & Johnson 1974; Estes, Kummel &

Arlian 1983). Other zoonoses that are directly transmitted from dogs to humans through close contact such as fleas and fungal pathogens can also cause itchiness that can lead to skin trauma, creating an opportunity for streptococcal infection.

Streptococci have also been isolated from the skin, pharynx and eyes of dogs in several Aboriginal Australian communities implicating dogs as possible sources of streptococci in these communities (Palmer & Presson 1990; Boon 2007) (Figure 2.1). Therefore, dogs may play both an indirect and direct role in the high rate of streptococcal disease in Aboriginal communities as described in Figure 2.1.

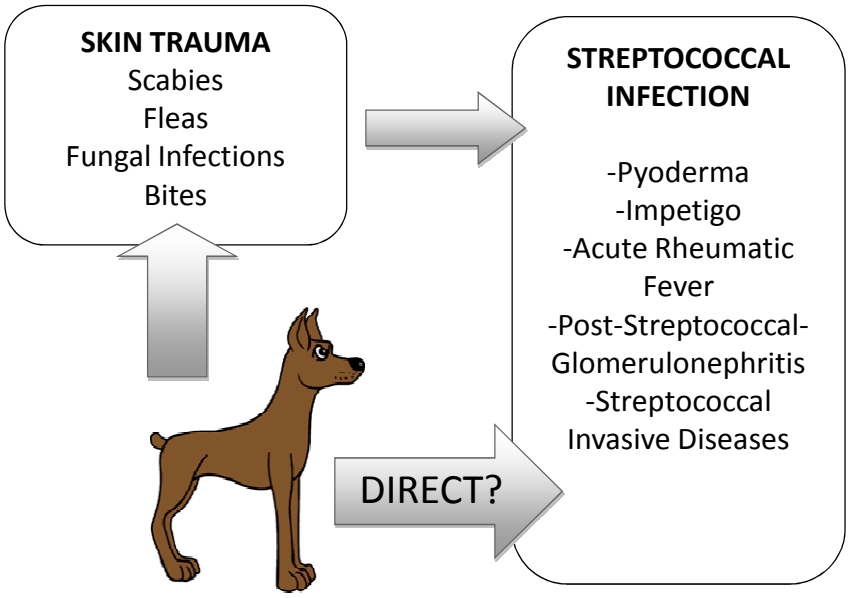

Figure 2.1. Dogs as contributors to streptococcal disease in Aboriginal Australian communities.

Aboriginal Australian populations are severely impacted by streptococcal associated disease with some northern communities experiencing the highest rates of ARF and rheumatic heart disease (RHD) in the world (Carapetis, Wolff & Currie 1996; Carapetis, Currie & Mathews 2000). Other streptococcal associated diseases are also overrepresented in Aboriginal populations including PSGN, streptococcal pyoderma and impetigo, and serious infections presenting as bacteraemia, necrotising fasciitis, myositis, scarlet fever and streptococcal toxic shock syndrome

(Streeton et al. 1995; Currie & Carapetis 2000; Norton et al. 2004; Valery et al. 2007; Whitehead, Smith & Nourse 2011).

2.6 History of streptococci

The term 'streptococcus' was first used to describe a chain-forming coccoid shaped bacteria present in wounds and erysipelas in humans and over the years that followed, the *Streptococcus* genus emerged to contain a number of significant pathogens of animals and humans (Billroth 1874; Jones 1978). This resulted in various attempts to classify the streptococci based on their pathogenicity, culture appearance on agar, cellular morphology, reactions on blood agar, reactions on milk culture, growth temperatures and fermentation of various sugar (Jones 1978). The following table summarises the most monumental early forms of classification of the *Streptococcus* genus (Table 2.3).

Table 2.3. History of classification schemes for the streptococci

Year	Classification Scheme	Reference
1903	Shottmuller inoculated blood agar plates with *Streptococcus* spp. and documented the reactions caused by the bacteria's ability to lyse red blood cells. Therefore the streptococci were split into two groups: β-haemolytic (complete haemolysis) Non β-haemolytic (no haemolysis)	Facklam (2002)
1906	Andrewes & Horder documented the reactions of streptococci with fermentation tests (clotting of milk, neutral red, saccharose, lactose, raffinose, inulin, salicilin, coniferin and mannite), growth on gelatine, morphological observations and pathogenesis in mice, and found variants within each group classified as: *S. pyogenes*: variable within groups but mainly defined by negative reactions with milk, neutral red, raffinose and inulin, vigorous growth on gelatine and extremely pathogenic to mice. *S. salivarius*: short chains, variable reactions of fermentation with no mannite or inulin and rarely pathogenic to mice. *S. anginosus*: long chained form of *S. salivarius* *S. faecalis*: differentiated by mannite production (now known as the genus *Enterococcus*). And the pneumococci: those with a well defined capsule (now known as *S. pneumonia).*	Andrewes & Horder (1906)
1919	Brown furthered the haemolytic tests and divided the streptococci into groups: β-haemolytic (complete haemolysis) α-haemolytic (partial haemolysis) γ-haemolytic (no haemolysis)	Brown (1919) available in Taranta & Moody (1971)
1933	Rebecca Lancefield conducted immunological assays on β-haemolytic streptococci and grouped them based on the presence of specific carbohydrate antigens present in their cell wall. The Lancefield grouping system extended from groups A to W but now is only used for a few species that possess the antigen A, B, C, F and G.	Lancefield (1928; 1933)
1937	Sherman organized the streptococci into groups based on haemolytic reactions, group carbohydrate antigens and phenotypic tests, resulting in the streptococci being separated into four groups: Pyogenic division Viridians division Lactic division Enterococci	Sherman (1937)
1984	The Enterococci were separated from the *Streptococcus* forming the *Enterococcus* genus	Schleifer & Kilpper-Balz (1984)
1986	The Lactic Division was separated from the *Streptococcus* forming the *Lactococcus* genus	Schleifer et al. (1985)

Today, streptococci are still identified based on complex characteristics including; colony size, haemolytic reaction, biochemical reactions and Lancefield group carbohydrate antigen. The Lancefield grouping system was a rapid method of identifying streptococci of clinical significance (mainly β-haemolytic streptococci [BHS]) (Murray, Rosenthal & Pfaller 2005). As a result, it became common to only identify BHS isolates according to group antigen and further tests to confirm species were not frequently performed. For example, the human pathogen *S. pyogenes* possesses the group A Lancefield antigen. Since this organism was frequently recovered from human infections, and at the time appeared to be the only species that produced the Lancefield group A antigen, it became known as 'Group A Streptococci (GAS)'. This resulted in the assumption that all isolates possessing the group A antigen were *S. pyogenes* and no further tests were conducted (Jones 1978; Facklam 2002). However, S*treptococcus dysgalactiae* subsp. *equisimilis* (SDSE) has also been isolated from human infections and can also (although infrequently) possess the group A antigen (Brandt et al. 1999; Tanaka et al. 2008). The heavy reliance on only one form of classification has severely hindered our understanding of streptococcal pathogenicity and relationships between the species within the *Streptococcus* genus (Jones 1978; Hardie & Whiley 1997; Facklam 2002). It is now clear that many BHS share Lancefield group antigens and depending on species, can be retrieved from both animals and humans (Table 2.4).

Table 2.4. β-haemolytic streptococci and corresponding Lancefield group (Facklam 2002).

Species	Lancefield group	Host
S. pyogenes	A	Human
S. agalactiae	B	Human, bovine
S. dysgalactiae		
subsp. *dysgalactiae*[*]	C	Animals
subsp. *equisimilis*	A, C, G, L	Human, animals
S. canis	G	Dog, human
S. equi		
subsp. *equi*	C	Animals
subsp. *zooepidemicus*	C	Animals, human
S. anginosus	A, C, G, F, None	Human
S. constellatus ssp. *pharyngis*	C	Human
S. porcinus	E, P, U, V, None	Swine, human
S. iniae	None	Dolphin, fish, human
S. phocae	C, F	Seal
S. didelphis	None	Opossum

* not β-haemolytic

2.7 Current classification and identification of streptococci

A clear definition of taxa is essential if studies on epidemiology, pathogenicity and resistance to microbial agents are to be successful (Hardie & Whiley 1997). After a review of the literature it is evident that the definition of many species within the *Streptococcus* genus is fluid and ever changing.

The most recent review of the *Streptococcus* genus was based primarily on traditional phenotypic characteristics complemented with genotyping techniques such as 16s rRNA gene sequence comparisons (Facklam 2002). In summary, 56 species of streptococci were identified; of which 11 species and 5 sub species are β-haemolytic. Attempts to identify the non-β-haemolytic streptococci have largely been unsuccessful because of the biochemical heterogeneity within this group. Facklam (2002) described the identification schemes of 26 species of streptococci, 5 related species within the *S. bovis* group, 5 species of nutritionally deprived streptococci, and 3 new genera of gram positive cocci in chains (*Dolosicoccus* spp., *Facklamia* spp. and *Globicatella* spp.). In addition, the author noted that one of the main issues in the identification of species within the *Streptococcus* genus is that new species have not been included in commercial streptococcal identification kits

and systems, reflecting a need for genetic procedures to accurately identify new and emerging streptococcal pathogens.

Molecular methods of species identification such as DNA-DNA hybridisation and 16S rRNA (also known as 16S rDNA) gene sequencing are becoming more popular and have offered a deeper insight into identification and phylogenetic relationships between the *Streptococcus* species (Kawamura et al. 1995; Hardie & Whiley 1997; Facklam 2002). The genome structure or genetic sequence of bacteria can be used to distinguish a family, genus, species, sub-species and strain of a particular isolate (Murray, Rosenthal & Pfaller 2005). Sequencing of the 16S rRNA gene seems to be the most widely used part of DNA for all bacterial identification including, the streptococci (Hardie & Whiley 1997; Brandt et al. 1999; Facklam 2002; Clarridge 2004). The 16S rRNA gene is an important housekeeping gene, encoding the 16s rRNA molecule which is the main RNA molecule of the small 30s ribosomal sub-unit of prokaryotes and thereby integral in facilitating bacterial protein synthesis and cellular function. The gene is approximately 1,550 bp long and contains both conserved (family or genus specific) and variable (species or sub-species specific) regions (Clarridge 2004). The 16S rRNA gene is the ultimate taxonomic chronometer because it is functionally constant and less prone to mutations, large enough to provide meaningful information but small enough to provide rapid identification and is present in all bacterial organisms (Woese 1987; Woese, Kandler & Wheelis 1990; Clarridge 2004).

The accuracy of 16S rRNA sequencing to speciate has recently been questioned when compared to DNA-DNA hybridization because it is believed that 16S rRNA sequencing may be unable to distinguish between closely related or recently diverged species (Fox, Wisotzkey & Jurtshuk 1992; Fraser et al. 2009). However, a further study found that the intragenomic sequence heterogeneity of the 16S rRNA gene was limited and would not have a significant effect on classification (Coenye & Vandamme 2003). Furthermore 16S rRNA identification is effective in identifying species using a cut-off value of 99% sequence similarity and 97% for identification to the genus (Drancourt et al. 2000). Therefore, I have chosen

to display the current species within the *Streptococcus* genus by phylogenetic relationship based on their 16S rRNA gene sequence. The minimum evolutionary tree includes all GenBank 16S rRNA reference sequences available for the *Streptococcus* genus (Figure 2.2).

The use of genetic identification techniques such as 16S rRNA coupled with biochemical test and phenotypic observations should result in a comprehensive identification scheme for the *Streptococcus* genus (Fraser et al. 2009).

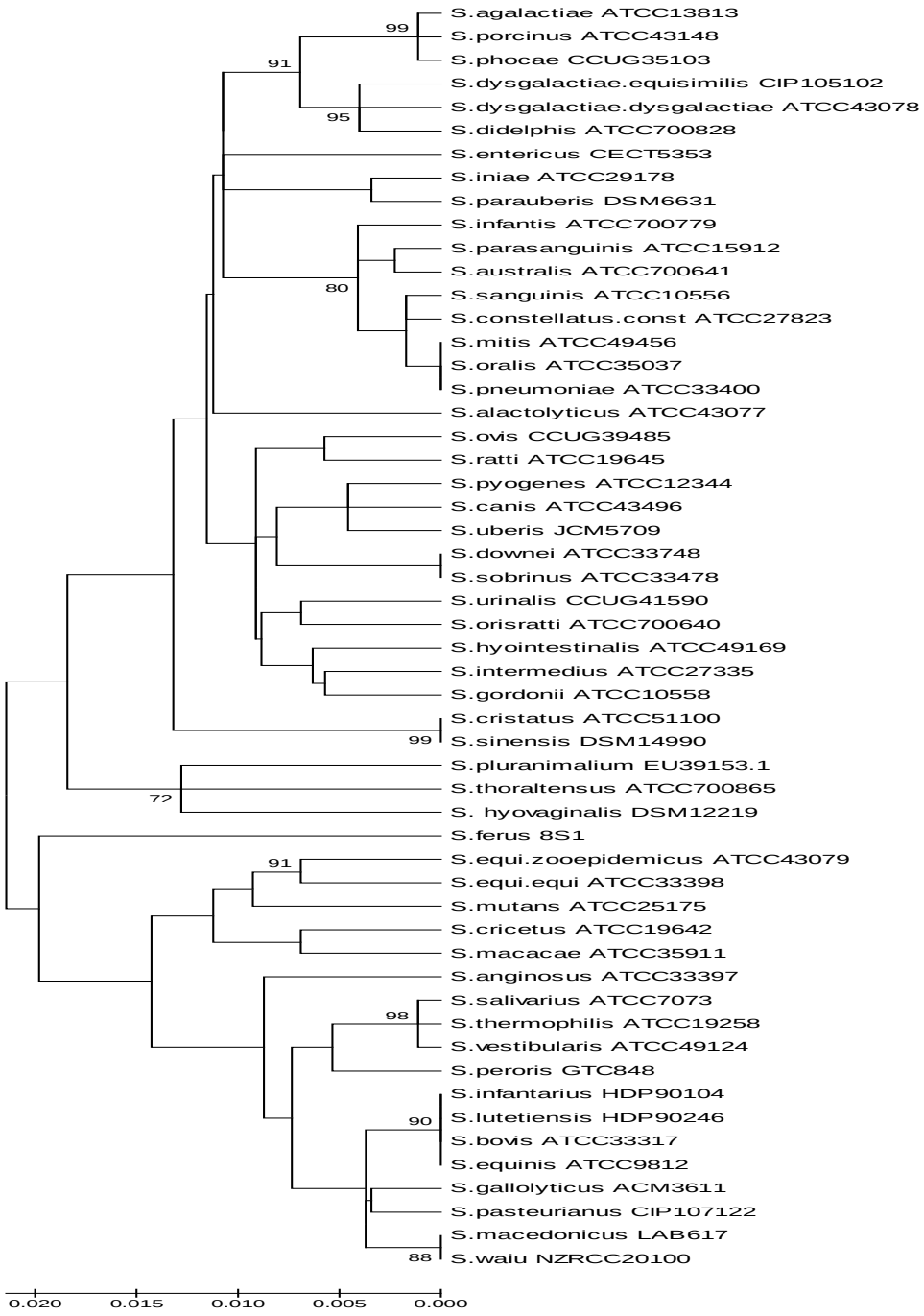

Figure 2.2. 16S rRNA Minimum Evolutionary tree of 54 *Streptococcus* spp. Sequences were aligned using ClustalW. Tree was produced in MEGA 4. Bootstrap values greater than 70% are shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.

2.8 Streptococcal disease in Aboriginal Australian communities

In Aboriginal Australian communities, *S. pyogenes* is the leading cause of ARF which can often lead to RHD. The pathway for disease after streptococcal infection is not fully understood but is believed to be a result of an auto-immune response (Cunningham 2000). ARF appears to be a multifactorial disease, largely dependent on host susceptibility. While the exact genes that predispose a host remain unidentified, it is believed that components of *S. pyogenes* initiate the disease. These are believed to include cross reactive antibodies with streptococcal antigens, molecular mimicry and T-cell autoreactivity (Cunningham 2000). Recurrent streptococcal infections were seen to be associated with severe, irreversible valve damage known as RHD (National Heart Foundation of Australia & Cardiac Society of Australia and New Zealand 2006). RHD and ARF are the major causes of cardiac morbidity and mortality in younger people (Haidan et al. 2000). Northern Australian Aboriginal populations suffer the highest rates of RHD and ARF in the world (McDonald, Currie & Carapetis 2004). Aboriginal Australian and Torres Strait Islanders are eight times more likely to be hospitalised for ARF and RHD than non-indigenous Australians and are ten times more likely to die as a result of these diseases (National Heart Foundation of Australia & Cardiac Society of Australia and New Zealand 2006).

PSGN is a disease of the kidneys that occurs when the glomeruli become inflamed. The damaged glomeruli do not function normally, resulting in protein and red blood cells passing into the urine (albuminuria, proteinuria and haematuria). PSGN is associated with an auto-immune response that usually occurs in children after throat or skin infection with group A BHS (Steer, Danchin & Carapetis 2007). PSGN occurs as a result of a complex interaction between the hosts immune response and streptococcal virulence factors. The exact pathways remain unknown but components of *S. pyogenes* are believed to be associated with the development of PSGN through a number of postulated immunomodulatory mechanisms. These include deposition of immune complexes, cross reactive antibodies with streptococcal and glomerular antigens, glomerular tissue alteration by *S. pyogenes*

products and *S. pyogenes* components deposited into the glomeruli mediating direct complement activation (Cunningham 2000).

A recent study showed that Aboriginal people of the Northern Territory with a history of childhood PSGN are at higher risk of developing end stage renal disease (ESRD) later in life (White, Hoy & McCredie 2001). Australian Aboriginal and Torres Strait Islander people are 9 times more likely to develop ESRD than non-Indigenous Australians, but are also, on average, 10 years younger when diagnosed with ESRD (McDonald & Russ 2003; Haysom et al. 2007; Haysom et al. 2009).

As previously mentioned, streptococcal pyoderma that can lead to PSGN is hyper-endemic among Aboriginal Australian children with up to 50% of children in some communities affected (Currie & Carapetis 2000). Consequently PSGN epidemics have occurred in many Aboriginal communities (Gogna, Nossar & Walker 1983; Carapetis 1995; Streeton et al. 1995). Previously it was believed that 'rheumatogenic' *S. pyogenes* strains associated with pharyngitis could exclusively cause ARF, whilst 'nephritogenic' *S. pyogenes* strains that could cause PSGN were believed to originate from streptococcal pyoderma (Wannamaker 1973). However, increasing evidence suggests that this theory is not applicable to Australian Aboriginal and Torres Strait Islander populations. In Aboriginal communities where ARF and RHD are predominant, streptococcal pharyngitis is rare but streptococcal pyoderma is hyper-endemic (McDonald, Currie & Carapetis 2004).

S. pyogenes are also associated with a wide range of serious diseases including streptococcal toxic shock syndrome, bacteraemia, cellulitis, erysipelas and necrotising fasciitis (Bisno & Stevens 1996). A recent study found that Aboriginal children residing in Queensland had a crude incidence of invasive disease due to group A streptococci (assumed *S. pyogenes*) at 13.5/100,000 compared to 3.5/100,000 for the total Queensland population (Whitehead, Smith & Nourse 2011). However, significant under reporting was acknowledged and previous reports of invasive group A streptococcal disease in Aboriginal communities of north Queensland and Northern Territory have been as high as 82.5/100,000 and

23.8/100,000 per year (Carapetis et al. 1999; Norton et al. 2004). In addition, the study found that the annualized incidence of invasive group A streptococcal disease in 2008 was 123.34/100,000 for Indigenous infants aged 0-1 year, contrasting with an incidence of 12.23/100,000 for non-Indigenous infants (Whitehead, Smith & Nourse 2011).

While *S. pyogenes* appears to be the main source of streptococcal disease in Aboriginal communities, other species of streptococci are being implicated for their role in causing disease. Current research conducted in northern Aboriginal populations where ARF is hyper-endemic has shown that throat carriage of *S. pyogenes* is exceeded by SDSE (identified based on biochemical characteristics, presence of Lancefield group G or C antigen and *emm* type) (McDonald et al. 2007). Since the development of ARF correlates with streptococcal throat infection, studies have investigated the potential of SDSE to cause ARF by investigating the ability of SDSE to invade oral epithelial cells (Haidan et al. 2000). The study found that both Lancefield group C and G SDSE had extremely high invasive potential and therefore may have adopted rheumatogenic factors through horizontal gene transfer (HGT) from *S. pyogenes*. The ability of SDSE to illicit streptococcal auto-immune sequelae such as ARF is speculative and has not yet been proven, probably made more difficult by the frequent co-infections of SDSE and *S. pyogenes* in Aboriginal populations.

2.9 Increasing pathogenicity of Group G and Group C streptococci

BHS possessing the Lancefield G or C antigen can be separated into those that form large or small colonies on blood agar (Ruoff, Whiley & Beighton 2003). They include a variety of *Streptococcus* spp. that are morphologically similar and not easily differentiated through biochemical testing, especially the sub-species of *S. dysgalactiae* (Table 2.5).

Table 2.5. Biochemical identification of β-haemolytic species of streptococci producing the Lancefield group G and C antigen (modified from Facklam 2002).

Species	Lancefield group	Bac	Cam	VP	Hip	Arg	Esc	Str	Sbl	Tre	Rib	Host
S. dysgalactiae												
subsp. *dysgalactiae**	C	-	-	-	-	+	v	-	v	+	+	Animals
subsp. *equisimilis*	A, C, G, L	-	-	-	-	+	+	-	-	+	+	Human, animals
S. equi												
subsp. *equi*	C	-	-	-	-	+	v	+	-	-	n/a	Animals
subsp. *zooepidemicus*	C	-	-	-	-	+	v	+	+	v	n/a	Animals, human
S. canis	G	-	+	-	-	+	+	-	-	+	n/a	Dog, human
S. anginosus (group)	A, C, G, F, None	-	-	+	-	+	+	-	-	+	n/a	Human
S. constellatus subsp. *pharyngis*	C	-	-	+	-	+	+	-	-	+	n/a	Human
S. phocae	C, F	+	-	-	-	-	-	-	-	n/a	n/a	Seal

Abbreviations: sensitive to Bac, bacitracin; Cam, CAMP reaction; VP, Voges-Proskauer reaction; Hip, hydrolysis of hippurate; Arg, deamination of arginine; Esc, hydrolysis of esculin; Str, hydrolysis of starch; Sbl, acid production in Sorbitol; Tre, acid production in trehalose; Rib, acid production in ribose. (+) denotes positive reaction in >95%; (-) denotes negative reaction in >95%; (v) denotes variable reaction with 6-94% positive; (n/a) not applicable.* *S. dysgalactiae* subsp. *dysgalactiae* is not β-haemolytic.

In the last decade, taxonomic changes to *S. dysgalactiae* have caused confusion relating to the biochemical and serological variability of this species (Efstratiou et al. 1994; Vandamme et al. 1996; Viera et al. 1998) (Table 2.6). Having no clear classification scheme has resulted in incomplete identifications and an unclear epidemiology of the diseases associated with this species and their potential hosts with strains either classified as *S. dysgalactiae* or *S. equisimilis*. The current classification is that *S. dysgalactiae* subsp. *equisimilis* are large colony forming, β-haemolytic, produce Lancefield group A, C, G or L antigens and have several ecovars within the subspecies that represent strains that infect humans and strains that infect animals (Viera et al. 1998; Facklam 2002; Kawata et al. 2003; Boon 2007; Preziuso et al. 2010; Karstrup et al. 2011).

Table 2.6. History of classification schemes separating the *S. dysgalactiae*.

Year	Classification scheme	Reference
1936	*S. equisimilis* was proposed for β-haemolytic, Lancefield group C streptococci.	Frost & Engelbrecht (1940)
1983	*S. dysgalactiae* proposed for α-haemolytic, Lancefield group C streptococci of bovine origin.	Garvie, Farrow & Bramley (1983)
1984	DNA hybridisation revealed high similarity between *S. equisimilis*, *S. dysgalactiae* and other large colony streptococci of Lancefield group G and L and therefore the *S. dysgalactiae* were expanded to include these species.	Kilpper-Balz & Schleifer (1984)
1996	Chemotaxonomic and phenotypic examination separates the *S. dysgalactiae* into two subspecies: subsp. *equisimilis*: large colony forming, β-haemolytic, Lancefield group C or G, isolated from humans and subsp. *dysgalactiae*: large colony forming, α, β, or no haemolyisis, Lancefield group C and L, isolated from animals.	Vandamme et al. (1996)
1998	Multilocus enzyme electrophoresis and DNA hybridisation revealed sub-clusters in which the β-haemolytic, Lancefield group C and L strains isolated from animals clustered with human strains and were divergent from the α-haemolytic, Lancefield group C strains isolated from animals. Therefore *S. dysgalactiae* subspecies were modified to: subsp. *equisimilis*: large colony forming, β-haemolytic, Lancefield group C, G, and L, isolated from both animals and humans, and subsp. *dysgalactiae*: large colony forming, α, or non haemolytic, Lancefield group C strains isolated from animals only.	Viera et al. (1998)

Large colony forming streptococci that produce the group G or C antigen isolated from humans are usually SDSE but occasionally *S. canis* and *S. equi* subsp. *zooepidemicus* (SESZ) are found. SDSE are usually considered commensal organisms in humans, but SDSE, *S. canis* and SESZ have been implicated in a wide spectrum of disease including many known to be caused by *S. pyogenes*. These include, pharyngitis, PSGN, cellulitis, necrotizing fasciitis, septicaemia and streptococcal toxic shock syndrome (McCue 1982, Francis et al. 1993, Bert & Lambert-Zechovsky 1997, Takeda et al. 2001, Whatmore et al. 2001, Sylvetsky et al. 2002, Korman et al. 2004, Galperine et al. 2007, Lam et al. 2007, Broyles et al. 2009, Abbott et al. 2010). Population surveillance studies have found that there has been an increase in prevalence of invasive disease caused by Lancefield group G streptococci (assumed to be SDSE) (Table 2.7). The majority of patients infected were elderly and had underlying medical conditions (Woo et al. 2001; Takahashi, Ubukata & Watanabe 2010). The predominant mode of invasion was through skin or soft tissue injuries (Hindsholm & Schonheyder 2002; Broyles et al. 2009).

Table 2.7. Frequency of invasive disease caused by SDSE in comparison to *S. pyogenes* worldwide.

Location	Study period	Identification method	Annual incidence of SDSE as causative agent	Associated disease	patients with no underlying disease	Mortality rate	Annual incidence of *S. pyogenes* as causative agent	Reference
Israel, Jerusalem.	1990-1999	*Lancefield group, Rapid ID 32 Strep.	0- 41.3 cases per 100,000.	Bacteraemia	0%	15%	30-70 cases per 100,000.	Sylvetsky et al. (2002)
Jerusalem.	1989-2000	*Lancefield group, 56 isolates *emm* typed.	0.0-20.0 cases per 100,000.	Bacteraemia	8.5%	5.3%	20.0-48.0 cases per 100,000.	Cohen- Poradosu et al (2004)
Atlanta, Georgia, California.	2002-2004	Lancefield group, Rapid ID 32 Strep, *emm* types	1.38 cases per 100,000 per year	Bacteraemia, cellulitis, peritonitis, appendicitis etc.	4%	15%	2.89 cases per 100,000.	Broyles et al. (2009)
Finland, Pirkanmaa.	1995-2004	*Lancefield group, Rapid ID32 Strep	1.81-4.32 per 100,000.	Bacteraemia	U	15%	0.9- 3.5 per 100,000	Rantala et al. (2009)
*Denmark	1981-1999	*Lancefield group	0.3- 1.72 per 100,000	Bacteraemia	11.9%	13%	1.8 per 100,000 (1999 only data)	Hindsholm & Schonheyder (2002)
*Denmark	1999-2002	*Uknown, assumed Lancefield group only	approximately 1.5-2.2 per 100,000.	Bacteraemia, erysipelas, NF, STSS, pneumonia, meningitis, erysipelas etc.	0%	18%	approximately 2.7 per 100,000.	Ekelund et al. (2005)
Canada, Calgary region.	1999-2004	Lancefield group (collated GGS/GCS)	2.24 per 100,000.	Termed invasive if found in a normally sterile site.	U	U	4.27 per 100,000	Laupland et al. (2006)

*Only Group G Streptococci or group G SDSE were used in the study. NF, necrotising fasciitis; STSS, streptococcal toxic shock syndrome. U, unknown

Literature searches failed to locate population surveillance studies of invasive disease caused by Lancefield group G or C streptococci in Australia including Aboriginal Australian communities. Therefore, it is unknown whether Aboriginal populations (that already experience extremely high rates of invasive disease caused by *S. pyogenes*) are also experiencing an increase in invasive disease caused by SDSE or other Lancefield group G or C streptococci.

A 14 year study of hospital isolates from the Townsville region found that bacteraemia episodes caused by BHS were those that produced the Lancefield group A (49%), B (28%), C (7%) and G (16%) respectively (Harris et al. 2010). In contrast to other studies, invasive disease caused by group G or C streptococci in this region was stable and did not appear to be predominant in the Indigenous population. However, there may be significant undercounts because the study used hospital isolates and the data collection methods relied heavily on the precision of hospital staff to identify Aboriginal and Torres Strait Islander people presenting to the hospital with illness.

As previously described, in some Northern Territory Aboriginal populations throat carriage of SDSE exceeds that of *S. pyogenes*. In these communities the throat carriage of SDSE is not often symptomatic and skin disease are often associated with *S. pyogenes* or *Staphylococcus aureus* infections (McDonald et al. 2007). Low rates of pharyngitis but high rates of SDSE throat carriage have also been reported in India which also has high rates of streptococcal disease (Bramhachari et al. 2010). The circulation of SDSE in areas with high rates of *S. pyogenes* disease, combined with the apparent increase in SDSE disease has resulted in investigations into the presence of *S. pyogenes* virulence genes in SDSE.

Streptococcus pyogenes is a significant human pathogen because it has a wide variety of virulence genes that enable it to successfully adhere to host tissues, invade the host and avoid being destroyed by the host immune response (Murray, Rosenthal & Pfaller 2005). Recent studies have found that HGT is occurring between

the streptococci that produce the group A, G and C antigens (Simpson, Musser & Cleary 1992; Schnitzler et al. 1995; Kalia et al. 2001; Sachse et al. 2002; Davies et al. 2005; Davies et al. 2006; Barroso et al. 2008).

Many SDSE strains have virulence factors that are nearly identical to known virulence genes of *S. pyogenes* encoding various adhesins, toxins and enzymes thought to improve the species fitness and capacity to cause disease in the host. Current evidence suggests that HGT is occurring between species of this genus in which both virulence and housekeeping genes are being transferred (Kalia et al. 2001). In regards to SDSE, it appears that much of the HGT is from *S. pyogenes* donors to SDSE recipients, which has been used as a plausible explanation for the gradual increase of SDSE virulence (Kalia et al. 2001; Kalia & Bessen 2003; Davies et al. 2005; Davies et al. 2007).

Horizontal acquisition of genomic DNA is a survival tactic for bacteria. The bacteria acquire the genes that have already been proven successful within a host (Davies et al. 2005). In locations where *S. pyogenes* is hyper-endemic, SDSE clones with *S. pyogenes* virulence genes are more common (Davies et al. 2006). Furthermore, because SDSE and *S. pyogenes* are frequently found in the same human host at the same time *S. pyogenes* may also be adopting genes from the SDSE, and possibly other Lancefield group G and C streptococci.

Molecular studies have found that some SDSE strains have alleles coding for virulence genes nearly identical to *S. pyogenes* virulence genes. These include, M protein, fibronectin binding proteins, plasminogen binding proteins, streptococcal surface enolase, vitronectin binding protein, glyceraldehydes-3-phosphate dehydrogenase, laminin binding protein, streptolysins O and S, capsule, streptokinase, C5a peptidase and streptococcal pyogenic exotoxins (superantigens) speA, speC, SpeG, SpeM, ssa (streptococcal superanitgen) and Smez (streptococcal mitogenic exotoxin Z) (Cleary et al. 1991; Schnitzler et al. 1995; Sriprakash & Hartas 1996; Sachse et al. 2002; Kalia & Bessen 2003; Towers et al. 2004; Brandt &

Spellerberg 2009). These proteins function in enabling bacteria to adhere to host tissues, invade and spread within the host and avoid host responses.

The most comprehensive study of *S. pyogenes* virulence gene presence in SDSE was conducted by Davies et al. (2007). The study assessed the virulence profile of 58 SDSE from both invasive and non-invasive streptococcal infections in humans within Australia by using a targeted microarray of 216 *S. pyogenes* virulence genes. It was found that the SDSE isolates harboured genes that encoded for up to 50% of *S. pyogenes* virulence genes probed for in the microarray. Furthermore, no association was found between any virulence gene repertoire and disease manifestation, tissue site or whether they were Lancefield group G or C isolates. Therefore, identifying pathogenic strains of streptococci that produce Lancefield group G and C antigens is difficult because of the biochemical and genetic variability within these species.

In *S. pyogenes* genetic methods of typing to identify virulent strains have included *emm* typing and multi-locus sequence typing (MLST) which have also been used to identify SDSE strains. The M-protein of *S. pyogenes* is an important virulence factor that aids in adhesion to host tissues, binds to factor H and fibrinogen avoiding host responses and phagocytosis, and has also been implicated in rheumatic fever through molecular mimicry of cardiac myosin (Cunningham 2000; Murray, Rosenthal & Pfaller 2005; Guilherme, Kalil & Cunningham 2006). The *emm* gene of *S. pyogenes* encodes the M-protein and sequencing of this gene has proven to be a valuable tool in identifying virulent strains of *S. pyogenes* often causing epidemics of streptococcal disease (Beall, Facklam & Thompson 1996). SDSE also harbour an *emm*-like gene which has also functioned to identify strains of SDSE (Schnitzler et al. 1995). Currently there are more than 50 SDSE *emm* types (*emm* sub-types) contrasting with over 200 *S. pyogenes emm* types recognised by the Centers for Disease Control and Prevention (http://www.cdc.gov/ncidod/biotech/strep/strepblast.htm). In northern Aboriginal Australian populations where SDSE throat carriage exceeds *S. pyogenes*, there are few *emm* sub-types of SDSE defined therefore suggesting that the SDSE population

may not be highly diverse (McDonald et al. 2007). However, research has shown that because *emm* is subject to selective pressures and HGT it may not be a good indicator of diversity and therefore other methods such as MLST may be required to fully explore SDSE population diversity (Kalia et al. 2001; McDonald et al. 2007; McMillan et al. 2011)

MLST is a nucleotide sequence based method used to characterise strains within a single species based on the seven core housekeeping genes; glucose kinase (*gki*), glutamine transport protein (*gtr*), glutamate racemase (*murI*), DNA mismatch repair protein (*mutS*), transkelotase (recP), xanthinine phosphoribosyl tranferase (*xpt*) and acetoacteyl coathioloase (*atoB*) . The resulting sequence of each of the seven loci is given an allelic number, which then combines to generate an allelic profile of the seven loci. Each new allelic profile is then designated a sequence type number (ST) (Feil et al. 2004). Analysis of the allelic profile and ST of isolates has been used extensively to investigate the genetic relationships of *S. pyogenes*, *S. agalactiae* and recently SDSE. MLST combined with *emm* typing can provide information of clonal complexes within species (described in detail in chapter 3) (Enright et al. 2001). In summary, a clonal complex describes the formation of a cluster of isolates that have identical profiles at six of the seven housekeeping loci (Feil et al. 2004). At present there are 80 recognised sequence types (ST's) within the SDSE MLST database (http://sdse.mlst.net/). MLST has also provided evidence of recent genetic exchange of housekeeping genes between *S. pyogenes* and SDSE. It has been postulated that the close interaction between humans (preferred *S. pyogenes* hosts) and animals (preferred Lancefield group G and C streptococci hosts) may have provided opportunity for a few clones of animal origin to adapt to human hosts increasing interspecies genetic exchange and ultimately resulting in human adapted lineages (Kalia et al. 2001).

2.10 Dogs and streptococcal disease

Many of the previous studies conducted on streptococci carriage in dogs have only identified species based on their Lancefield group (Kurek & Rutkowiak

1971; Svartman et al. 1972; Crowder, Dorn & Smith 1978; Biberstein, Brown & Smith 1980; Palmer & Presson 1990; Wilson, Maroney & Gander 1995; Falck 1997). Boon (2007) was an exception, highlighting the importance of complete species identification. The majority of BHS that have been isolated produced the group G antigen, but Lancefield group C streptococci, group E streptococci and group A streptococci have also been found (Kurek & Rutkowiak 1971; Palmer & Presson 1990; Boon 2007). In most studies, since identification was performed using only Lancefield grouping it is impossible to identify the exact species of streptococci that were isolated. Furthermore, the majority of epidemiological studies have been focused on the presence of Lancefield group A streptococci because the pathogenic potential of the streptococci producing group G and C antigens was not yet fully appreciated (Svartman et al. 1972; Crowder, Dorn & Smith 1978; Wilson, Maroney & Gander 1995; Falck 1997).

Most species of streptococci found in animals are normal flora of the lower genital, alimentary and upper respiratory tract (Hirsh & Biberstein 2004). In dogs, the majority of BHS have been identified as; *Streptococcus canis* but SDSE, SDSD and SESZ have also been identified. Although these species can be found in dogs without causing disease they have also been found to be associated with septicaemia, necrotising fasciitis, streptococcal toxic shock syndrome, abortion, dermatitis and hemorrhagic pneumonia (Miller et al. 1996; Hirsh & Biberstein 2004; Songer & Post 2005; Priestnall & Erles 2011). Isolates retrieved from dogs have also been found to have genes homologous to *S. pyogenes* virulence genes (DeWinter, Low & Prescott 1999; Boon 2007; Rato et al. 2011).

Molecular studies comparing virulence of streptococci only identified by their Lancefield group G antigen isolated from animal and human sources have concluded that the animal isolates lack *S. pyogenes* virulence genes (Cleary et al. 1991; Simpson, Musser & Cleary 1992; Schnitzler et al. 1995). These studies failed to distinguish between *S. canis* and SDSE both possessing the Lancefield group G antigen. In contrast, other studies have found *S. pyogenes* virulence genes in *S. canis* isolated from infected dogs or humans (DeWinter, Low & Prescott 1999; Igwe

et al. 2003; Fulde et al. 2011). Virulence genes encoding streptolysin O, streptokinase, streptococcal fibronectin binding protein I, streptococcal pyogenic exotoxins; *smeZ* and *speG*, and a novel M-like protein have been found in some *S. canis* strains (Miller et al. 1996; DeWinter, Low & Prescott 1999; Igwe et al. 2003; Boon 2007; Fulde et al. 2011). *S. canis* has also been found to be associated with cases of soft tissue inflammation (cellulitis), bacteraemia/septicaemia, urinary infections, osteomyelitis and pneumonia in humans (Bert & Lambert-Zechovsky 1997; Takeda et al. 2001; Whatmore et al. 2001; Galperine et al. 2007; Lam et al. 2007). All of these streptococcal diseases were concurrent with underlying medical conditions in the patient and in many of these cases the patient had close contact with a dog (Bert & Lambert-Zechovsky 1997; Takeda et al. 2001; Lam et al. 2007).

Barroso et al. (2008) tested whether animal isolates of the species SDSE, SDSD, *S. equi* subsp. *equi*, SESZ and *S. canis* had the potential to cause rheumatic fever by their ability to cleave human collagen IV. They found that while some animal isolates could cleave collagen IV they did not aggregate the collagen compared to a previous study looking at human isolates of SDSE. However, none of the animal isolates were from an endemic *S. pyogenes* area where human and animals live in close proximity potentially having ample opportunity for HGT between the species.

In Aboriginal Australian communities where *S. pyogenes* infection is hyper-endemic and humans and animals live in close proximity, dogs have been found to carry strains of SDSE and *S. canis* that expressed virulence genes homologous to *S. pyogenes* encoding streptokinase, streptococcal pyrogenic exotoxin G and streptococcal fibronectin binding protein I (Boon 2007). Unlike previously mentioned surveys, these species were determined by biochemical tests, phenotypic observations and sequencing of the 16Sr RNA gene.

In conclusion, the pathological relevance of SDSE and perhaps other Lancefield group G and C streptococci in Aboriginal populations may be increasing. The fact that unhealthy dogs live in close proximity to people that are often infected

with species of streptococci (mainly *S. pyogenes*) suggests the potential for dogs to play a role in circulating streptococci within a community and/or facilitating HGT in which normally commensal streptococci are increasing in virulence.

2.11 Aims of the thesis

This thesis had four aims:

Aim 1: Isolate streptococci from dogs and characterise the strains;

Aim 2: Investigate associations between health and social parameters of dogs and isolation of streptococci;

Aim 3: Isolate streptococci from children with skin sores and determine if there was any indication of the strains being shared with dogs;

Aim 4: Translate scientific knowledge, including the new information from this study into community action to improve the health of dogs.

The next chapter describes the methods used to engage with the community and the process of collecting samples. It also describes the procedures for the identification and molecular methods used to genetically characterise strains of streptococci. The chapter ends by discussing the methodology used to document and develop strategies to improve dog health in the Yarrabah community.

Chapter 3: Materials and Methods

3.1 Introduction

The previous chapter outlined the role of dogs in Aboriginal communities and their potential associations with spreading infectious disease. The epidemiology of streptococcal disease in Aboriginal communities was described and the potential of dogs playing a role in the increasing pathogenicity of Lancefield group G and C streptococci.

As described in chapter 1, whilst the main aim of the research project was to investigate the carriage of streptococci by dogs, a parallel process of implementing strategies to improve dog health and ownership within the community was extremely important in engaging with the community and providing tangible outcomes of the research to the community.

This chapter begins with the process of community engagement and describes the methods of sampling, data collection and identification of streptococcal isolates, including PCR protocols. The chapter ends with a description of the methodology and processes used to document, develop and implement strategies to improve dog health in the community. The feedback of research findings to the community is also described.

3.2 Community engagement

The research project was presented to and approved by the Yarrabah Aboriginal Shire Council and a group of community members involved in health and wellbeing within the community including the community controlled health service (Yarrabah Local Managers Forum). Initial introductions to community organisations occurred in the first year of research and included; relevant Elders and family

members, Gurriny Yealamucka Aboriginal Health Service (community controlled health service), Yarrabah hospital, Yarrabah Aboriginal Shire Council and Yarrabah Primary School. Engagement with individuals and organisations was an ongoing process throughout the research project.

Posters and pamphlets were developed designed specifically for engaging community participation in the research project. The posters and pamphlets described the researchers' family ties, and the purpose of the research project in community language. These were assessed by family members and once approved were placed around the community in the waiting room of the community controlled health service, the hospital and the main shop.

3.3 Participant recruitment, consent and ethics

A picture book that graphically described the research project, methods of swabbing, information transfer and storage of bacteria was developed and used to explain the research project to prospective participants and households with dogs (Appendix 1).

All participants and/or dog owners were required to sign a consent form previously approved by the Human Research Ethics Committee of Cairns and Hinterland through the Cairns Base Hospital (Reference: HREC/10/QCH/19-648). Further ethical approval was provided by the University of Sydney Animal Ethics Committee (Number: N00/3-2010/3/5272) and the University of Sydney Human Ethics Committee (Reference: PB/KR, database number: 13000).

3.4 Sampling techniques and procedures

3.4.1 Community dog samples

Animals were chosen for the study by convenience. The majority of dogs were strays or unwanted dogs collected by the AMW for disposal and kept in the community pound. Other dogs (and owners) were referred to the researcher by the

AMW, were volunteered by their owners in response to community engagement processes previously described, or referred by the doctor who also recruited participants.

Pharyngeal swabs were a priority over sampling any other body location or data collection. Previous researchers have found that pharyngeal carriage of streptococci in dogs is high in comparison with other body locations (Wilson, Maroney & Gander 1995; Boon 2007). Dogs were either restrained by their owners, the AMW or the researcher and the mouth was held open. Sterile cotton-tipped swabs were used to obtain samples from each animal either by the researcher or the AMW. The swab was then placed immediately into Amie's transport medium (either plain or charcoal) (Biomerieux Pty Ltd) and placed in an esky with ice for transport back to the laboratory for processing.

3.4.2 Human samples

Attempts to recruit an Aboriginal health worker to accompany the researcher to households to take throat swabs of children were unsuccessful because the health workers were uncomfortable taking throat swabs from patients and preferred the doctor to do this. Therefore, discussions with the health service resulted in the doctor offering to recruit participants with purulent sores and frequent contact with a dog. Once informed consent of participant's parents was received a pharyngeal swab and skin sore swab were taken from the child. The doctor then (with the permission of the participant) referred the researcher and AMW to the household to swab the dog. Swabs taken by the doctor from children were placed in Amie's transport medium and refrigerated until collected by the researcher. Once collected by the researcher (within 48 hours) the swabs were transported back to the laboratory in an esky with ice.

The researcher also recruited children into the study only if at the time of sampling the dog a child was also present with purulent or dry sores. In this case, a swab was taken of the sores once informed consent from parents was received. Further information was collected from the parents for the purpose of relaying

information back to the participant in cases where medical attention was required. The swabs were then placed in transport medium and transported to the laboratory by the researcher within 24 hours.

If there were no children present, the swabs were collected from adult dog owners who self-swabbed their own nasal cavity.

3.4.3 Sampling difficulties and potential sources of bias

The researcher recognises that within this project there are issues of selection bias. Samples were taken by convenience and not randomised. The overall population of dogs and mean health of dogs within the community was largely unknown; therefore, it was difficult to take a representative sample of the entire dog population.

In addition, the manner in which children were recruited into this study also contributed to sample bias as participants were only eligible if they had skin sores and were in close contact with a dog. Therefore, asymptomatic carriage of streptococci species in dog owners would have been largely missed.

3.4.4 Demographic data

Demographic data were collected for each dog sampled and included; location of dog (pound or household number), number of dogs residing in the household, sex and age of dog categorised as either; puppy (if still suckling), juvenile (young yet independent of mother) or adult (had a litter or appears aged and fully grown). The general health status of dogs were also determined by designating a Purina body condition score which grouped dogs into three categories; Too thin (numbered 1-3); ideal (numbered 4-5); too heavy (numbered 6-9); and skin condition score, assessing the percentage of body surface affected by lesions or balding (numbered 0-5; number 0 having no lesions and 5 having complete hair loss) (Appendix 2).

3.4.5 Wild dog samples

Wild dogs were either retrieved by trapping or found deceased. Pharyngeal swabs were taken with the sterile swabs and placed in Amie's transport medium and transported to the laboratory within 48 hours. A dog was classed as a 'wild dog' if it appeared to be either a dingo or dingo hybrid and had no signs of human ownership (no micro-chip, collar, traces of surgical procedures etc.).

3.5 Bacterial culture and identification

All bacterial culture and identification were performed by the researcher.

3.5.1 Culturing and isolating streptococci from swabs

Upon arrival at the laboratory each sample was inoculated onto two separate culture media: 5% Horse blood agar (HBA) (a non-selective culture medium) and a commercial streptococci selective medium called Columbia CNA Agar which consists of 10 mg colistin and 10 mg nalidixic acid per litre in a Columbia agar base enriched with 5% sheep blood. Columbia CNA agar is used to suppress a variety of Gram negative bacteria allowing other species such as streptococci and staphylococci to grow. The used swabs were then stored in the refrigerator at 4°C.

All swab inoculated plates were incubated in 5% CO_2 at 35°C. The plates were examined for colony growth at 24 hours. Large colonies (>0.5 mm), with complete haemolysis (β-haemolytic) and a grey to white appearance were repeatedly subcultured onto HBA plates and incubated under the same conditions until a pure culture was obtained (Ruoff, Whiley & Beighton 2003). The morphological features of the colony were recorded: size of colony, haemolysis (complete, incomplete or nil) and any other distinguishing features ie. pigment etc. Each culture was then subjected to routine microbiological tests (catalase and Gram stain) to determine if they were streptococci. Gram positive and catalase negative isolates demonstrating complete haemolysis were classified as β-haemolytic streptococci. However, colonies that had complete haemolysis and appeared to be

streptococci but were catalase positive were kept due to the unreliability of the catalase tests when bacteria is grown on blood containing agar, and due to the recent description of a catalase positive streptococci, *Streptococcus didelphis* (Rurangirwa et al. 2000). Both confirmed and suspect streptococci isolates were then inoculated into microscopic beads (Biomerieux Pty Ltd) storage solution in duplicate, one kept at -20°C as a working stock the other kept at -80°C for long term storage.

3.5.2 Lancefield grouping

Classification by Lancefield grouping was conducted for all confirmed and suspect streptococci isolates. Each isolate was re-inoculated onto HBA plates by taking one microscopic bead in a loop (directly from the -20°C working stock) and streaking it onto the plate. The inoculate plates were incubated in 5% CO_2 at 35°C and examined after 24 hours to ensure pure cultures were obtained. Each isolate was classified according to their Lancefield serogroup using the streptococcal grouping kit (Oxoid Diagnostics) according to the manufacturer's instructions. Briefly, 3-4 colonies were inoculated into a test tube with 0.4 mL of extraction enzyme using a disposable pipette. Test tubes were then placed in a water bath at 36°C. After 5 minutes the content of the tubes were mixed with a pipette and placed back into the water bath for a further 5 minutes. The serogroup test reagents A, B, C, D, G and F were allowed to warm to room temperature. One drop of each reagent was dispensed onto the cards provided and one drop of bacteria mixture was added to each reagent and mixed. Rapid agglutination between 30 seconds and 1 minute was recorded as a positive result.

3.5.3 Streptococcal identification using the API20 Strep system

All confirmed and suspect streptococci isolates were identified to the species level using the API20 Strep system (Biomerieux Pty Ltd) according to the manufacturer's instructions. In short, an isolated colony was picked up from the purified HBA subculture and resuspended in 0.3 mL of sterile water. This suspension was then aseptically swabbed onto a HBA plate and incubated in an anaerobic

chamber at 35.5°C for 24 hours. The bacterial lawn was swabbed using a sterile cotton tip and dissolved in 2 mL of sterile water to generate a turbidity of greater than 4 McFarland standards. Test strips were prepared and trays filled with 5 mL of distilled water. Approximately 100 µL of the bacterial suspension was distributed using a pipette into each cupule to test for the following reactions/ enzymes; Voges Proskauer test (measuring acetoin production), hydrolysis (hipuric acid), β-glucosidase hydrolysis (esculin), pyrolidonyl arylamidase, α-galactosidase, β-glucuronidase, β-galactosidase, alkaline phosphate and leucine aminopeptidase. The bacterial suspension was then used to fill the tube for the arginine dihydrolase test. The remaining bacterial suspension was mixed with the provided API GP Medium, consisting of a mixture of L-cystine, tryptone, sodium chloride, sodium sulphide, phenol red and demineralised water which functions to rehydrate the sugar substrates. This mixture of bacterial suspension and GP medium was then distributed into the tubes of the fermentation tests evaluating the bacterial ability to ferment the following sugars; ribose, arabinose, mannitol, sorbitol, lactose, trehalose, inulin, raffinose, amidon and glycogen. Sterile mineral oil was then distributed on top of each tube forming a convex meniscus from the arginine dihydrolase test through all the fermentation tests to create anaerobic conditions.

The lid was placed on the tray and incubated anaerobically at 35.5°C. The first reading was made between 4 and 4.5 hours and the second reading at 24 hours. Test results were interpreted using the API website (http://apiweb.biomerieux.com).

All isolates that were not identified to 99.9% were subcultured and re-tested.

3.5.4 Bacterial isolation difficulties and recommendations

Pharyngeal swabs of dogs have previously been shown to retrieve larger amounts of BHS than any other body area presumably because dogs lick themselves. However, research also suggests that human throat swabs should avoid the tongue and other areas because saliva can inhibit the growth of streptococci producing the Lancefield group A antigen (Carroll & Reimer 1996; Murray,

Rosenthal & Pfaller 2005). During the pharyngeal swabbing of dogs difficulties were experienced restraining dogs and therefore many swabs would have come into contact with the tongue and saliva. Whether canine saliva also inhibits the growth of Lancefield group A streptococci is unknown. The study conducted by Boon (2007) retrieved one group A streptococci from the skin of a dog. Therefore, the addition of canine skin swabs and careful pharyngeal swabbing could possibly have resulted in further isolations of streptococci producing the Lancefield group A antigen.

The morphological similarities and shared Lancefield group of *S. canis*, SDSE and SESZ made it difficult to distinguish between the species on blood agar and in the laboratory. Only one representative colony of each morphological type and of each Lancefield group was purified from the initial culture from a given swab. This was because it was assumed that repetitive purification would result in duplicates of the same strain. However, after 16S rRNA sequencing and MLST it became apparent that there may have been several species and strains in the one swab. Therefore, the numerical data regarding species and strain types may not be an overall true reflection but an under-estimation of the BHS flora in dogs of this community.

In retrospect, isolating several morphologically similar colonies from each culture plate may have provided a more accurate depiction of the BHS species and strains within this population.

3.6 Bacterial speciation and genetic characterisation

3.6.1 DNA extraction

Isolated bacterial colonies were inoculated on HBA at 37°C under anaerobic conditions overnight. Bacterial DNA was extracted using QIAamp DNA mini kit (QIAGEN Pty Ltd, V ictoria, Australia) by the researcher, according to the manufacturer's instructions and stored at a final concentration of ~0.5 µg/mL, at -20°C for further use.

3.6.2 PCR template reaction mixture for 16S rRNA, *emm* and MLST

PCR amplifications for 16S rRNA, *emm* and MLST were performed using the same reaction volume of 50 µL comprising of 49 µL of mastermix and 1 µL of bacterial DNA. Mastermix was prepared by the researcher using 5 µL of 10X PCR buffer with $MgCl_2$, 1 µL of forward and reverse primers (described in their corresponding chapters) (25 µM), 5 µL dNTPs to a final concentration of 0.2 mM and 0.1 µL Taq (5U/µL) then adding pyrogen free H^2O to 49 µL.

3.6.3 PCR cycle conditions

PCR amplifications for 16S rRNA, *emm* and MLST were performed using the same cycle conditions by Dr. Rebecca Towers of Menzies School of Health Research, Darwin, Australia. PCR amplification was performed on a Rotor-Gene 6000 Real-Time Rotary Analyser, version 1.7 (Corbett Life Science; QIAGEN Pty Ltd, Victoria, Australia) using the cycle parameters; 94°C for 1 min followed by 10 cycles of 94°C for 15s, 46.5°C for 30s, 72°C for 1 min 15s followed by 20 cycles of 94°C for 15s, 46.5°C for 30s, 72°C for 1 min 15s with 10s increment for each of the subsequent 19 cycles followed by 72°C for 10 mins then storage at 4°C, PCR products were stored at -20°C until use.

Amplified PCR products were purified with QIAquick PCR purification kit according to the manufacturer's instructions (QIAGEN Pty Ltd, Victoria, Australia). PCR products were visualised by gel electrophoresis using a 1% SYBR-Safe stained agarose gel and compared to known markers in order to determine and validate the expected product size.

3.6.4 Real-time PCR (qPCR) reaction mixture for detection of virulence genes

qPCR reactions were performed by the researcher in a reaction volume of 10 µL comprising of 2 µL of commercially available Platinum® SYBR® Green qPCR Super-Mix-UDG (Invitrogen, California, USA) with 1 µM each of forward and reverse

primers of the virulence genes described in chapter Five, 1 µL of a 1/10 dilution of template DNA and DNAse-free distilled water to a final reaction volume of 10 µL.

3.6.5 qPCR amplification and high resolution melt analysis for detection of virulence genes

qPCR amplification and high resolution melt (HRM) were performed by the researcher on the Rotor-Gene 6000 Real-Time Rotary Analyser, version 1.7 (Corbett Life Science) using the same cycling conditions for all primers. Cycling conditions were 1 min at 95°C, 40 cycles of 5s at 95°C, 5s at 55°C, 2 min at 72°C a further 2 min at 72°C for amplification and a further 30s at 50°C then ramp from 68-90°C rising by 0.1°C at each step and pausing for 2s prior to each temperature rise to perform the high resolution melt phase.

The sampling, culture, isolation and identification of streptococci used in this study have been described. The major aim of the entire thesis was to assess the carriage of streptococci in dogs and their association with streptococci typically infecting humans. However, members of the community expressed their concerns with dog health, stray dog numbers and issues of dog ownership. Therefore, a second component was included in the study aimed at improving responsible dog ownership. This adds value to the study as it highlights that community members have many suggestions and solutions to solving some of the broader issues relating to dog health in the Yarrabah community.

The next section discusses the use of qualitative methods used to document strategies to improve dog health and ownership and a participatory action research methodology used to implement these strategies within the Yarrabah community.

3.7 Qualitative methodology to document and implement knowledge translation strategies to improve dog health in the research community

As previously described, this component of the study was important in providing tangible outcomes for the Yarrabah community. The aim of this component was to document the issues of dog health and ownership perceived by Yarrabah community members and strategies for improvements, suggested by community members. These strategies were then developed and implemented through collaboration with the AMW. A qualitative methodology suited the aim of this component of the study because the aim was to understand the perceptions, expectations and ideas of community members for improvements in their current situation. Quantitative methods that use numbers and counting would not have provided this deeper insight. Furthermore, the use of this methodology also promotes relationships between the researcher and participants that are empowering, collaborative and participatory (Finlay 2007).

3.7.1 Development of interview questions and aims

The understanding of current issues and strategies was achieved by interviewing (or 'yarning') with community members. The interviews were first developed to focus on documenting the knowledge of zoonoses by members of the community and strategies to improve knowledge. However, as the interviews progressed it became clear that the needs of the community were not always concerned with knowledge of zoonoses but also about basic dog care and healthy dog ownership. In this study, the term healthy dog ownership relates to the ability and capacity of dog owners to care for their dogs.

Further discussions with the EHO and AMW resulted in the modification of the aim of the interviews to focus on community members perceptions of dog issues in the community that may be impeding the ability of dog owners to practice healthy dog ownership. Participants were also encouraged to suggest strategies

that would be successful in improving dog health and ownership in the Yarrabah community.

3.7.2 Interview technique

Semi-structured interview questions were initially designed and piloted on family members. These trials revealed that the use of direct questioning was not culturally appropriate because it often made both the researcher and participants uncomfortable. This is partly because it was an unfamiliar method of conversation between the researcher and participants who were often already known to each other. Therefore a yarning protocol was used to discuss dog issues in the community and strategies that would be successful to aid in the resolving of some of these issues.

"Yarning" is emerging as a legitimate and culturally appropriate method of gathering information during an interview process, especially in Indigenous communities (Bessarab & Ng'andu 2010; Fletcher et al. 2011). In my view, a 'yarn' differs from an interview in that there are no set questions and the subject matters are introduced in a deliberately open manner within the conversation between researcher and participant. In this study, participants often talked around subjects but given time returned to the subject. In addition, at times during the research it seemed that the responses were irrelevant but after analysis it was evident that many of these responses were in fact extremely relevant but differed from the researcher's preconceived conclusions. The yarning process not only achieves an outcome but also builds a relationship between participant and researcher. This method was used because in many of these interviews the participant already had a relationship with the researcher. Therefore, a western view of an 'interview', in which conversations are led by the researcher solely for the purpose of the research outcome, would have been culturally inappropriate and disrespectful.

3.7.3 Participatory action research for the implementation of strategies to improve healthy dog ownership

Previous dog health programs have focussed on capacity building for individuals within the community to take on a role of instituting and promoting dog care and welfare for their community. In this case, the Yarrabah community already employed an AMW who was being trained on a regular basis by Queensland Health. The EHO and AMW expressed their desire to build the capacity of dog owners to care for their own animals and therefore the results of these interviews were used to identify barriers inhibiting healthy dog ownership and to implement suggested strategies to build capacity of dog owners.

Once the interviews had been collated and thematically analysed (described further in the corresponding chapter), the results were presented to the EHO and AMW for the planning of strategies. A participatory action research (PAR) methodology was used for this component of the study. PAR is a qualitative methodology that has previously been accepted as an appropriate methodology by the Yarrabah community (Tsey et al. 2002; Mayo, Tsey & Empowerment Research Team 2009). PAR is a successful method in engaging with Aboriginal people because they are not viewed as "subjects" of a researcher's agenda but participants and collaborators in a research journey taken by both researcher and community (Hughes et al. 1994; Wadsworth 1998; Dickson 2000; Silka 2010). In addition, the outcomes of PAR are represented as actions, thereby minimising the time it takes for research to be translated into action (Hughes et al. 1994).

PAR methodology involves researching in cycles that begin by planning actions for change and moving to new actions through a cycle of reflection as shown in Figure 3.1 (Hughes et al. 1994; Wadsworth 1998).

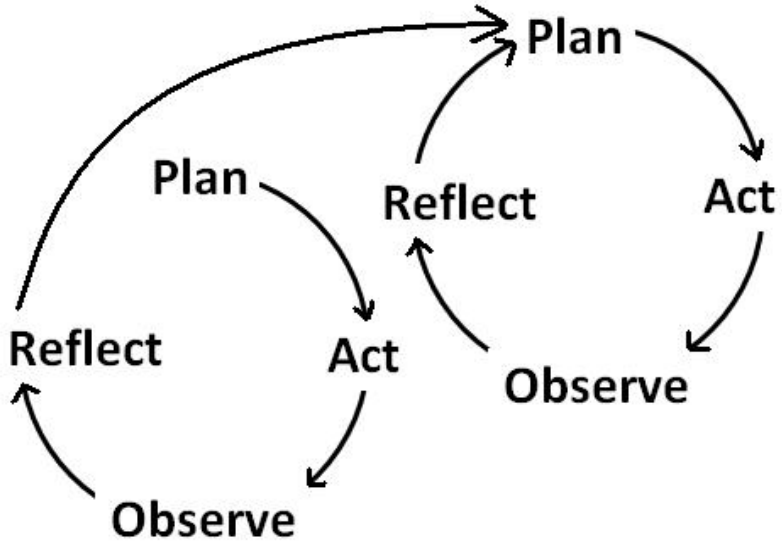

Figure 3.1. Cycles of participatory action research methodology used for this study

In this study, the researcher, EHO and AMW moved through this process together. Strategies suggested by community members were discussed and planned according to those that corresponded with the EHO and AMW's priorities. These chosen strategies were also implemented, observed and reflected on to evaluate the strengths and weaknesses of these actions in order to improve and plan new actions.

3.8 Feedback of findings to the Yarrabah community

The feedback of all research findings to the Yarrabah community was extremely important in the research process and facilitated to provide continued engagement with community organisations and members.

The participatory action research methodology provided a partnership with the researcher, EHO and AMW and therefore the EHO and AMW were constantly informed of the progress and findings throughout the entire study.

All organisations within the community are connected to the same email network therefore emails were a relevant method of reporting findings to many organisations and individuals within the community.

A short lay report was written at half yearly intervals and sent (via email) to the Yarrabah Shire Council. The report discussed current findings and future activities. A one page report of the final findings was emailed to all relevant community collaborators and letters sent to all participants involved in the study. A poster describing the research findings was also created and displayed at the community controlled health service, council office and the dog care days implemented during this study (Appendix 3).

The researcher and AMW also met with the CEO to discuss the final findings of the study and future recommendations.

3.9 Further issues faced and recommendations

Other researchers working in Indigenous communities have experienced the difficulties of time taken to engage with community whilst meeting academic deadlines (Bailie & Paradies 2005). This is because the time taken to build relationships and inform the community of the research project is often lengthy and further extended by other community priorities and disruptions. During this project the AMW was replaced by another community member and the community controlled health service underwent significant organisational and structural changes resulting in this study becoming low priority.

In retrospect, participation may have been increased by early collaborations with community organisations because it may have resulted in the community being better informed of the research project. A previous study documenting the prevalence of rheumatic fever in the community had a participation rate of 89% (Neilsen et al. 1993). This was attributed to the education program conducted prior to the research project, informing the entire community of the project through the use of a short film delivered by local Aboriginal health workers (Cedric 1986; Neilsen et al. 1993). Whilst, it would not have been feasible to develop a film for this research project, it highlights the importance of community engagement, involvement and ownership in successful research projects.

Chapter 4: β-haemolytic streptococci in dogs and children in an Aboriginal community.

4.1 Introduction

The previous chapter described the methods used to engage community in the research process and the collection of samples. Isolation and identification techniques used to speciate and genetically characterise the streptococci were also described.

This chapter describes the *Streptococcus* spp. that were isolated from dogs and children in the Yarrabah community. It also investigates some physiological and social parameters in dogs that may increase the likelihood of streptococcal carriage.

The impact of streptococcal disease in Aboriginal populations has been described previously. In summary, streptococcal disease associated with *S. pyogenes* infections are over-represented in Aboriginal populations, causing significant morbidity and mortality (Carapetis, Currie & Mathews 2000; Currie & Carapetis 2000; Norton et al. 2004; Whitehead, Smith & Nourse 2011).

In some Northern Territory Aboriginal communities where ARF and PSFN is hyper-endemic, throat carriage of SDSE exceeds that of *S. pyogenes* (McDonald, Currie & Carapetis 2004; McDonald et al. 2007).

In the wider community, SDSE (and infrequently *S. canis* and SESZ) have been implicated in a variety of diseases usually linked to *S. pyogenes*. These include; pyoderma, pharyngitis, bacteraemia/septicaemia, cellulitis, osteomyelitis, meningitis, toxic shock syndrome, necrotising fasciitis, PSGN and perhaps ARF (McCue 1982; Reid et al. 1985; Yuen et al. 1990; Haidan et al. 2000; Nicholson et al. 2000; Woo et al. 2001; Sylvetsky et al. 2002; Williams 2003; Korman et al. 2004;

Galperine et al. 2007; Brandt & Spellerberg 2009). Furthermore, population surveillance studies suggest that invasive disease caused by the Lancefield group G and C streptococci may be increasing and therefore research has focussed on the potential increasing pathogenicity of these species (Laupland et al. 2006; Broyles et al. 2009).

Dogs in Aboriginal communities have previously been found to be colonised with streptococcal bacteria identified by 16S rRNA sequencing as SDSE, *S. canis*, *S. equi* subsp. *equi* and *S. agalactiae* (Boon 2007). Therefore, dogs may be a source of SDSE to humans in this community. Furthermore, dogs living in close proximity to people with frequent *S. pyogenes* infections may also be facilitating HGT between *S. pyogenes* and other species of streptococci.

Therefore, the aim of this chapter was to investigate the carriage of BHS in dogs and their owners within an Aboriginal community with a history of epidemic disease outbreaks associated with streptococcal infection (Davis & Morahan 1993; Neilsen et al. 1993; Minaur et al. 2004). The cross-sectional survey also attempts to investigate risk factors that increase the likelihood of dogs to carry streptococci.

4.2 Materials and Methods

4.2.1 Study site

The Yarrabah Aboriginal Shire is situated approximately 40 km from Cairns separated by a mountain range as described in chapter 1. Streptococcal diseases are common in this community with documented epidemics of PSGN and ARF. The community also has a large population of stray and unwanted dogs.

4.2.2 Sample population

Sixty one dogs (57 community dogs and 4 wild dogs) and 6 humans, from 9 households were sampled to investigate BHS infection in dogs and humans within the community. Of the dogs sampled, 57 resided in the community and were

termed 'community dogs'. The sampling of community dogs was largely based on convenience, comprising of; 26 dogs found abandoned or held in the pound (either stray or unwanted) sampled prior to euthanizing, 3 dogs recruited from households 2 and 4 during a desexing program and 24 dogs from households 1, 3, 5, 6 and 7 recruited during visits to promote desexing or to treat dogs for mange. Four dogs from household 8 and 9 were sampled because one child from each of these houses had presented at the community controlled health service with sores and was treated by the doctor. Four wild dogs found outside the community were also included in the study.

Sample techniques and further data collected are described in detail in chapter 3. In short, pharyngeal swabs were taken from dogs and data on the general health status of dogs was recorded using the Purina body condition score (1-3: too thin; 4-5: ideal; 6-9: too heavy) and skin condition score (0-5; 0: no lesions, 5: complete hair loss) (see Appendix 2). Other data collected included sex, number of dogs in the house and age category.

A total of six humans were sampled for streptococcal carriage. Two children with visible pyoderma lesions were swabbed from households 1 and 6 (n=2). Pharyngeal and skin swabs were collected from two children from households 8 and 9 by the doctor (n=2) and adult dog owners from households 5 and 7 self-swabbed their nasal cavities to detect streptococci (n=2) (see chapter 3).

4.2.3 Species identification

Streptococci were cultured and identified using routine microbiological techniques; described in detail in chapter 3. All isolates were identified to the species level using both the API20 Strep identification system and sequencing of the 16S rRNA gene.

4.2.4 16Sr RNA gene amplification and sequencing

PCR amplification of the 16S rRNA gene was performed using the forward primer 27F and reverse primer 1492R described in Table 4.1. PCR cycle conditions and reaction mixture are described in chapter 3.

Sequencing of the PCR product was performed by Macrogen Inc. Korea using forward primer 518F and reverse primer 800R described in Table 4.1, resulting in the reading and sequencing of 1358bp of the 1465bp 16S rRNA product.

Table 4.1. Primers used for the amplification and sequencing of the 16S rRNA gene (Macrogen Inc. Korea).

Primer Name	Primer sequence	Amplification	Sequencing
27F	5' AGAGTTTGATCMTGGCTCAG 3'	X	
1492R	5' TACGGYTACCTTGTTACGACTT 3'	X	
518F	5' CCAGCAGCCGCGGTAATACG 3'		X
800R	5' TACCA GGT ATCTAATCC 3'		X

X= primer used for procedure

Finally, the sequences were compared with 16S rRNA sequences available in GenBank using BLASTn (Basic Alignment Search Tool). Sequences with 100% query coverage and ≥99% maximum identities to a previously submitted strain were designated the same species.

4.2.5 Submission of 16S rRNA sequences

All new 16S rRNA sequences were sent to GenBank for submission (Accession numbers JN176316-JN176355. Displayed in Appendix 4).

4.2.6 Statistical analysis

Graphs describing the demographics and streptococcal carriage of dogs were created in SPSS version 18 (PASW statistics 18). Ninety-five percent confidence limits were calculated using the Binomial Confidence online tool, provided by the Southwest Oncology Group (SWOG) (accessed at http://www.swogstat.org/stat/public/binomial_conf.htm).

Chi-squared contingency tables were performed to evaluate any associations between the sample characteristics and presence or absence of BHS. Significance for the Chi-squared analysis was indicated by P values <0.05. Exact chi-squared values were used but Fischer's exact test was implemented for cases with values less than 5. Missing values (or unknown values) were excluded from the tests.

4.2.7 Sequence alignment and phylogenetic tree

Sequences were aligned and trimmed using the ClustalW alignment, using default parameters (gap opening penalty: 15, gap extension penalty: 6.66, transition weight: 0.5) in MEGA (Molecular Evolutionary Genetic Analysis) Version 4 Software (Tamura et al. 2007). A minimum evolution tree was constructed (bootstrap values of 500 replicates) using Mega4. The 16S rRNA sequences of reference strains from GenBank included in the phylogenetic tree are; *Streptococcus canis* (strain ATCC43496, accession number: NR_024633), *Streptococcus pyogenes* (strain ATCC 12344, accession number: NR_028598), *Streptococcus dysgalactiae* subsp. *equisimilis* (strain CIP105120, accession number: NR_043661), *Streptococcus dysgalactiae* subsp. *dysgalactiae* (strain ATCC43078, accession number: NR_027517), *Streptococcus agalactiae* (strain ATCC 13813 accession number: NR_040821), *Streptococcus equi* subsp. *zooepidemicus* (strain ATCC 43079 accession number: NR_036758).

Pair-wise sequence alignment was performed using ClustalW2 provided by the European Bioinformatics Institute (2012) server.

4.2.8 Ethical approval

Ethical approval and participant recruitment and consent are described in detail in chapter 3.

4.3 Results

4.3.1 Prevalence of BHS in sampled populations

The number of dogs and humans in which one or more BHS were isolated are shown in Table 4.2. BHS were isolated from 29 of the 57 community dogs (50.8%; 95% CI= 64.4, 37.3), 2 out of 4 dogs wild dogs (50.0%; 95%CI= 6.8, 93.2) and 4 out of 6 humans (66.7%; 95% CI= 22.2, 95.7).

Three of the four households with a BHS positive child also had at least one BHS positive dog.

Table 4.2. Number of dogs and humans carrying β-haemolytic streptococci.

Location	Number of Positive dogs	Number of dogs examined	Number of positive humans	Number of humans examined
Household 1	2	6	1	1
Household 2	2	2	0	0
Household 3	0	1	0	0
Household 4	1	1	0	0
Household 5	0	1	0	1[a]
Household 6	1	2	1	1
Household 7	9	14	0	1[a]
Household 8	1	2	1	1
Household 9	0	2	1	1
Pound/abandoned	13	26	-	-
Wild dogs	2	4	-	-
Total	31	61	4	6

[a] Adult nasal swab.

A total of 41 BHS isolates were recovered and tested from the total sample population (humans and dogs). A single streptococcal strain was recovered from 27 dogs but 4 community dogs had a mixed streptococcal infection with two strains of streptococci recovered from each dog. Two BHS strains were recovered from two of the 4 children tested; the other two had a single *Streptococcus* strain recovered. Therefore a total of 35 isolates of BHS were retrieved from dogs (33 isolates from community dogs and 2 isolates from wild dogs) and 6 isolates were retrieved from children.

4.3.2 Physiological and social parameters of community dogs sampled

Some physiological and social parameters of community dogs were documented to identify risk factors associated with a dog's likelihood of carrying BHS. The characteristics of community dogs (n=57) sampled in the study are represented graphically in Figure 4.1.

The majority of community dogs sampled in the study were in good condition with an ideal body condition score of 5 (Figure 4.1A) and skin clear of sores and mange-like signs (Figure 4.1B). In addition, dogs from all age categories and genders were represented (Figure 4.1C & D). However, a large number of puppies (n=10) were included without noting their sex (Figure 4.1D).

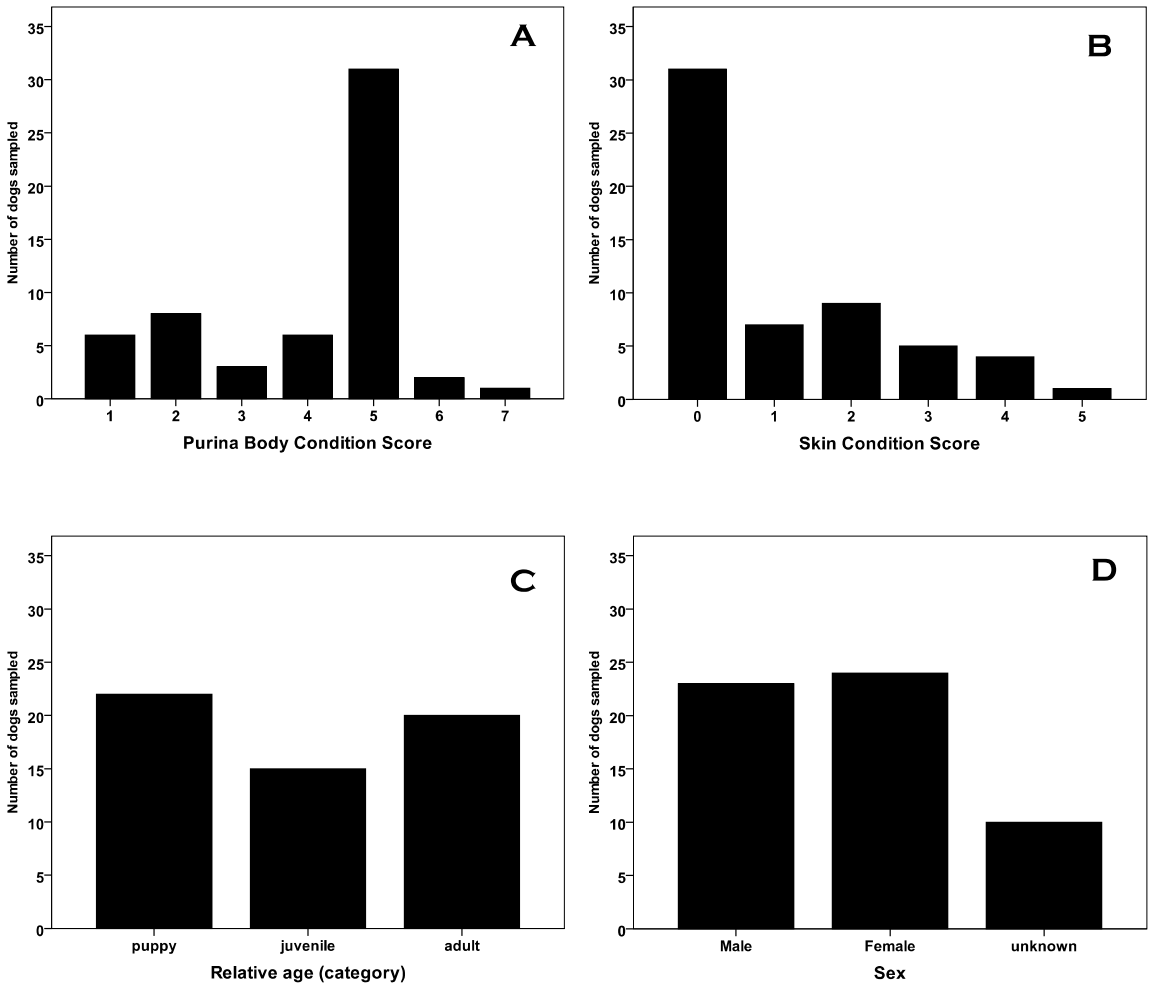

Figure 4.1. Characteristics of community dogs in this study (n=57): (A) Purina body condition score, (B) Skin condition score, (C) Relative age (category), (D) Sex.

4.3.3 Relationship between streptococcal carriage and some physiological and social parameters of community dogs.

The statistical results from a Chi-squared analysis investigating the relationship between the presence of BHS and some physiological and social parameters to investigate risk factors are described in Table 4.3. Using the data from this study it appears that puppies, male dogs and dogs from houses with a dog population greater than 10, have a higher prevalence of BHS. However, the sample size was too small to detect any significant differences.

Body or skin condition did not significantly influence whether a dog was BHS positive or negative. In fact, it appeared that dogs with an ideal body condition were found more frequently with BHS but this association was not statistically significant.

Table 4.3. Bivariate analysis of β-haemolytic streptococcal carriage, dog health and social parameters.

Characteristics	Total (n=57)	BHS positive (n=29)	p-value
Dogs' age			P=0.563
Puppy	22 (38.6%)	13 (59.1%)	
Juvenile	15 (26.3%)	6 (40.0%)	
Adult	20 (35.1%)	10 (50.0%)	
Dogs' sex			P=0.147
Male	23 (48.9%)	13 (56.5%)	
Female	24 (51.1%)	8 (33.3%)	
*Unknown	10		
Number of dogs in household			P=0.457
1-2	7 (17.9%)	2 (28.6%)	
3-4	2 (5.1%)	1 (50.0%)	
5-9	16 (41.0%)	7 (43.8%)	
10 or more	14 (35.9%)	9 (64.3%)	
Body condition			P=0.743
Too thin (1-3)	17 (29.8%)	8 (47.1%)	
Ideal (4-5)	37 (64.9%)	20 (54.1%)	
Too heavy (6-9)	3 (5.3%)	1 (33.3%)	
Skin condition/ mange			P=0.792
No (score 0)	31 (54.4%)	15 (48.4%)	
Yes (score >=1)	26 (45.6%)	14 (53.8%)	

*missing values not included in statistical testing

4.3.4 Efficacy of the API20 Strep system in identification of BHS

Of the 41 isolates retrieved in the study, 6 had a slight catalase positive reaction, further investigation of Lancefield group and species identification revealed that they were in fact streptococci. No further investigations into whether these isolates were truly catalase positive were conducted but it is likely that the catalase reaction was a result of growth on blood agar. The API20 Strep system was sufficient in identifying 22 out of the 41 isolates to a 99.9% species level. Fifteen of these isolates were identified as *S. canis*, 4 isolates were identified as *S. pyogenes* (only isolated from humans), 2 isolates as *S. agalactiae* and one as SESZ. The remaining 19 isolates could not be identified to a 99.9% species level using the API20 Strep system. The biochemical results and corresponding species designation using 16S rRNA sequencing are described later and shown in Table 4.4. Five isolates were suspected *S. canis*; 13 as SDSE and 1 as a "group L streptococci". According to Facklam's biochemical characteristics of *S. canis* and SDSE (see chapter 2) neither species hydrolyses hippurate, both species hydrolyse esculin and SDSE ferments trehalose, whilst not all *S.canis* strains ferment trehalose. These isolates differ from typical *S. canis* and SDSE in that 3 hydrolysed hippurate, none of them hydrolysed esculin, and all isolates had variable abilities to ferment trehalose. Furthermore, the API20 Strep differentiates the "group L streptococci" from other SDSE, although this is now included in the one SDSE subspecies (Viera et al 1998).

Therefore, it is evident that the API20 Strep system failed to identify all SDSE of both human and canine origin and some strains of *S. canis*.

4.3.5 Species identification using 16S rRNA sequencing

Because of the biochemical variability of the BHS, all isolates underwent 16S rRNA sequencing for species identification. According to 16S rRNA sequencing, the BHS recovered belong to one of five streptococci taxa, namely *Streptococcus canis*, *Streptococcus pyogenes* (found only from human samples), *Streptococcus dysgalactiae* (SD) with some strains further identified to SDSE, *Streptococcus agalactiae* and SESZ.

The phylogenetic relationships of these isolates are illustrated in Figure 4.2. This tree also includes reference sequences curated by GenBank to facilitate a more extensive taxonomic comparison of the isolates obtained. It is clear that while the isolates separate into species they also separate into sub-groups.

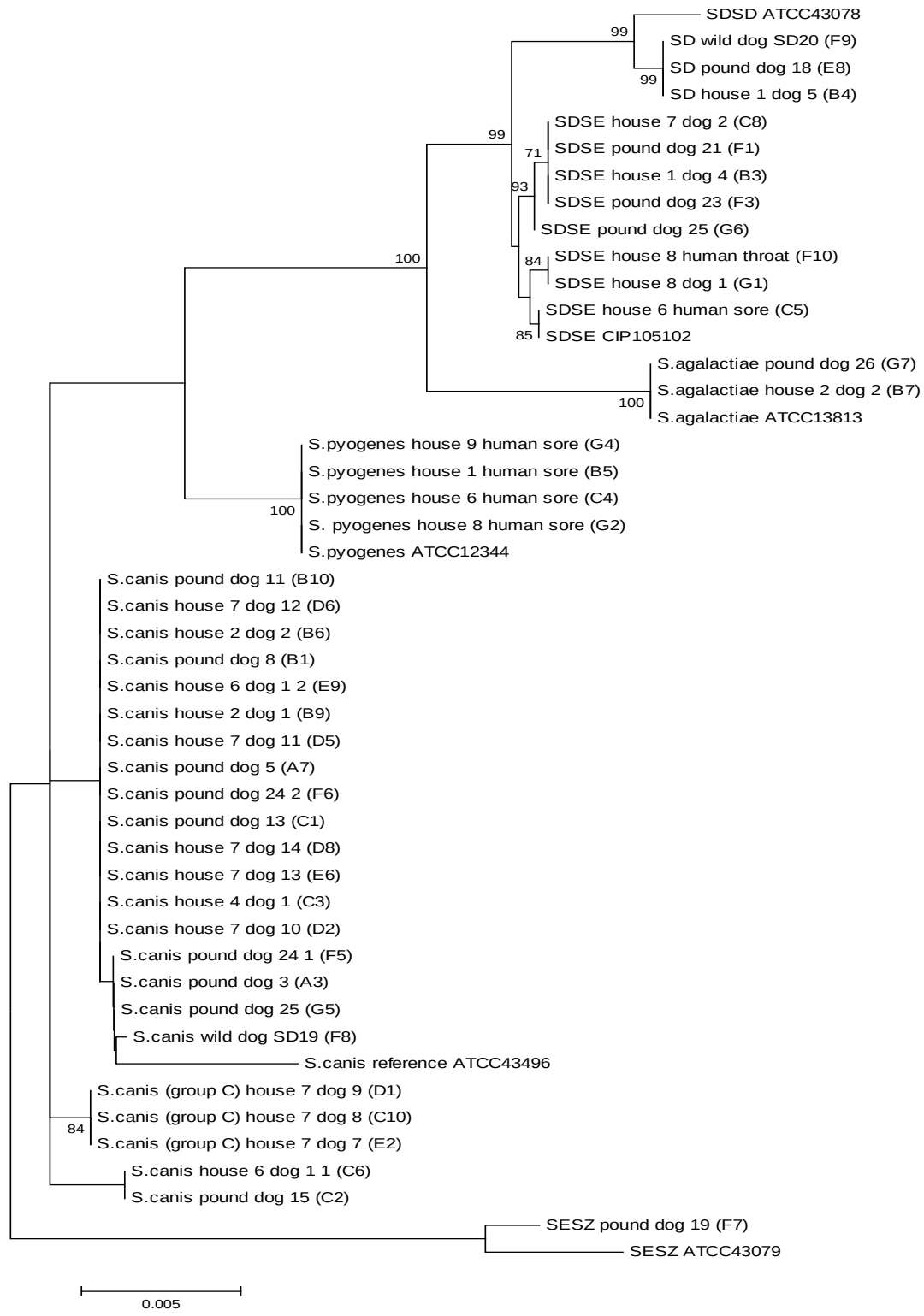

Figure 4.2. Minimum evolutionary tree of 16Sr RNA of BHS isolated from dogs and children from this study with selected GenBank reference sequences. The phylogenetic tree was linearized assuming equal evolutionary rates in all lineages. The tree is drawn to scale with, branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Bootstrap values less than 70% are not shown. Location and dog number are described and code in parenthesis represents the isolate identification number.

All isolates identified to a 99.9% species level using the API20 Strep system were confirmed as those same species by 16S rRNA sequencing.

The 19 isolates that had biochemical characteristics which failed to clearly identify them using the API20 Strep system were speciated further using 16S rRNA sequencing (Table 4.4). Eight isolates were identified as *S. canis*; five of these isolates were Lancefield group G, were negative for β-glucoronidase and clustered in the phylogenetic tree with other *S. canis* strains identified to 99.9% using the API20 Strep system (Table 4.4 & Figure 4.2), therefore confirming their classification as *S. canis*. All five isolates had differing biochemical properties, including those that clustered together in the 16S rRNA tree (Table 4.4 & Figure 4.2).

Three *S. canis* isolates were found to produce the Lancefield group C antigen. The strains (C10, D1 and E2) were found in 3 puppies belonging to one household (Household 007). The API20 Strep could not identify the strains because their biochemical properties differed from typical *S. canis* in that they hydrolysed hippurate, were positive for the enzyme β-glucoronidase (a property usually used to differentiate SDSE from *S. canis*) (Facklam 2002), and fermented lactose (Table 4.4). The 16S rRNA sequence was identical for all three isolates and the BLAST had query coverage of 100% and 99% maximum identity with the GenBank 16S rRNA sequence of an *S. canis* isolated from a human ear infection (Whatmore et al. 2001) (accession number: AJ413205.1; see Appendix 5 for full sequence alignment). The isolates also clustered with the other *S. canis* isolates and were clearly diverged from the other species therefore verifying their classification as *S. canis* (Figure 4.2).

Another 8 isolates were identified as SDSE; these also included a human Lancefield group G and human Lancefield group C isolate. All isolates fermented trehalose but not sorbitol and clustered in a separate cluster to the SDSD reference sequence in the phylogenetic tree (Table 4.4 & Figure 4.2). Although the isolates were all identified as SDSE the 16S rRNA tree shows that there are sub-clusters which may represent different strains. The human group G isolate clustered with the reference sequence but was diverged from the other SDSE isolated from dogs.

However, isolates F10 and G1 clustered together; these isolates were retrieved from a child and dog within the same household. The strains appear to be homologous; clustering together in the 16S rRNA tree (Figure 4.2), with an identical biochemical profile (Table 4.4), and identical 16S rRNA sequence (Figure 4.3). In contrast to *S. canis*, strains of SDSE appeared to have highly similar biochemical properties if they clustered together in the 16S rRNA tree (Table 4.4 & Figure 4.2).

Table 4.4. Biochemical profile of 19 isolates of BHS using the API20 Strep system and corresponding 16S rRNA identification.

ID	Group[a]	Origin[b]	VP	HIP	ESC	PYRA	αGAL	βGUR	βGAL	PAL	LAP	ADH	RIB	ARA	MAN	SOR	LAC	TRE	INU	RAF	AMD	GLYG	API[c]	%ID[d]
***S. canis*[e]**																								
E6	G	Dog	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	+	-	canis	67.6
E9	G	Dog	-	-	-	-	+	-	-	+	+	+	+	-	+	-	+	+	+	-	+	-	canis	0
F5	G	Dog	-	-	-	-	-	-	+	+	+	+	+	-	-	-	+	+	+	-	+	-	canis	0
F6	G	Dog	-	-	-	-	+	-	-	+	+	+	+	-	-	-	-	-	-	-	+	+	canis	81.6
G5	G	Dog	-	-	-	-	+	-	-	+	+	+	+	-	-	-	+	+	-	-	+	-	canis	97.5
C10	C	Dog	-	+	-	-	-	+	-	+	+	+	+	-	-	-	+	-	-	-	+	-	SDSE	74.5
D1	C	Dog	-	+	-	-	-	+	-	+	+	+	+	-	-	-	+	-	-	-	+	-	SDSE	74.5
E2	C	Dog	-	+	-	-	-	+	-	+	+	+	+	-	-	-	+	-	-	-	+	-	grpL	49.8
SDSE[e]																								
B3	C	Dog	-	-	-	-	-	+	-	+	+	+	+	-	-	-	-	+	-	-	+	+	SDSE	68.4
C8	C	Dog	-	-	-	-	+	+	-	+	+	+	+	-	-	-	-	+	-	-	+	+	SDSE	95.7
F1	C	Dog	-	-	-	-	-	+	-	+	+	+	+	-	-	-	-	+	-	-	+	+	SDSE	68.4
F3	C	Dog	-	-	-	-	-	+	-	+	+	+	+	-	-	-	-	+	-	-	+	+	SDSE	68.4
G6	C	Dog	-	-	-	-	-	+	-	+	+	+	+	-	-	-	-	+	-	-	+	+	SDSE	68.4
G1	C	Dog	-	-	-	-	-	-	-	+	+	+	+	-	-	-	+	+	-	-	+	-	SDSE	91.9
F10	C	Hum	-	-	-	-	-	-	-	+	+	+	+	-	-	-	+	+	-	-	+	-	SDSE	91.9
C5	G	Hum	-	-	-	-	-	+	-	+	+	+	+	-	-	-	-	+	-	-	+	-	SDSE	98.5
SD[e]																								
F9	A/G	WDog	-	-	-	-	-	+	-	+	+	+	+	-	-	-	-	+	-	-	+	+	SDSE	68.4
B4	C	Dog	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	+	+	SDSE	75.1
E8	C	Dog	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	+	+	SDSE	75.1

[a] Lancefield group antigen; [b] origin: Dog: community dogs, WDog: wild dog, Hum: human; [c] Species/subspecies according to API20 Strep: canis= *S. canis*, SDSE: *S. dysgalactiae* subsp. *equisimilis*; [d] Identification percentage according to API20 Strep;[e] Species/subspecies according to 16S rRNA sequence. Abbreviations: VP, Voges Proskauer; HIP, hippurate hydrolysis; ESC, esculin hydrolysis; PYRA, pyrolidonylarylamidase; αGAL, α galactosidase; βGUR, β glucoronidase; βGAL, β galactosidase; PAL, alkaline phosphatise; LAP, Leucine aminopeptidase; ADH, arginine dihydrolase; acidification of: RIB, ribose; ARA, arabinose; MAN, mannitol; SOR, sorbitol; LAC, lactose; TRE, trehalose; INU, inulin; RAF, raffinose; AMD, amidon and GLYG, glycogen.

```
Child           TGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTAGAACGCTGAGGACTGG 60
Dog             TGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTAGAACGCTGAGGACTGG 60
                ************************************************************

Child           TGCTTGCACCGGTCCAAGGAGTTGCGAACGGGTGAGTAACGCGTAGGTAACCTACCTCAT 120
Dog             TGCTTGCACCGGTCCAAGGAGTTGCGAACGGGTGAGTAACGCGTAGGTAACCTACCTCAT 120
                ************************************************************

Child           AGCGGGGGATAACTATTGGAAACGATAGCTAATACCGCATAAAAGTGTTTAACCCATGTT 180
Dog             AGCGGGGGATAACTATTGGAAACGATAGCTAATACCGCATAAAAGTGTTTAACCCATGTT 180
                ************************************************************

Child           AAACATTTAAAAGGTGCAATTGCATCACTATGAGATGGACCTGCGTTGTATTAGCTAGTT 240
Dog             AAACATTTAAAAGGTGCAATTGCATCACTATGAGATGGACCTGCGTTGTATTAGCTAGTT 240
                ************************************************************

Child           GGTGAGGTAACGGCTCACCAAGGCGACGATACATAGCCGACCTGAGAGGGTGATCGGCCA 300
Dog             GGTGAGGTAACGGCTCACCAAGGCGACGATACATAGCCGACCTGAGAGGGTGATCGGCCA 300
                ************************************************************

Child           CACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCA 360
Dog             CACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCA 360
                ************************************************************

Child           ATGGACGGAAGTCTGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAG 420
Dog             ATGGACGGAAGTCTGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAG 420
                ************************************************************

Child           CTCTGTTGTTAGAGAAGAATGATGGTGGGAGTGGAAAATCCACCATGTGACGGTAACTAA 480
Dog             CTCTGTTGTTAGAGAAGAATGATGGTGGGAGTGGAAAATCCACCATGTGACGGTAACTAA 480
                ************************************************************

Child           CCAGAAAGGGACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTCCCGAGCGTT 540
Dog             CCAGAAAGGGACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTCCCGAGCGTT 540
                ************************************************************

Child           GTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTCTTTAAGTCTGAAGTTAAAGGC 600
Dog             GTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTCTTTAAGTCTGAAGTTAAAGGC 600
                ************************************************************

Child           AGTGGCTCAACCATTGTACGCTTTGGAAACTGGAGAACTTGAGTGCAGAAGGGGAGAGTG 660
Dog             AGTGGCTCAACCATTGTACGCTTTGGAAACTGGAGAACTTGAGTGCAGAAGGGGAGAGTG 660
                ************************************************************

Child           GAATTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAACACCGGTGGCGAAAGCG 720
Dog             GAATTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAACACCGGTGGCGAAAGCG 720
                ************************************************************

Child           GCTCTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGAT 780
Dog             GCTCTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGAT 780
                ************************************************************

Child           ACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAGGTGTTAGGCCCTTTCCGGGGCTTA 840
Dog             ACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAGGTGTTAGGCCCTTTCCGGGGCTTA 840
                ************************************************************

Child           GTGCCGGAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTC 900
Dog             GTGCCGGAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTC 900
                ************************************************************

Child           AAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGC 960
Dog             AAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGC 960
                ************************************************************

Child           GAAGAACCTTACCAGGTCTTGACATCCTCCTGACCGGTCTAGAGATAGGCTTTCCCTTCG 1020
Dog             GAAGAACCTTACCAGGTCTTGACATCCTCCTGACCGGTCTAGAGATAGGCTTTCCCTTCG 1020
                ************************************************************

Child           GGGCAGGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTA 1080
Dog             GGGCAGGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTA 1080
                ************************************************************

Child           AGTCCCGCAACGAGCGCAACCCCTATTGTTAGTTGCCATCATTAAGTTGGGCACTCTAGC 1140
Dog             AGTCCCGCAACGAGCGCAACCCCTATTGTTAGTTGCCATCATTAAGTTGGGCACTCTAGC 1140
                ************************************************************

Child           GAGACTGCCGGTAATAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTA 1200
Dog             GAGACTGCCGGTAATAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTA 1200
                ************************************************************

Child           TGACCTGGGCTACACACGTGCTACAATGGTTGGTACAACGAGTCGCAAGCCGGTGACGGC 1260
Dog             TGACCTGGGCTACACACGTGCTACAATGGTTGGTACAACGAGTCGCAAGCCGGTGACGGC 1260
                ************************************************************

Child           AAGCTAATCTCTCAAAGCCAATCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAA 1320
Dog             AAGCTAATCTCTCAAAGCCAATCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAA 1320
                ************************************************************

Child           GTCGGAATCGCTAGTAATCGCGGATCAGCACGCCGCGG 1358
Dog             GTCGGAATCGCTAGTAATCGCGGATCAGCACGCCGCGG 1358
                ***************************************
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Figure 4.3. Sequence alignment of 16Sr RNA of SDSE isolated from child and dog from the same household. (*) along the bottom line denotes identical nucleotides.

Three isolates (F9, B4 and E8) could not be identified to a 99.9% species level using the API20 Strep. Using 16S rRNA sequencing these isolates had 100% query coverage and 99% maximum identity with both SDSD and SDSE reference strains but appeared to be related to the SDSD reference sequence in the 16S rRNA phylogenetic tree (Figure 4.2).

Isolate F9 agglutinated both the Lancefield group A and G antigens despite being subcultured and re-tested. This isolate also differed from B4 and E8 in that it was positive for β-glucoronidase and fermented trehalose (Table 4.4). All 3 isolates appeared to be SDSE based on their β-haemolysis and ability to ferment glycogen (Viera et al. 1998; Facklam 2002). However, given their apparent sequence homology to the SDSD reference strain and uncertainty of the classification of this subspecies they were classified as *S. dysgalactiae* (SD) until further characterisation could be performed (Figure 4.2).

4.3.6 *Streptococcus* spp. found in study populations

In the community dogs BHS recovered were *S. canis*, SD/SDSE, *S. agalactiae* and SESZ (Figure 4.4). Twenty two isolates of *S. canis* were retrieved from 20 of the 57 community dogs (35.1%; 95%CI= 22.9, 48.9); 6 isolates of SDSE (excluding the 2 SD isolates) were isolated from 6 community dogs (10.5%; 95% CI= 3.9, 21.5); 2 isolates of *S. agalactiae* were retrieved from 2 community dogs (3.5%; 95% Cl= 0.43, 12.1); and a single isolate of SESZ was retrieved from 1 community dog (1.7%; 95% Cl= 0.04, 9.3).

Dogs from all health ranges (emaciated to ideal and no skin lesions to complete hair loss) were found with SD/SDSE but no puppies were found with SDSE.

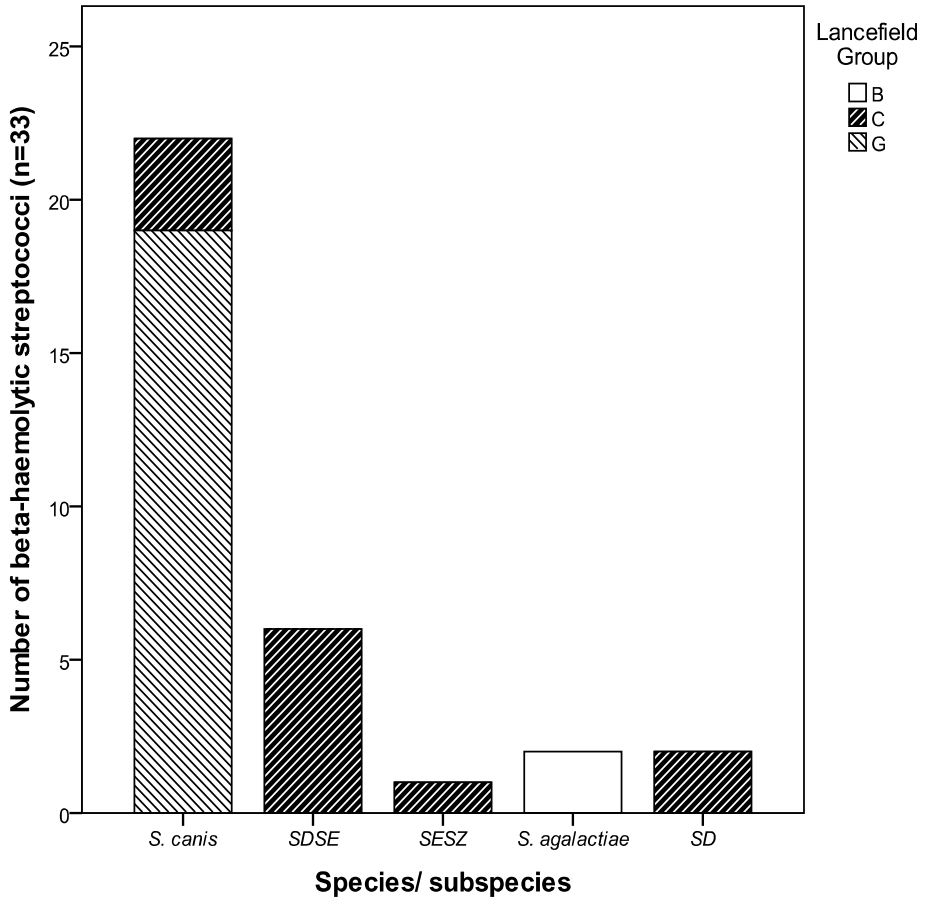

Figure 4.4. Species/subspecies and corresponding Lancefield group of β-haemolytic streptococci recovered from community dogs.

The BHS retrieved from the wild dogs sample were *S. canis* (Lancefield group G) and the atypical SD previously described which agglutinated both Lancefield group A and G antigens (Table 4.5).

Table 4.5. Characteristics of wild dogs sampled for the presence of streptococci.

Dog ID	Sex	Age	Skin Condition	PBS[a]	Distance from community (Km)[b]	*Streptococcus* spp. (group antigen)[c]	Isolate ID
TD01	Male	5-6 yrs	No mange	5	44.7	None found	-
TD03	Male	2 yrs	No mange	4	15.7	None found	-
SD19	Female	12 wks	No mange	4	34.7	*S.canis* (G)	F8
SD20	Male	16 wks	Demodex	3	15.7	SD (A/G)	F9

[a] PBS: Purina Body Condition Score. [b]Distance of wild dog location by road, from Aboriginal community researched according to Google maps. [c]*S.canis*; *Streptococcus canis*, SD; *Streptococcus dysgalactiae.*

In humans the BHS retrieved were found to be *S. pyogenes* and SDSE; all sores sampled were found to contain *S. pyogenes*, with 1 skin sore containing a mixed infection of *S. pyogenes* and SDSE. The SDSE recovered in this case produced a Lancefield group G antigen (Table 4.6). The Lancefield group C SDSE retrieved from the throat appeared to be of no clinical relevance. This isolate (F10) had an identical biochemical profile and 16S rRNA sequence as a group C SDSE retrieved from a dog within the same household, as previously described (isolate G1) (Table 4.4).

Table 4.6. Species and location of BHS retrieved from Aboriginal children in the study site.

Child ID	Household	Location of swab	Species (Group antigen)	Isolate ID
001-1H	001	Skin sore	*S. pyogenes* (A)	B5
006-1H	006	Skin sore	*S. pyogenes* (A)	C4
			SDSE (G)	C5
008-1H	008	Skin sore	*S. pyogenes* (A)	G2
		Throat	SDSE (C)	F10
009-1H	009	Skin sore	*S. pyogenes* (A)	G4

4.4 Discussion

This study has found that dogs in this community carried a variety of *Streptococcus* spp. some of which can sometimes infect humans (*S. canis*, SDSE, *S. agalactiae* and SESZ). No risk factors were identified that increased the likelihood of isolating BHS from dogs and therefore it is unknown whether increasing the health of dogs in this community would inhibit the carriage of streptococci. However, even without any statistical significance, some tendencies can be observed and given a larger study size results may have been significant. For example, it appeared that puppies, male dogs, dogs from houses with greater than 10 dogs and dogs with skin sores or mange-like signs had a greater percentage of BHS positive dogs. On the other hand, associations may not have been identified because bacterial load was not investigated, only the presence or absence of BHS.

In this community it was found that a little over 10% of dogs were found with species identified by 16S rRNA sequencing as SDSE. The finding of a group C SDSE strain with identical 16S rRNA sequence and biochemical profile in both a child and dog from the same household suggests that some strains are zoonotic and can be shared between dog and human hosts.

The 16S rRNA sequence tree and biochemical properties of the SD subspecies suggest that there are strains that differ both biochemically and genetically from other strains. Further characterisation could provide evidence as to whether these strains are diverged based on host specificity and/or zoonotic ability.

Three β-haemolytic SD isolates found in the dogs in this study clustered with the SDSD reference strain according to 16S rRNA sequencing and therefore could not be identified to the subspecies level. Previous studies have found that 16S rRNA sequencing could not separate strains of SDSE from SDSD and other recently diverged species (Fox, Wisotzkey & Jurtshuk 1992; Kawata et al. 2003). Multilocus sequence analysis (using a different seven housekeeping genes from MLST) of SDSE from humans and SDSD from strains of animal origin have shown that SDSD and SDSE

separated into clusters which further diverged into sub-clusters. The BHS of animal origin that were assumed to be SDSE (based on β-haemolysis) actually clustered with the other β-haemolytic SDSD. This finding resulted in the conclusion that all BHS isolated from animals originally believed to be SDSE may in fact be SDSD, and that SDSE is probably a human specific organism (Jensen & Kilian 2012). Further characterisation of our dubious strains classified as SD should provide further information as to whether they are actually SDSD, or that they are in fact, SDSE strains that are able to transmit between animal and human host species.

One of these SD isolates (F9) produced a Lancefield group antigen that reacted with both group A and G antibodies. Lancefield antigen variation (or 'cross-reactivity') in which a single strain of faecal streptococci (now known as *Enterococcus faecalis*) producing both the D and G Lancefield antigens has been previously documented (Birch, Keaney & Ganguli 1984). The Lancefield antigen variation seen with this *E. faecalis* strain is believed to have occurred because the substance that reacts with the group D antigen (a glycerol teichoic acid) is a different substance to that which reacts with the group G antisera (a cell wall carbohydrate). Therefore the presence of both substances in a cell would produce a reaction with both groups (Lancefield 1933; Jones & Shattock 1960). There have been no documented cases of streptococci that produce both the A and G antigen reported in the literature.

The cell wall of group G streptococci resembles that of the group A and C streptococci in that they all have a mucopeptide matrix of N-acetyl-glucosamine, N-acetyl muramic acid and four amino acids (McCarty 1956; Araujo & Krause 1963; Curtis & Krause 1964b). However, the group specific carbohydrates differ. The Lancefield group G cell wall carbohydrate consists of L-rhamnose terminal to N-acetyl-galactosamine and galactose. The antigenic determinant of the group G carbohydrate is the L-rhamnose (Curtis & Krause 1964b). In contrast, the group A streptococci carbohydrate contains N-acetyl-glucosamine residue terminal to a rhamnose moiety, with the antigenic component being N-acetyl-glucosamine (McCarty 1956). Furthermore, the group C cell wall carbohydrate consists of N-acetyl-galactosamine terminal to a rhamnose moiety, in which the antigenic determinant is the N-acetyl-

galactosamine (Araujo & Krause 1963). The L-rhamnose of group G is different to the rhamnose moiety of group A and C in that it is terminal to the other sugars in the carbohydrate and was not found to cross react with other groups experimentally (Curtis & Krause 1964b). In contrast, the group B streptococci carbohydrate have a similar rhamnose substance to the group G carbohydrate in their cell wall which has previously resulted in cross-reactions between group B and G streptococci (Curtis & Krause 1964a).

The group A/G SD found in this study may be somehow incorporating both the L-rhamnose terminal and an N-acetyl-glucosamine (instead of an N-acetyl-galactosamine) in the cell wall carbohydrate. Further investigations into the cell wall properties of this strain are required. Experimental passaging of group A streptococci through mice have resulted in a loss of reactivity of the group A antigen in some strains. These A-variant strains differ in cell wall carbohydrate components in that the rhamnose occurs at a proportion higher than the N-acetyl-glucosamine; therefore, no antigen reaction occurs (McCarty & Lancefield 1955). The reason changes in the cell wall component associated with laboratory passaging of streptococci through animals is not known but are believed to be a consequence of a rare mutational event with selection in the animal tissues (McCarty & Lancefield 1955). It is conceivable that, if some strains are able to pass through various host species, it may result in changes in the cell wall carbohydrate through mutation and possible swapping of relevant genes via HGT to enable strain adaptation to suit particular host(s) in a population. Therefore, the possible adoption of an N-acetyl-glucosamine within this SD strain may function to enhance the strains fitness in a particular host species, or may be a simple reflection of a heterogeneous SD population which is evolving and adaptable to human and canine hosts.

The finding of three atypical *S. canis* isolates producing the Lancefield group C antigen is novel and has not been documented elsewhere. However, other atypical species of SDSE which produced a group A antigen have been found in human infections (Brandt et al. 1999; Tanaka et al. 2008). The antigenic determinant of the group C streptococci as previously mentioned is the N-acetyl-galactosamine residue

found in the cell wall carbohydrate. As previously mentioned, the group G carbohydrate also contains this terminal amino sugar residue but the antigenic determinant for group G is the L-rhamnose. It is possible that the group C antigen production in these strains may be a result of chemical changes in which the carbohydrate components may have altered resulting in the N-acetyl-galactosamine (group C determinant) being expressed at a larger proportion than the L-rhamnose (group G determinant), thereby resulting in a group C antigenic reaction. Like the A/G variant, this change in carbohydrate components may enhance the strains fitness and adaptability to hosts and could possibly have been facilitated through HGT from other group C streptococci shared in the canine host, including SDSE isolates seen in man.

The heterogeneity of Lancefield groups amongst strains of streptococcal species in this study further demonstrates that Lancefield grouping should not be used as the sole identification technique for streptococcal species (Facklam 2002). In fact, many of the BHS isolated from dogs within this sample varied biochemically and serologically resulting in the inability to make valid identifications based on Lancefield grouping or using the API20 Strep system further reinforcing the need to utilise sequence identification techniques.

The vast majority of BHS recovered from dogs in this study were species that produce the Lancefield group C or G antigen (excluding the novel Lancefield group A/G SD variant). Many species of BHS that produce group C and/or G antigens (such as *S. canis*, SDSE, SESZ and SDSD) are considered normal flora of the mucous membranes of animals and are therefore considered opportunistic pathogens that rarely cause disease in humans or animals (Efstratiou 1997; Brandt & Spellerberg 2009). Although extensive clinical examinations of dogs in this study were not performed, none of these streptococci isolated from dogs appeared to be causing obvious illness at the time of sampling. However, some dogs were severely emaciated and covered in skin lesions and therefore without a proper clinical examination no assumptions can be made as to the pathogenesis of streptococci recovered in this population of dogs.

In dogs *S. canis,* although considered to be commensal, has been associated with abortion, polyarthritis, neonatal septicaemia, streptococcal toxic shock syndrome and necrotising fasciitis, with strains found to possess *S. pyogenes* virulence genes encoding the M-protein and streptolysin O (DeWinter, Low & Prescott 1999; Fulde et al. 2011). In humans, strains of *S. canis* have been implicated in a variety of diseases ranging from cellulitis, bacteraemia, septicaemia, urinary tract disease, osteomyelitis and pneumonia (Bert & Lambert-Zechovsky 1997; Takeda et al. 2001; Whatmore et al. 2001; Galperine et al. 2007; Lam et al. 2007). Recent epidemiological studies have shown that *S. canis* was associated with approximately 1% of human hospitalised streptococcal infections in France. However, the source of the infections could not always be traced back to an animal and most people infected had underlying medical conditions (Galperine et al. 2007). In the 16S rRNA phylogenetic tree, the *S. canis* strains diverge into separate clusters. Whether some of these clusters represent strains that are more prone to cause disease in humans and others in dogs needs to be investigated. The significance of *S. canis* within Aboriginal Australian populations has yet to be explored, but may be potentially significant given the underlying poor general health status of many Aboriginal people combined with an abundant dog population co-existing in the same community.

S. agalactiae was found in the pharynx of two dogs in this study. In the veterinary literature, *S. agalactiae* is considered an obligate pathogen of the udder of cows, causing contagious bovine mastitis. However, the organism has also been isolated from various other animals including cats, dogs, monkeys, fish, frogs and crocodiles (Amborski et al. 1983; Keefe 1997; Lammler, Abdulmawjood & Weib 1998; Evans et al. 2002; Yildirim et al. 2002; Bishop et al. 2007).

In humans, *S. agalactiae* is considered a commensal of the vagina and peri-anal area but is also associated with diseases in pregnant women and newborn children, being the leading cause of sepsis in neonates (Schuchat 1998; Gotoff 2002). Aboriginal babies suffer from early onset (occurring in the first week of life) infection of *S. agalactiae* at a rate 3 times higher than the rest of Australia (Australasian Study Group for Neonatal Infections 1995). While rare, *S. agalactiae* may also cause disease in

adults; in north Queensland this species was recovered in 28% of bacteraemia episodes in non-neonatal cases (Harris et al. 2010).

Zoonotic sources of *S. agalactiae* have been investigated and epidemiological studies of strains isolated from bovine and human sources revealed that a strain, ST17 (frequently found in humans and significantly associated with human neonatal disease) has arisen from a bovine *S. agalactiae* ancestor. It appears in this case that the strain has crossed the species barrier and is circulating within the human population (Bisharat et al. 2004).

One dog in the community was found to carry SESZ. SESZ is commonly found in mucous membranes of horses and has been associated with opportunistic respiratory disease and reproductive disease (Oikawa et al. 1994; Smith et al. 2003). It has also been associated with disease in a wide variety of other animal species including pigs, goats and chickens and recently implicated as an emerging cause of haemorrhagic pneumonia in dogs (Carter & Wise 2004; Priestnall & Erles 2011). SESZ has been occasionally associated with zoonotic outbreaks of PSGN, septicaemia and streptococcal toxic shock syndrome in humans via consumption of contaminated milk or other animal products (Yuen et al. 1990; Francis et al. 1993; Korman et al. 2004; Beres et al. 2008). A recent case study found that SESZ infection had been transmitted from a dog to its handler, proven through MLST analysis (Abbott et al. 2010). It is believed that the dog became infected through contact with an infected horse. At the time of sampling for this study there were over 50 horses in the community that roam freely; therefore, the dog may have contracted the SESZ from one of these horses.

All human participants in this study had skin lesions infected with *S. pyogenes*. One SDSE was isolated from a throat swab, with no obvious associated respiratory illness, and the other SDSE from a skin sore in which *S. pyogenes* was also isolated. Therefore, no assumptions can be made as to the pathogenic significance of these SDSE strains.

SDSE, as previously described, are not unusual colonizers of the skin, female genital upper respiratory or gastrointestinal tract of humans (Brandt & Spellerberg 2009). However, they are clearly emerging as important human pathogens having seen to be associated with diseases comparable to those traditionally thought to be caused by the frequent human pathogen, *S. pyogenes* (Williams 2003; Broyles et al. 2009; Takahashi, Ubukata & Watanabe 2010; Kittang et al. 2011). High rates of throat carriage of SDSE in northern Aboriginal Australian, Trinidad and Indian communities have resulted in SDSE being implicated in ARF and PSGN (Reid et al. 1985; McDonald et al. 2007; Bramhachari et al. 2010). The perceived increase in pathogenicity of SDSE has sparked investigations into the genetic profiles of commensal and pathogenic SDSE, which has provided evidence to suggest HGT between *S. pyogenes* and SDSE in humans (Kalia et al. 2001; Davies et al. 2005; Davies et al. 2007).

Epidemiological studies of SDSE in Northern Territory Aboriginal populations found that the SDSE asymptomatically carried were mostly Lancefield group G at a ratio of about 3:1 (257 GGS /80 GCS) (McDonald et al. 2007). This is also reflected in human invasive diseases caused by SDSE in which isolates were found to be either Lancefield group G or C at a ratio of 6:1 (Broyles et al. 2009). None of the SDSE retrieved from the dogs in this study produced the Lancefield group G antigen (excluding the A/G variant). A previous study investigating streptococci in dogs from two other Aboriginal communities also failed to find any Lancefield group G SDSE (Boon 2007). Therefore, although there appears to be no association between Lancefield group and virulence of SDSE strains (Davies et al. 2007), Lancefield group C SDSE are epidemiologically less frequently found in humans with or without streptococcal disease.

The small and opportunistic nature of the sample has limited the findings of this study and further studies should attempt to sample a larger population of dogs and humans including asymptomatic individuals. Further studies that investigate the bacterial load may also be beneficial in identifying the potential of risk factors associated with BHS carriage in dogs.

In conclusion, this study has shown that dogs in this Aboriginal community carry species of BHS that are associated infrequently with disease in humans. However, the combination of a fluctuating classification system, the variability observed in these taxa and the small human sample in this study has made it difficult to reach any solid conclusions about the frequency and significance of strain sharing. Novel heterogenous Lancefield antigens within the species seen in this study could reflect HGT events between streptococci that may enable adaptation to particular hosts and could facilitate the ability of certain strains to cross more easily between canine and human host. Further genetic characterisation using techniques such as *emm* typing and MLST typing should provide further evidence as to the correct identification of these species (especially the suspected SDSE) and the role specific strains play in the epidemiology of streptococcal infection and the ecology of SDSE and the other group G and C streptococci within Aboriginal communities. Further characterisations of these strains of streptococci are described in chapter 5.

Chapter 5: Genetic characterisation of β-haemolytic streptococci from dogs and humans in an Aboriginal community.

5.1 Introduction

In the previous chapter, phenotypic characteristics and 16S rRNA gene sequencing were used to investigate BHS isolated from dogs and humans in an Aboriginal community. Dogs were found to carry 4 streptococci species *S. canis*, SD, *S. agalactiae* and SESZ. All but 3 of the canine SD isolates could be classified as SDSE. Both humans and dogs carried SDSE strains and a Lancefield group C SDSE, with identical 16S rRNA sequence, was isolated from both a child and dog from the same household. The remaining human isolates were *S. pyogenes*.

The strains of streptococci isolated from dogs in this community expressed considerable variability; 3 strains of *S. canis* producing the Lancefield group C antigen were found and one SD that could not be classified further, producing both the A and G antigens. The API20 Strep system also had difficulty identifying SD/SDSE and *S. canis* isolates. Furthermore, whilst the 16S rRNA sequences could identify species it was clear from the phylogenetic tree that the SDSE and *S. canis* further diverged into clusters of different strains.

Therefore, this chapter uses more detailed genetic sequencing techniques to further explore and identify these strains. Further investigations into the presence of *S. pyogenes* virulence genes within these strains are also performed.

Epidemiological studies of SDSE have utilised both *emm* typing (encoding the M-protein) and MLST to identify potentially pathogenic strains within the sub-species (Kalia et al. 2001; Ahmad et al. 2009; McMillan et al. 2010; McMillan et al. 2011). MLST

has previously been used to deduce genetic relationships between human SDSE isolates of clinical and non-clinical relevance, via an online database (Aanensen & Spratt 2005). Each housekeeping loci is designated a number based on their nucleotide sequence. The resulting seven numbers (of the seven housekeeping gene sequences) make up an allelic profile. Each allelic profile is designated a Sequence Type (ST) base on this profile. Therefore, isolates with identical STs have identical nucleotide sequences at all of the seven housekeeping loci. This method has been used in epidemiological studies of SDSE in Australia, Portugal, USA and India (Ahmad et al. 2009; McMillan et al. 2010; McMillan et al. 2011).

In this chapter *emm* typing and MLST are used to genetically characterise strains of *S. dysgalactiae* (SDSE and SD) isolated from dogs and children in this study population. Since MLST has also been used to characterise strains of *S. canis* (Ahmad et al. 2009), this method was also used in this study to investigate the genetic relationships of *S. canis* isolates recovered in this study with particular focus on the atypical Lancefield group C strains of *S. canis* isolates. All canine and human streptococci isolates recovered from the community in this study also underwent Real-time (q) PCR exploration probing for *S. pyogenes* virulence genes. The 7 chosen virulence genes encode well known adhesins (collagen type I binding protein, fibronectin binding protein F1, fibronectin binding protein F2, fibronectin binding protein X), phage associated virulence proteins (mitogenic factor, streptodornase) and an exotoxin (pyrogenic exotoxin G). All of which have been previously shown to be variably distributed among SDSE of human origin (Davies et al. 2007).

5.2 Materials and Methods

5.2.1 Bacterial isolates

Forty one streptococcal isolates were recovered from 31 out of 61 dogs (29 of 57 community dogs and 2 of the 4 wild dogs) and 4 of 6 humans sampled in an Aboriginal community as described in the previous chapter. Speciation was previously determined through Lancefield grouping, biochemical characteristics and 16S rRNA

sequencing (as illustrated in chapter 4). Dogs were found to be infected with *S. canis*, SD/SDSE, *S. agalactiae* and SESZ. Humans were found to be infected with both *S. pyogenes* and SDSE. A group C SDSE isolate was cultured from a dog and a child sharing the same house (Isolate ID F10 & G1). Table 5.1 illustrates the further genotyping investigations performed on isolates recovered in this study.

Table 5.1. Streptococci isolated from dogs and humans and further genotyping analysis conducted in this study.

					Genotyping methods		
Isolate ID	**Species**	**Group***	**Isolation site**	**Source**	***emm* typing**	**MLST**	**Virulence gene PCR**
B5	*S. pyogenes*	A	skin sore	Human			+
C4	*S. pyogenes*	A	skin sore	Human			+
G2	*S. pyogenes*	A	skin sore	Human			+
G4	*S. pyogenes*	A	skin sore	Human			+
C5	SDSE	G	skin sore	Human	+	+	+
F10	SDSE	C	throat	Human	+	+	+
B3	SDSE	C	throat	Dog	+	+	+
C8	SDSE	C	throat	Dog	+	+	+
F1	SDSE	C	throat	Dog	+	+	+
F3	SDSE	C	throat	Dog	+	+	+
G1	SDSE	C	throat	Dog	+	+	+
G6	SDSE	C	throat	Dog	+	+	+
B4	SD	C	throat	Dog	+	+	+
E8	SD	C	throat	Dog	+	+	+
F9	SD	A/G	throat	Wild dog	+	+	+
A3	*S. canis*	G	throat	Dog		+	+
A7	*S. canis*	G	throat	Dog		+	+
B1	*S. canis*	G	throat	Dog		+	+
B6	*S. canis*	G	throat	Dog		+	+
B9	*S. canis*	G	throat	Dog		+	+
B10	*S. canis*	G	throat	Dog		+	+
C1	*S. canis*	G	throat	Dog		+	+
C2	*S. canis*	G	throat	Dog		+	+
C3	*S. canis*	G	throat	Dog		+	+
C6	*S. canis*	G	throat	Dog		+	+
C10	*S. canis*	C	throat	Dog		+	+
D1	*S. canis*	C	throat	Dog		+	+
D2	*S. canis*	G	throat	Dog		+	+
D5	*S. canis*	G	throat	Dog		+	+
D6	*S. canis*	G	throat	Dog		+	+
D8	*S. canis*	G	throat	Dog		+	+
E2	*S. canis*	C	throat	Dog		+	+
E6	*S. canis*	G	throat	Dog		+	+
E9	*S. canis*	G	throat	Dog		+	+
F5	*S. canis*	G	throat	Dog		+	+
F6	*S. canis*	G	throat	Dog		+	+
F8	*S. canis*	G	throat	Wild dog		+	+
G5	*S. canis*	G	throat	Dog		+	+
B7	*S. agalcatiae*	B	throat	Dog			+
G7	*S. agalactiae*	B	throat	Dog			+
F7	*SESZ*	C	throat	Dog			+

* Lancefield group antigen; += genotyping performed.

5.2.2 *Emm* typing

Emm typing was performed on extracted DNA (see chapter 3) of all SD/SDSE and *S. canis* isolates listed in Table 5.1. PCR amplifications for *emm*-like genes were performed using the forward and reverse primers recommended by the CDC as shown in Table 5.2 (Beall, Facklam & Thompson 1996; Beall 2005). PCR protocol, template and purification of amplified products are described in chapter 3.

Sequencing of the PCR product was performed using the single sequencing primer (emmseq2) recommended by the CDC as shown in Table 5.2. Sequencing was performed by Macrogen Inc. Korea, using BigDye terminator cycle sequencing kit (Applied BioSystems, USA). The sequencing products were then resolved on an Applied Biosystems model 3730XL automated DNA sequencing system.

Table 5.2. Sequences used for the amplification and sequencing of *emm* in this study.

		Amplification	Sequencing
Forward	5' TATT(C/G)GCTTAGAAAATTAA 3'	x	
Reverse	5' GCAAGTTCTTCAGCTTGTTT 3'	x	
emmseq2	5' TATTCGCTTAGAAAATTAAAAACAGG 3'		x

x=procedure performed.

The achieved *emm* sequences were subject to a homology search via the CDC streptococci group A subtyping request form, BLAST 2.0 online server (http://www.cdc.gov/ncidod/biotech/strepblast.html) to determine *emm* type. Sequences that matched 100% over the entire 180 bases were designated identical *emm* types. Sequences that differed from the M protein reference strain encoding the first 50 residues of the mature M protein were assigned to a subtype. Sequences that did not match *emm* types or were not assigned to a subtype were submitted to the CDC and assigned a new *emm* type/subtype.

5.2.3 Multilocus Sequence Typing (MLST)

PCR amplification of the seven housekeeping genes (MLST); glucose kinase (*gki*), glutamine transport protein (*gtr*), glutamate racemase (*murI*), DNA mismatch repair protein (*mutS*), Transketolase (*recP*), xanthinine phosphoribosyl transferase

(*xpt*) (alternative *xpt* primers used for *S. canis*) and acetoacetyl-coathioloase (*atoB*) was performed on all SD/SDSE and *S. canis* isolates described in Table 5.1, using a combination of alternative primers recommended by the CDC and *S. pyogenes* primers recommended by the MLST database (http:www.mlst.net.) as shown in Table 5.3.

PCR protocol, template and purification of amplified products are described in chapter 3. Sequencing of the PCR product was performed by Macrogen Inc. Korea as previously described for *emm* typing. All housekeeping genes were sequenced using only the forward primer from Table 5.3. All new MLST were confirmed by repeat sequencing using the reverse primer.

Table 5.3. Primers used for amplification and sequencing of the seven multilocus housekeeping genes (MLST) for *S. dysgalactiae*, SDSE and *S. canis* isolates.

Gene	**Forward Primer** (5' to 3')	**Reverse Primer** (5' to 3')	**Product Size**
gki (glucose kinase)	GCAGATTTTATTGGTATCGGTATGG	TCTCCTGCTGCTGACAC*	498bp
gtr (Glutamine transport ATP-binding protein)	GGAATTGATTTAAACATTATGTCAGGAG	CACAATAACGCCGCCATCCATA	450bp
murI (glutamite racemase)	TTATGGTCCAAGACCTGCTGAGC	TTTCAGGACTTGCCGTTGTGTAAAA	438bp
mutS (DNA mismatch repair, HexA)	AGGTCAGATGTTAGAGGCTAGG	CCTAGTTCATCAAATAGAATAAGGGAA	405bp
recP (Transketolase)	TGTCCGCACCCTATCAATGGAT	CATCTTTCACAAGGATATGTTGCC	459bp
xpt (Xanthine phosphoribosyltransferase)	ATGCAGTTACTTGAAGAACGCATCTTAAC	GCCTCCAAGAAGTTTAGATTACCA	450bp
atoB (Acetoacetyl-coathiolase, *yqiZ*)	CAGATGCTTTTAACAATTACCACATGG	CCCATTCACATTCACGATTTCTGG	434bp
***S. canis* MLST genes**			
Same primers as above except for *xpt*			
xpt (Xanthine phosphoribosyltransferase)	TTACTTGAAGAACGCATCTTA*	ATGAGGTCACTTCAATGCCC*	450bp

All primers recommended by the Centers for Disease Control and Prevention (http://www.cdc.gov/ncidod/biotech/strep/alt-MLST-primers.htm) except for those denoted with (*) which were recommended for *S. pyogenes* by the MLST database (http://spyogenes.mlst.net/misc/info.asp).

Allelic profiles of each SD/SDSE isolate were determined by submitting each housekeeping gene sequence into the SDSE MLST database (Aanensen & Welcome Trust 2012). Complete allelic profiles were then submitted to determine sequence type (ST). All new MLST alleles were sent to the SDSE MLST curator for allele assignment and new MLST sequence types were also submitted for assignment. Dr. Rebecca Towers of Menzies School of Health Research also provided access to further SDSE allele codes to enable ST designation of these isolates, the SDSE MLST database with ST allelic codes and sequences for each locus are included in Appendix 6.

An unpublished *S. canis* MLST database was also developed using the isolates from this study, from the study of Ahmad et al. (2009) and other *S. canis* isolates from dogs collected in the Northern Territory. This is included in Appendix 7.

5.2.4 Data analysis

Simpsons Index of Diversity places a value on diversity by assessing the probability that two organisms randomly selected from a population will be different (Simpson 1949). Therefore the higher the D value the more diverse the population. Simpsons Index of Diversity was used to determine the diversity of SD/SDSE STs, SD/SDSE *emm* subtypes and *S. canis* STs by using the online tool (http://darwin.phyloviz.net/ComparingPartitions/index.php).

5.2.5 Phylogenetic analysis

A concatenated sequence of all 7 genes representing each ST, were used to construct a minimum evolutionary tree (including bootstrap test with 500 replicates) as implemented by MEGA (Molecular Evolutionary Genetic Analysis) Version 4 Software (Tamura et al. 2007). The concatenated tree included STs found in this study, the 80 STs available from the SDSE MLST online database and further SDSE isolates from dogs and humans collected previously from the Northern Territory (Appendix 8). A concatenated sequence was extracted from a non β-haemolytic SDSD ATCC 27957 isolated from an udder infection in a cow and was included in the analysis for

comparison (accession number: AEGO01000001.1, AEGO01000002.1) (McDonald & McDonald 1976). The STs 81-133, from India, were not included in this analysis as they were previously reported as being largely unique to India (McMillan et al. 2011).

Clonal Complexes (CCs) of the STs included in this analysis were determined by GoeBURST by accessing the online tool at (http://goeburst.phyloviz.net/). CCs in this study are defined as groups of STs that share identical alleles at six of the seven housekeeping loci, further described as single locus variants (SLV). Singletons describe STs that differ at two or more alleles from every other ST in the sample (Feil et al. 2004).

Individual minimum evolutionary trees were also constructed for all housekeeping loci as previously described and implemented by Mega4. All SD/SDSE STs from the database in Appendix 6 (including India STs) were used in the analysis. The extracted SDSD ATCC 27957 housekeeping alleles and *S. canis* alleles were also included for comparison. The multiple alignment for the comparison of *S. canis gki* allele 6 was performed by using ClustalW2 online sequence alignment tool (European Bioinformatics Institute 2012).

Split decomposition analysis was generated using all of the STs from this study, those of the database and the SDSD reference strain (ATCC27957) in SplitsTree4 software (version 4.12.3). SplitsTrees are believed to be more accurate at visualising evolutionary data because real evolutionary data often contains conflicting signals that do not support a tree like network (Huson 1998). Split decomposition analysis was also used to generate a splitstree of the dog derived SD/SDSE STs in comparison to their closest relatives of SDSE and the SDSD reference strain (ATCC27957) to visualise the separation of dog derived SD/SDSE STs from the SDSD reference strain.

The *S. canis* ST sequences were also concatenated and used to construct a minimum evolutionary tree in Mega4. CCs of the *S. canis* STs were also determined using GoeBurst.

5.2.6 qPCR for the detection of *S. pyogenes* virulence genes

qPCR for the detection of selected virulence genes was conducted on all 41 isolates recovered from dogs and humans in this study (Table 5.1). *S. pyogenes* virulence genes used in this study were chosen from a group of well characterized *S. pyogenes* virulence genes previously shown to be variably distributed among SDSE isolates in a microarray of 216 GAS virulence genes (Davies et al. 2007). Four of these genes represent cell surface proteins that function in adhesion to host cells; these are collagen type I binding protein (*cpa*), fibronectin binding protein F1 (*sfbI*), fibronectin binding protein F2 (*prtF2*) and fibronectin binding protein X (*sfbX*). Isolates were also tested for the presence of genes encoding streptococcal pyrogenic exotoxin G (*SpeG*) and phage associated genes, mitogenic factor (*sdaB*) and streptodornase (*sdn*).

Primers were designed based on the probe regions used by the *S. pyogenes* microarray kindly provided by Dr. Mark Davies (Appendix 9). Forward and reverse primers are described in Table 5.4. Four SDSE isolates previously described as positive or negative for at least one of the designated virulence genes were used as controls also provided by Dr. Mark Davies (Davies et al. 2007) (Table 5.5). A no template control of pyrogen free H_2O was also included in the analysis.

Methodology related to bacterial DNA extraction, qPCR protocol, template DNA and high resolution melt analysis (HRM) are all described in chapter 3.

The results were viewed by Rotor-Gene 6000 Series Software version 1.7. The positive SDSE controls did not react as strongly as the *S. pyogenes* isolates and therefore the determination of a positive reaction was based on the amplification curve threshold cycle (C_T) of the positive SDSE controls and the C_T of the positive *S. pyogenes* isolates.

The HRM Analysis curves were normalised and viewed with the Rotor-Gene 6000 Series Software version 1.7. Isolates were collated according to the HRM curves, in which isolates clustered into groups.

Table 5.4. Primers used for amplification of virulence genes.

Virulence gene	Forward Primer (5' to 3')	Reverse Primer (5' to 3')	Amplicon Length
cpa (collagen type I binding protein)	ACAGTTACAGATGGAAAAGCTACCTTCACA	GAAGGTAGCCCCTCAACAATCAATGATTC	71bp
sfb1 (fibronectin binding protein F1)	CCTCTAGCGGGTGAGTCTGGTG	GGGTTCTGTTGATTTCCATAGACTTCGG	65bp
prtF2 (fibronectin binding protein F2)	CAGATGGACAAGTGAAAGATTTCTACCTGA	GCTGCGGTTTCGACAAATGTATATTTTCCT	70bp
sfbX (Fibronectin Binding protein X)	AACTTGCTCCTCATGATAGCCACACAAC	AGGCAAGTGAGGGGCTGCTGTATC	70bp
speG (pyrogenic exotoxin G)	AAAGGGGATAAGGTCGATGTTTTTGGTCTC	GCTCCCCGATGTATAACGCGATTC	297bp
sdaB (mitogenic factor)	GTTGTTCTAAATGATGGCGCAAGCAAGTAC	GGACTGTCATTGAATGTCCAAGCTAATGC	70bp
sdn (streptodornase- phage associated)	GAGTAACCGTATCTGATGTAGTTTATAACC	TAAGCGCCTGTTGAACGTTTATAGCCATC	70bp

Table 5.5. SDSE controls and corresponding positive virulence genes.

Control ID	NS752 (42)	NS3396 (43)	MD08 (44)	G121 (45)	GGS48 (46)
Group	G	G	G	G	G
***emm*-type**	stG6	stG480	stG2078	stC74A	stG4831
ST	44	39	17	29	-
Virulence gene profile					
cpa		x			
sfb1	x			x	
prtF2					x
sfbX					x
speG	x	x		x	x
sdaB		x			
sdn			x		

(x) denotes a previous positive reaction.

5.3 Results

5.3.1 SD/SDSE *emm* subtype and MLST

The 8 isolates of SDSE and 3 SD belonged to one of 6 STs with each ST corresponding to an *emm* subtype (Table 5.6). The diversity of ST and *emm* subtype of isolates collected in this study was 85.5% (D=0.855, 95%CI (0.719-0.990)).

A new SDSE *emm* subtype stG839.5 was found in the child and dog SDSE isolates from the same household. These isolates also had identical housekeeping alleles at all seven loci (ST3). The majority of SDSE isolated from dogs belonged to the new ST 142 with corresponding *emm* type stC9431.0.

The three dubious isolates identified as SD were designated new ST's 134 and 143. These STs also shared alleles with the other canine SDSE STs and ST 143 had identical alleles with SDSE ST10 at five of the seven housekeeping loci.

Table 5.6. *Emm* type and MLST (ST) allelic profile of 8 SDSE (isolate ID: G1- B3) and 3 SD (isolate ID: F9-B4) isolated from dogs and children.

				Allelic Profile							
ID	**Origin**	**Lancefield Group**	***emm* subtype**	***gki***	***gtr***	***murI***	***mutS***	***recP***	***xpt***	***atoB***	**ST**
G1	dog	C	stC839.5*	5	3	4	1	6	2	1	3
F10	human	C	stC839.5*	5	3	4	1	6	2	1	3
G6	dog	C	stC7505.0	10	4	7	7	12	13	8	10
C5	human	G	stG10.0	3	3	2	2	9	8	2	15
F3	dog	C	stC9431.0	10	5	17*	6	12	13	9	142*
F1	dog	C	stC9431.0	10	5	17*	6	12	13	9	142*
C8	dog	C	stC9431.0	10	5	17*	6	12	13	9	142*
B3	dog	C	stC9431.0	10	5	17*	6	12	13	9	142*
F9	dog	A/G	stC46.1*	17*	14*	16*	7	12	13	9	134*
E8	dog	C	stL1929.0	10	13*	16*	7	12	13	8	143*
B4	dog	C	stL1929.0	10	13*	16*	7	12	13	8	143*

*new allele or ST from this study. ST; designated Sequence Type

5.3.2 Evolutionary relationships of SD/SDSE STs

The phylogenetic relationships of the SDSE STs isolated from dogs and people from Aboriginal communities (Northern Territory and Yarrabah) and invasive disease

from various locations in USA and Portugal (MLST database) are shown in Figure 5.1. The minimum evolutionary tree shows that the majority of STs isolated from dogs form a separate sub-cluster from the majority of human SDSE STs, clustering with a small number of STs that have been found in humans (ST1 and ST10), including the SD isolates (shaded area in Figure 5.1). SDSE isolates from humans belonging to the STs within this dog cluster (ST1 and ST10) were isolated from cases of invasive streptococcal disease and had the same *emm* types as the dog isolates; stL1929, stC7505 and stC9431. These *emm* types were not found in any of the STs within the human cluster.

The two animal isolates that cluster with the human STs include the SDSE strain found in both dog and child from the same household (ST3), and an SDSE strain isolated from a foot ulcer in a possum from the Northern Territory (ST12).

The SDSE STs form 18 CCs at the SLV level. STs in the dog cluster do not form CCs with any STs from the human cluster (Figure 5.1).

The split decomposition analysis of all SDSE STs show that the dog cluster of STs appear diverged from both the human STs and the SDSD strain (Figure 5.2). However, the split decomposition of STs from the dog cluster in relation to its closest relatives shows evidence of recombination between both human and dog clusters but not the SDSD reference strain, evident by the net-like appearance of the splitstree (Figure 5.3).

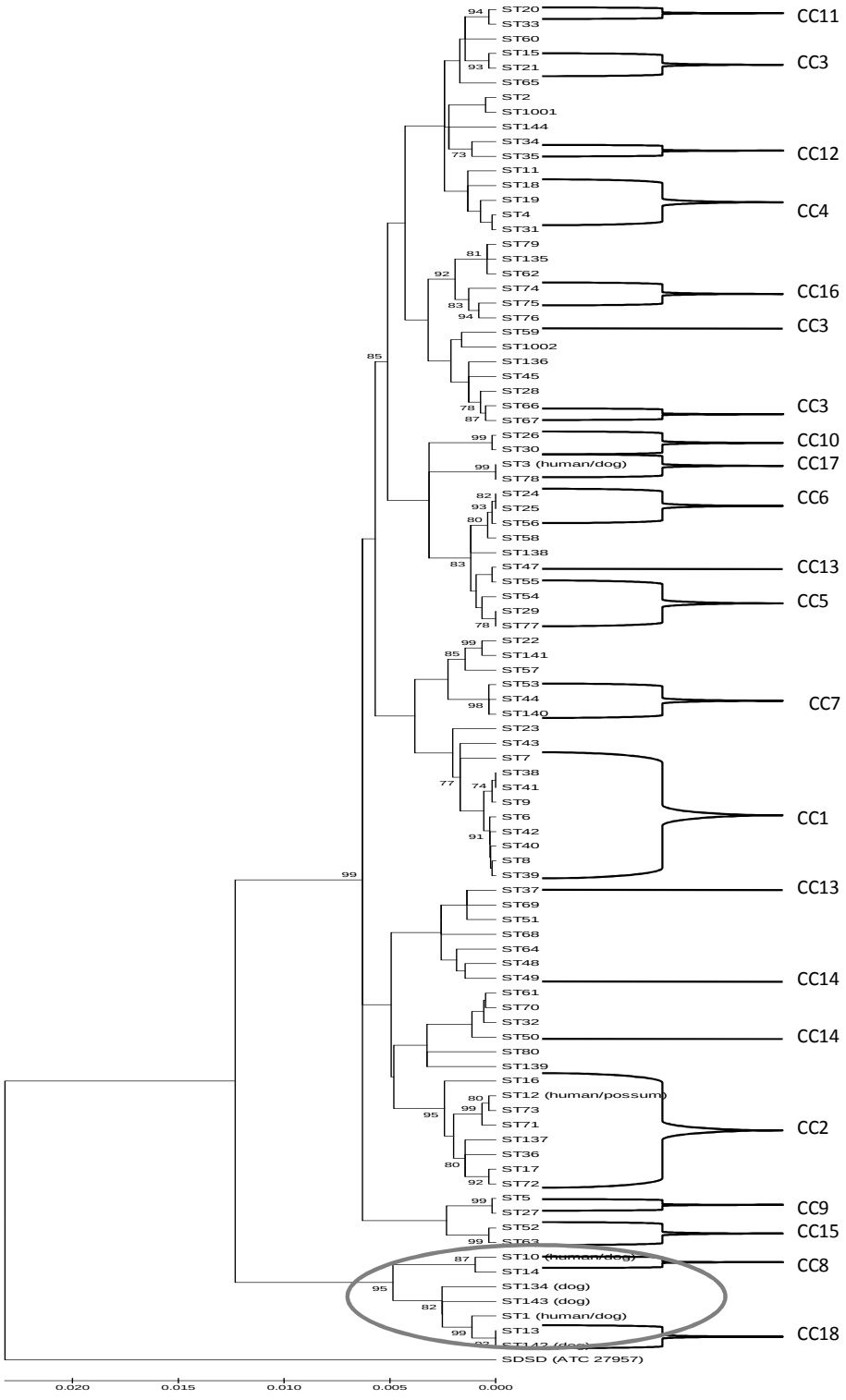

Figure 5.1 Minimum evolutionary tree of concatenated SDSE MLST loci excluding Indian ST's 81-133. Only bootstrap values greater than 70% are shown. CC= clonal complexes at SLV level as determined by goeBURST. Circled area illustrates the dog cluster. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.

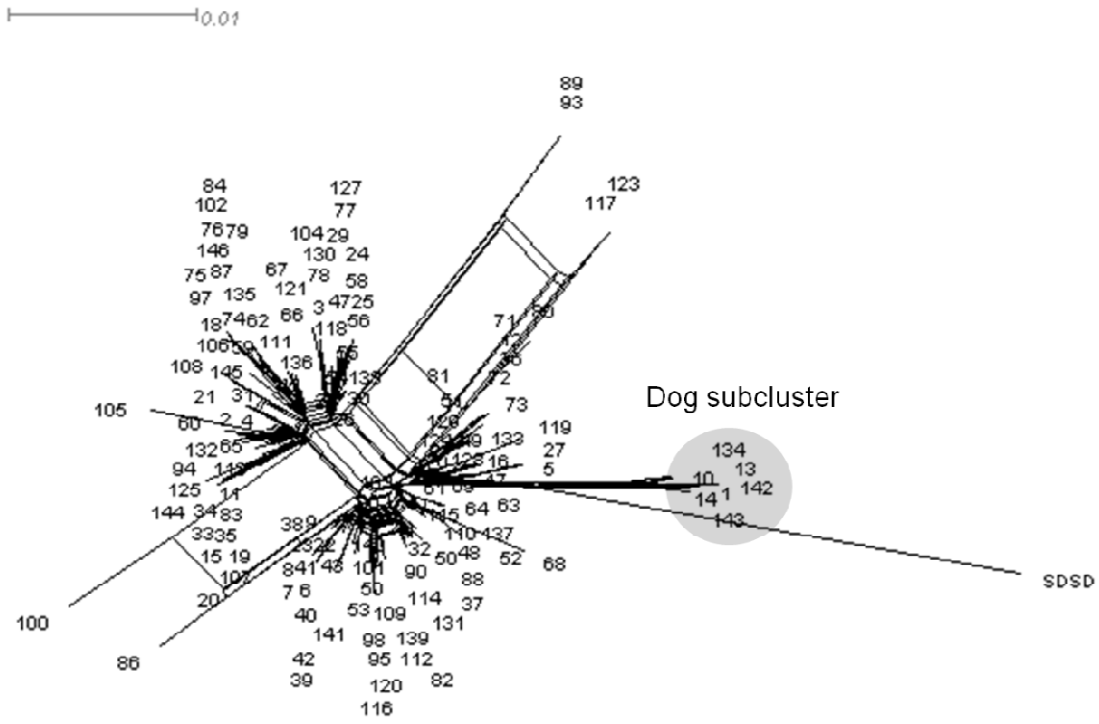

Figure 5.2. Split decomposition analysis of SDSE STs (excluding outliers). Numbers indicate STs. Shaded circle represents the dog cluster.

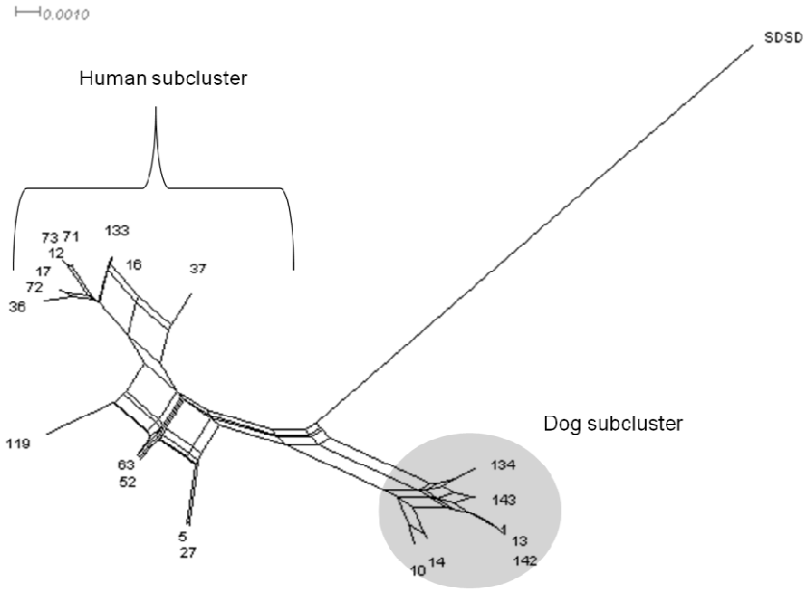

Figure 5.3. Split decomposition analysis of dog ST cluster in relation to closest human ST relatives and SDSD. Numbers indicate STs. Shaded circle indicates dog cluster.

5.3.3 Allelic variation of housekeeping loci of STs within the dog cluster

Two housekeeping loci alleles are shared by STs from the human and dog sub-clusters; glutamine transport protein allele 4 (*gtr*4) and transketolase allele 12 (*recP*12) (Table 5.7). The distribution of *gtr*4 appears more commonly in the human cluster of STs whilst *recP*12 is more commonly associated with the dog cluster of STs being present in only one of the human cluster of STs.

Table 5.7. Allelic variation of seven housekeeping loci of SD/SDSE STs found in dogs and humans.

Loci	Allele	Dog cluster STs	Human cluster STs
gki	10	ST1,ST10, ST13, ST14,ST142, ST143	-
	17*	ST134	-
gtr	4	ST10, ST14	ST2, ST12, ST16, ST17, ST36, ST68, ST72, ST73, ST144
	5	ST13, ST142	-
	6	ST1	-
	13*	ST143	-
	14*	ST134	-
murI	6	ST1, ST13	-
	7	ST10, ST14	-
	16*	ST134, ST143	-
	17*	ST142	-
mutS	6	ST1, ST13, ST14, ST142	-
	7	ST10, ST143, ST134,	-
recP	12	ST1, ST10, ST13, ST14, ST134, ST142, ST143	ST5
xpt	13	ST1, ST10, ST13, ST14, ST134, ST142, ST143	-
atoB	8	ST1, ST10, ST14, ST143	-
	9	ST13, T134, ST142,	-

*new alleles from this study

The minimum evolutionary trees of each housekeeping locus showed that alleles from the dog STs were often divergent from the SDSD alleles. The alleles that clustered closely with the SDSD reference strain were *gtr*14 (new allele from ST134) and *recP*12 which was present in all canine SDSE isolates. No alleles were shared with the SDSD reference strain or *S. canis* (see Appendix 10 for individual housekeeping loci trees). However, interestingly the *gki*6 *S. canis* allele separated from the other *S. canis* alleles and clustered with the canine SDSE *gki* alleles (Appendix 10).

Further multiple sequence alignment of the *S. canis gki*6 allele in comparison with other *S. canis* and SDSE *gki* alleles suggest that this allele may be chimeric (Figure 5.4). From the alignment it appears that the junction position is between 91bp and

104bp (outlined region in Figure 5.4) which has appeared to permit a homologous recombination event and exchange of sequence. Prior to this junction *S. canis gki*6 appears to be a typical *S. canis gki* allele, but afterwards it has more sequence homology to the SDSE *gki* alleles. Subsequent alignment of the *S. canis gki*6 allele after removal of the sequence prior to the junction (using the sequence after the junction) showed that it clusters with the SDSE *gki* alleles from the dog cluster (Figure 5.5). This suggests that a recombination event has previously taken place between *S. canis* and dog derived SDSE strains. The *S. canis gki*6 allele is a less prevalent allele among the *S. canis* STs, but SDSE *gki*10 and SDSE *gki*17 are quite common among the SDSE STs of the dog cluster.

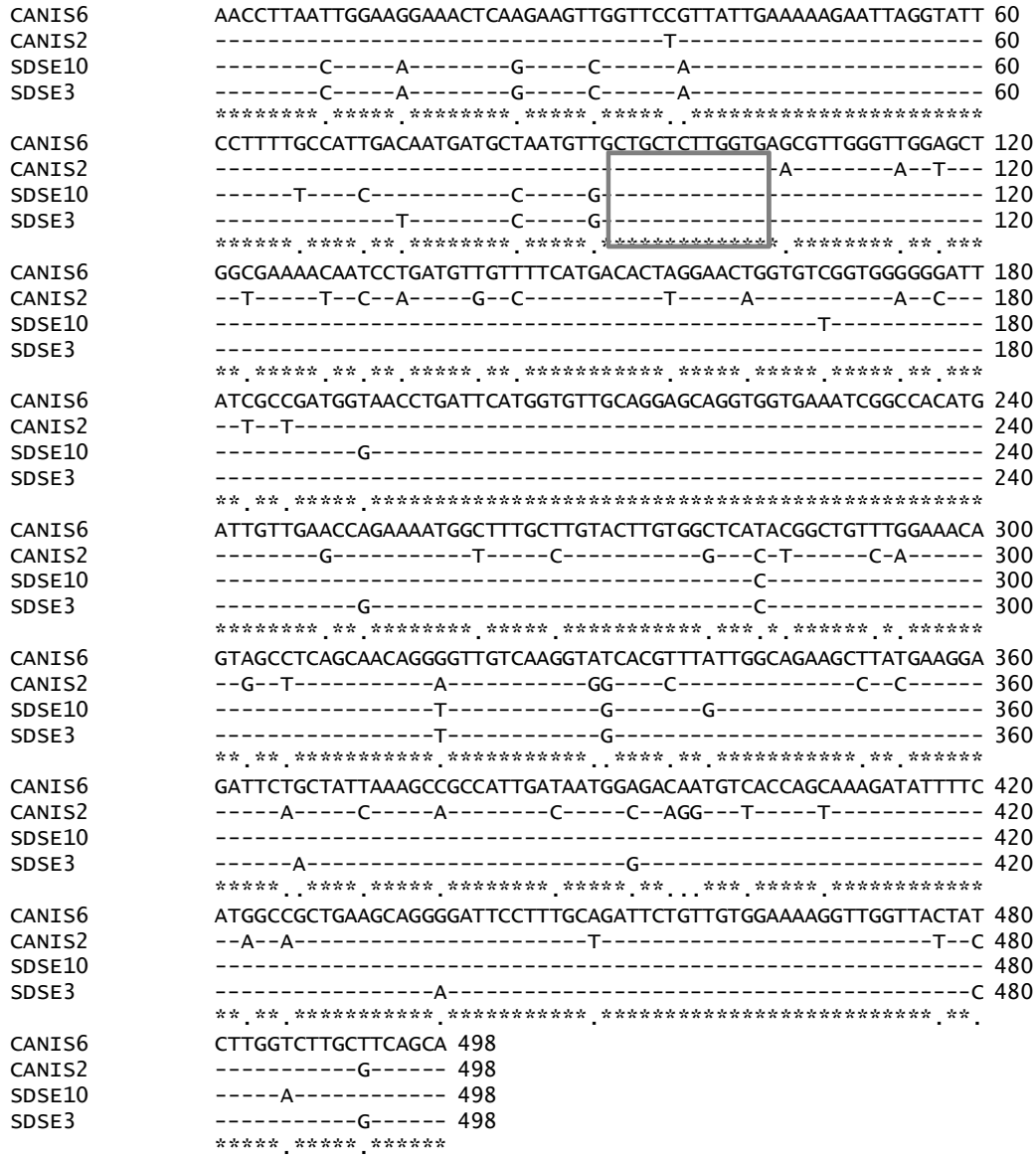

Figure 5.4. Multiple sequence alignment of *S. canis gki* 6 allele in comparison with other *gki* alleles. CANIS6= *S. canis gki*6 allele; CANIS2= *S. canis gki*2 allele; SDSE10= SDSE *gki*10 allele; SDSE3= SDSE *gki*3 allele. Outlined section indicates proposed junction of homologous DNA between *S. canis gki*6 allele and SDSD *gki* alleles. (*) along the bottom row and (-) within the sequence indicate identical nucleotides.

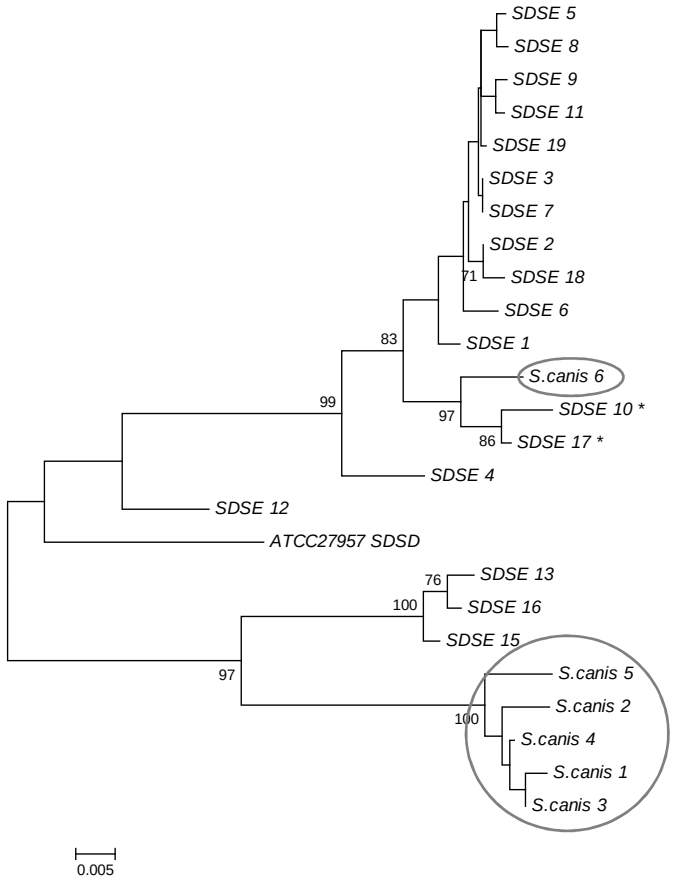

Figure 5.5. Minimum evolutionary tree of *S. canis gki* allele 6 (positions 105-498) and *gki* alleles from SDSE and an SDSD strain. Bootstrap values less than 70% are not shown. Circled areas indicate *S. canis gki* alleles showing that *S. canis gki* allele 6 is diverged from other *S. canis* alleles. (*) indicates SDSE *gki* alleles that are found in STs within the dog sub-cluster.

5.3.4 *S. canis* MLST

The 23 *S. canis* isolated from dogs in the study community separated into 4 distinct allelic profiles which were all new STs (Table 5.8). The 3 atypical Lancefield group C *S. canis* isolates (C10, D1, E2) all had identical allelic profiles at all of the loci implying that the isolates are likely to be from a single clonal origin (i.e., the same strain). The majority of *S. canis* isolates (69.57%) were ST6. The diversity of *S. canis* STs collected in this study was 50.2% (D=0.502, 95%CI (0.280-0.724)).

Table 5.8. MLST profile of 23 *S. canis* isolates from dogs in an Aboriginal community.

		Allelic profile							
ID	**Group**	***gki***	***gtr***	***murI***	***mutS***	***recP***	***xpt***	***atoB***	**ST***
A3	G	1	1	1	1	4	1	1	**5**
F5	G	1	1	1	1	4	1	1	**5**
G5	G	1	1	1	1	4	1	1	**5**
A7	G	4	2	4	4	5	4	4	**6**
B1	G	4	2	4	4	5	4	4	**6**
B6	G	4	2	4	4	5	4	4	**6**
B9	G	4	2	4	4	5	4	4	**6**
B10	G	4	2	4	4	5	4	4	**6**
C1	G	4	2	4	4	5	4	4	**6**
C2	G	4	2	4	4	5	4	4	**6**
C3	G	4	2	4	4	5	4	4	**6**
C6	G	4	2	4	4	5	4	4	**6**
D2	G	4	2	4	4	5	4	4	**6**
D5	G	4	2	4	4	5	4	4	**6**
D6	G	4	2	4	4	5	4	4	**6**
D8	G	4	2	4	4	5	4	4	**6**
E6	G	4	2	4	4	5	4	4	**6**
E9	G	4	2	4	4	5	4	4	**6**
F6	G	4	2	4	4	5	4	4	**6**
C10	C	5	4	5	3	4	5	5	**7**
D1	C	5	4	5	3	4	5	5	**7**
E2	C	5	4	5	3	4	5	5	**7**
F8	G	6	5	6	3	4	6	1	**8**

*according to unpublished *S. canis* MLST database, see Appendix 7.

5.3.5 Evolutionary relationships of *S. canis* STs

The minimum evolutionary tree of all *S. canis* STs is shown in Figure 5.6. Analysis using all *S. canis* STs from the database showed that 3 CCs were formed at the SLV level. No *S. canis* isolates collected in this study formed CCs with each other. The *S. canis* ST8 isolated from the throat of a wild dog appeared to be diverged from the other *S. canis* STs.

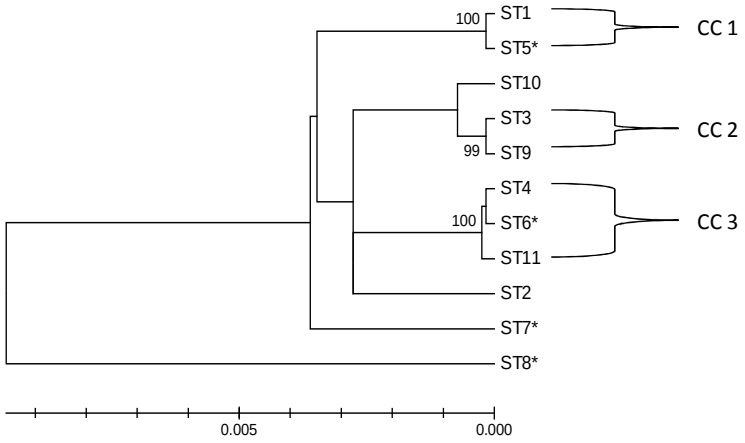

Figure 5.6. Minimum evolutionary tree of concatenated *S. canis* MLST loci. *Indicates STs found in this study. Bootstrap values less than 70% are not shown. CC= clonal complexes at SLV level as determined by goeBURST. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.

5.3.6 Virulence gene profile

All *S. pyogenes* and SD/SDSE isolates from the Aboriginal community were found to encode at least one of the *S. pyogenes* virulence genes (Table 5.9). Negative qPCR results for all 7 virulence genes was observed for all *S. canis*, *S. agalactiae* and SESZ isolates tested. Only *S. pyogenes* isolates were positive for virulence genes *cpa*, *prtF2*, *sfbX* and *sdaB*.

All SD/SDSE and *S. pyogenes* isolates were positive for *speG*. For the SD/SDSE isolates encoding of virulence genes was associated with given STs. Isolates belonging to ST3, ST10 and ST15 were only positive for the toxin *speG*. Isolates from ST142 were positive for *sfbI* and *speG*. Isolates from the dubious SD ST134 and ST143 were positive for *sfbI*, *speG* and *sdn*. No human SDSE isolates were positive for *sfbI* or *sdn* (Table 5.9).

The HRM analysis showed that isolates positive for *sfbI* and *speG* separated into 3 separate curves indicating that there were differences in the sequences of the

gene product amplified. For isolates positive for *sfbI*, human *S. pyogenes* and dog SDSE isolates from ST142 grouped together in curve 2. However, curve 3 consisted of only the dog derived SD from ST143 and ST134 (Table 5.9 & Figure 5.7A). The isolates positive for *speG* separated into 3 curves; curve 1 only contained *S. pyogenes* isolates, curve 2 contained SDSE from humans and dogs and curve 3 contained only SDSE isolated from dogs (Table 5.9 & Figure 5.7B). The 3 SD isolates of canine origin from ST143 and ST134 were the only isolates to produce an amplicon for *sdn* (Table 5.9 & Figure 5.7C).

Table 5.9. qPCR screening results of *S. pyogenes* and SD/SDSE isolates for selected *S. pyogenes* virulence genes.

Species	Origin	ID	Group	MLST	*cpa*	*sfbI**	*prtF2*	*sfbX*	*speG**	*sdaB*	*sdn*
S. pyogenes	human	B5	A		+	-	+	-	1	+	-
	human	C4	A		-	2	-	+	1	+	-
	human	G2	A		-	2	+	+	1	+	-
	human	G4	A		-	2	+	+	1	+	-
SDSE	human	F10	C	ST3	-	-	-	-	2	-	-
	canine	G1	C	ST3	-	-	-	-	2	-	-
	canine	G6	C	ST10	-	-	-	-	3	-	-
	human	C5	G	ST15	-	-	-	-	2	-	-
	canine	B3	C	ST142	-	2	-	-	3	-	-
	canine	C8	C	ST142	-	2	-	-	3	-	-
	canine	F1	C	ST142	-	2	-	-	3	-	-
	canine	F3	C	ST142	-	2	-	-	3	-	-
SD	canine	B4	C	ST143	-	3	-	-	3	-	+
	canine	E8	C	ST143	-	3	-	-	3	-	+
	canine	F9	A/G	ST134	-	3	-	-	2	-	+
Controls	NS752	42	G	ST44	-	2	-	-	2	-	-
	NS3396	43	G	-	+	-	+	-	2	-	-
	MD08	44	G	ST17	-	-	-	-	-	-	-
	G121	45	G	ST29	-	1	-	-	2	-	-
	GGS48	46	G	-	-	-	-	-	2	-	-

Adhesins: *cpa*: collagen type I binding protein; *sfbI*: fibronectin binding protein F1; *prtF2*: fibronectin binding protein F2; *sfbX*: fibronectin binding protein X. Toxins: *speG*; pyrogenic exotoxin G. Bacteriophage associated: *sdaB*; mitogenic factor (DnaseB). *sdn*; streptodornase.* Numbers are a positive reaction and reflect corresponding HRM curve from Figure 2A & 2B.

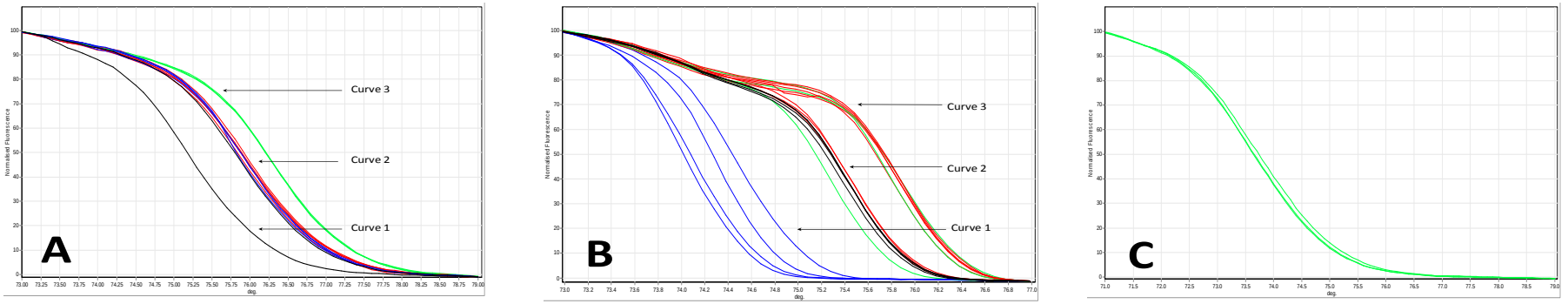

Figure 5.7. qPCR High Resolution Melt of A) *sfbI*; B) *speG*; C) *sdn*; ■SDSE controls; ■*S. pyogenes*; ■SDSE; ■SD.

5.3.7 SD/SDSE dog cluster and biochemical and virulence gene associations

The biochemical and virulence gene profiles of SD/SDSE isolates collected in this study were further investigated to determine whether there were any associations that may enable differentiation between the dog and human sub-clusters (Table 5.10). The only definitive biochemical property distinguishing the clusters was that isolates from the dog ST cluster fermented glycogen and all those from the human cluster did not.

No dog STs fermented lactose, but neither did ST15 from the human cluster. All of the STs within the human cluster failed to produce an amplicon for *sfbI* and grouped in curve 2 of the *speG* HRM analysis (with one ST from the dog cluster). None of the STs from the dog cluster produced a group G Lancefield antigen (except for the atypical A/G variant), including those from the SDSE MLST database.

Table 5.10. Biochemical and virulence gene profile of STs of SD/SDSE isolates from this study belonging to human and dog sub-clusters.

	Dog cluster								Human cluster		
Isolate ID	B3	C8	F1	F3	G6	B4[#]	E8[#]	F9[#]	C5	F10	G1
Origin	dog	dog	dog	dog	dog	dog	dog	dog	human	human	dog
***emm*ST**	stC9431.0	stC9431.0	stC9431.0	stC9431.0	stC7505.0	stL1929.0	stL1929.0	stC46.1*	stG10.0	stC839.5	stC839.5
ST	ST142*	ST142*	ST142*	ST142*	ST10	ST143*	ST143*	ST134*	ST15	ST3	ST3
GROUP	C	C	C	C	C	C	C	A/G	G	C	C
sfbI	2	2	2	2	-	3	3	3	-	-	-
speG	3	3	3	3	3	3	3	2	2	2	2
sdn	-	-	-	-	-	+	+	+	-	-	-
Vogues Proskauer (VP)	-	-	-	-	-	-	-	-	-	-	-
Hippuric acid (HIP)	-	-	-	-	-	-	-	-	-	-	-
β-glucosidase hydrolysis (ESC)	-	-	-	-	-	-	-	-	-	-	-
Pyrolidonyl arylamidase (PYRA)	-	-	-	-	-	-	-	-	-	-	-
α-galactosidase (αGAL)	-	+	-	-	-	-	-	-	-	-	-
β-glucoronidase (βGUR)	+	+	+	+	+	-	-	+	+	-	-
β-galactosidase (βGAL)	-	-	-	-	-	-	-	-	-	-	-
alkaline phosphate (PAL)	+	+	+	+	+	+	+	+	+	+	+
leucine aminopeptidase (LAP)	+	+	+	+	+	+	+	+	+	+	+
arginine dihydrolase (ADH)	+	+	+	+	+	+	+	+	+	+	+
ribose (RIB)	+	+	+	+	+	+	+	+	+	+	+
arabinose (ARA)	-	-	-	-	-	-	-	-	-	-	-
mannitol (MAN)	-	-	-	-	-	-	-	-	-	-	-
sorbitol (SOR)	-	-	-	-	-	-	-	-	-	-	-
lactose (LAC)	-	-	-	-	-	-	-	-	-	+	+
trehalose (TRE)	+	+	+	+	+	-	-	+	+	+	+
inulin (INU)											
raffinose (RAF)	-	-	-	-	-	-	-	-	-	-	-
amidon (AMD)	+	+	+	+	+	+	+	+	+	+	+
glycogen (GLYG)	+	+	+	+	+	+	+	+	-	-	-

*New ST or *emm*ST from this study. [#] Isolates identified as SD.

5.4 Discussion

This chapter has shown that strains of SD/SDSE diverge into separate clusters according to host species. SD/SDSE typically carried by dogs appeared to form a distinct subpopulation diverged from typical human strains. The finding that *emm* subtypes were not shared and clonal clusters were not formed between the two populations, despite the high rates of recombination in the human population of SDSE (McMillan et al. 2010; McMillan et al. 2011), suggests host niche separation between human and animal strains. Therefore, isolates with the *emm* types stL1929, stC9431, stC7505 and perhaps stC46, probably belong to the dog ST cluster. However, this study has also provided evidence of genetic recombination between these populations and alleles shared between typical human strains of SDSE, typical dog strains of SD/SDSE and *S. canis*.

Genetic transfer appears to have occurred in both directions; from human SDSE STs to dog SD/SDSE STs and vice versa; the alleles *gtr4* and *recP12* were present in both human and dog STs. However, *gtr4* is more common in human STs and *recP12* is more common among dog STs. This distribution suggests that *gtr4* is likely to be of human ST origin and *recP12* of dog ST origin, suggesting that while host niche separation is evident some level of genetic exchange between the niche adapted bacteria has occurred.

Whilst host specificity is evident among SD/SDSE, this study has found evidence of typical human strains of SDSE being isolated in dogs. This is supported by the observed isolation of a homologous strain of SDSE isolated from both a child and dog from the same household in the study community. Furthermore, documented cases of human streptococcal disease associated with strains of SDSE with *emm* types and STs that are seen with dog STs are further evidence of the zoonotic ability of SDSE (Ahmad et al. 2009; Beall 2012).

The evidence of zoonotic transmission and genetic exchange between human and animal SDSE suggest that the species have not diverged completely. Furthermore, if these subgroups of human and dog STs are frequently in an environment where they come into contact with each other and other species such as SDSD and *S. canis*, will recombination and genetic exchange be ongoing, further blurring the species/subspecies barrier?

Dog SD/SDSE STs may also function to facilitate recombination and genetic transfer events with other streptococcal species evident by the finding of a chimeric *S. canis* allele that begins as a typical *S. canis* allele and ends as a typical dog SD/SDSE allele. The *S. canis gki6* allele was only found in *S. canis* ST8, but SDSE *gki*10 and SDSE *gki*17 were found in all dog SD/SDSE STs. This suggests that the direction of HGT was from a dog SD/SDSE to an *S. canis* recipient. Other interspecies genetic exchange and recombination events between dog SD/SDSE and *S. canis* could also have facilitated the novel strains of Lancefield group C *S. canis* with biochemical homogeneity to typical SDSE strains.

The *S. canis* STs 1, 2, 3 and 4 have previously been found to be associated with diseases in humans (Ahmad et al. 2009). Whilst ST1 has been found in a dog sourced from the Northern Territory, these human infective STs did not cluster together and often formed CCs with STs found in isolates from dogs in this study. Therefore, in contrast to SDSE it does not appear that there are particular strains of *S. canis* that are more prone to colonising humans. Interestingly, ST8 and ST7 appeared to be further diverged from the other *S. canis* STs. The isolate that belonged to ST8 contained the chimeric *gki6* allele and isolates from ST7 produced the Lancefield group C antigen. The fact that the isolate that belonged to ST8 was found in a wild dog with minimal to no human contact further suggests that dog (or animal) SD/SDSE strains have facilitated interspecies recombination in this case. Further characterisation of *S. canis* from both domestic and wild dog populations with genetic comparisons to dog SD/SDSE are needed to investigate whether there are strains of *S. canis* with homology to SDSE that may be diverging from typical *S. canis* strains.

The continued ability of SDSE and other streptococci of both human and dog origin to come into contact with each other in shared environments will most likely result in further variability of the streptococci and emergence of other novel strains that are difficult to classify. The three strains of *S. dysgalactiae* that could not be identified to the subspecies level form part of the dog SDSE ST cluster and do not share alleles with the non β-haemolytic SDSD strain. In a previous study using multilocus sequence analysis (MLSA); using seven different housekeeping genes to MLST, a β-haemolytic stL1929.0 strain which also fermented glycogen was reclassified as SDSD (Jensen & Kilian 2012). In the MLSA analysis of that study the SDSE separated into two sub-clusters and the SDSD into two sub-clusters, one which only contained β-haemolytic isolates. The authors concluded that all β-haemolytic *Streptococcus dysgalactiae* of animal origin are in fact SDSD. Since dog STs do not appear to share *emm* types with human STs, strains with *emm* stL1929 would most certainly belong to the dog SD/SDSE STs. Therefore, under this classification isolates that belong to the dog cluster of STs would be identified as SDSD. The 3 strains of SD from this study, that appear more closely related to the SDSD may constitute strains of β-haemolytic SDSD that can infect humans; as both *emm* types stC46 and stL1929 have been found in invasive disease in humans (Ahmad et al. 2009; Beall 2012); or support the current classification scheme that there are SDSE ecovars that represent strains that are more suited to a human host and strains more suited to an animal host. Further studies using larger samples of non β-haemolytic SDSD, β-haemolytic SDSD/dog SDSE and human SDSE are required to determine a more definite classification scheme of SD subspecies.

The isolates from the dog SD/SDSE ST's in this study were positive for a number of virulence genes *speG*, *sfbI* and *sdn*, unlike those of the human cluster. Furthermore, isolates from the dog SD/SDSE STs encoded *sfbI* genes that appeared to have some level of sequence homology (same HRM curve) with the *sfbI* positive *S. pyogenes* strains. Further investigations are required to assess the level of sequence homology and phylogeny of the *sfbI* gene between the species to investigate the origin of this gene. Whether dog SD/SDSE STs encode more virulence genes than the human SDSE

ST cluster overall remains to be examined. The amplified *speG* product also differed between the isolates of the dog SD/SDSE STs and those of the human SDSE STs. In addition, only the 3 SD strains were positive for *sdn*. These differences in virulence gene profiles of dog and human STs are probably a result of host tropism in which the presence of particular virulence genes are important in the colonisation and pathogenesis of the particular host species.

Further comparisons of animal derived SD/SDSE with human SDSE, using techniques such as representational difference analysis used by Davies et al (2005) to detect genetic difference between related species or strains, could determine whether these animal isolates do contain more virulence genes. If they are found to contain more virulence genes, and determination of the direction of gene movement can be investigated, then it may provide further evidence as to whether animal strains could function to provide typical human SDSE with greater virulence capacity. On the other hand, perhaps the HGT of virulence genes in animal strains of SDSE to other species such as *S. canis* could also facilitate increasingly virulent strains of other normally commensal streptococci. Furthermore, investigations into antibiotic resistance in dog strains and other bacterial species such as *Staphylococcus aureus* which also occur in dogs and human skin sores with SDSE may provide further evidence of HGT.

The frequency at which zoonotic strains of SD/SDSE are found in humans is unknown and requires further investigation. Further characterisation and exploration of Group C and G strains (either SD/SDSE or *S. canis*) associated with invasive disease in humans in Aboriginal communities and carriage in dogs may provide further insights into the relevant zoonotic risks of these strains to Aboriginal communities.

From these findings one can hypothesise that the continued ability of SDSE to colonise different host species in communities that have high rates of streptococcal infection could result in ongoing recombination and HGT resulting in further variability among the streptococci. It is also possible that this continued swapping of genes and heterogeneity could result in the emergence of highly virulent clones that could potentially cause disease in both or either humans and dogs.

In conclusion, this study has confirmed that there are strains of SD/SDSE that can be shared between dogs and humans in this community. Strains of SDSE appear to be relatively host specific, but this is not absolute. This study has provided genetic evidence to suggest that SDSE strains and other species of streptococci are exchanging genetic information within either human or canine host. The finding of an essentially identical SDSE strain in a dog and a child sharing the same home environment supports the hypothesis of zoonotic transfer of streptococci within communities. The continued exchange and swapping of genetic material between the *S. dysgalactiae* and other species such as *S. canis* may have further facilitated strains to transfer between host species. Therefore, longitudinal-population based studies sampling dogs and humans for streptococci over time are important in establishing the epidemiology, origin and adaptation of SDSE STs and other strains of streptococci. Studies will also provide further information as to which species (and SDSE ST's) frequently colonise humans or animals for extensive periods and which are merely short-term transients. Further investigations into the epidemiology, genomics and speciation of β-haemolytic *Streptococcus dysgalactiae* should include humans, dogs and potentially other animals (i.e., horses and wildlife) within a given population to gain further knowledge about host tropism, genetic exchange and virulence capacity of *Streptococcus* spp. in communities.

Chapter 6: Knowledge translation and strategies to improve dog health and ownership in the study community.

6.1 Introduction

The findings of this thesis have provided evidence of a shared SDSE strain in a child and dog and genetic exchanges between *Streptococcus* spp. infecting dogs and humans within this Aboriginal community. Whilst this finding has relevance in the scientific arena and possible implications in the classification of strains within the *Streptococcus* genus the ability to translate this knowledge in context with the study community is extremely important in providing outcomes for the community.

Knowledge translation (KT) describes the dissemination of knowledge between researchers and users with the expectation that knowledge results in the capacity to act (Landry et al. 2006). Therefore the use of KT is extremely important in providing outcomes and action especially for research that would otherwise be observational.

In the context of this study, KT can be used to both disseminate the research findings of the streptococcal component but also to develop strategies and disseminate information or resources to improve dog health within the community. Implementation of KT can face many problems that relate to the applicability and transferability of information and how they are suited to the context of the community. In many cases KT is often confused with knowledge transfer which is largely a one-way linear process in which knowledge is passed from researcher to user without privileging the knowledge of the study community. KT differs in that it is a process that is non-linear, two-way and ongoing (Estey et al. 2008). Therefore, in order to ensure that KT within this study would be suitable the community discussed their concerns around dog health and suggested KT strategies that they believed would be easily applied and successful within their own community.

Therefore, the aim of this chapter is to gather information around perceived issues of dog health faced by the community and suggested strategies of improvements. The results of this chapter will be used to identify strategies of KT that will be used for the purpose of disseminating research findings in an applicable and transferrable way, and sharing knowledge with community members to improve healthy dog ownership with the assumption that it will result in healthier dogs.

6.2 Methodology

A qualitative methodology, described in detail in chapter 3, was used in this component of the study. A combination of methods was used in this chapter for the purpose of achieving KT outcomes. These methods are shown in Figure 6.1 and can be separated into two components.

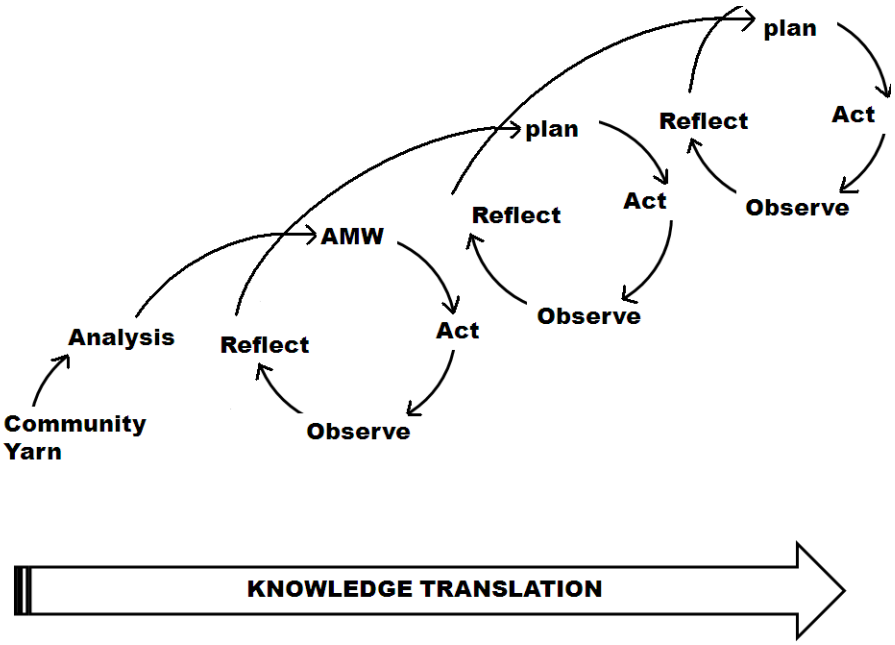

Figure 6.1. Qualitative methods used in this study.

The first component represented by the cyclical illustration involves the process in which the community yarns (interviews) around the topics of dog health and dog health strategies were used to inform the researcher and AMW collaboratively, and consequently plan actions around these suggested strategies that were implemented, observed, reflected and modified accordingly to result in better planning and more efficient actions.

The results of the first component were used to determine the KT content and the strategies suggested by community members that would be best suited to deliver KT. These strategies of KT were also used to feedback results of the streptococcal project.

The components of the methodology are described in further detail below.

6.2.1 Community yarning

Trialling of semi-structured interview questions as described in chapter 3 resulted in the adaption of a semi-structured interview to a yarning protocol. The yarning methodology has been described in chapter 3 and has been validated as a culturally appropriate method with Aboriginal participants (Fletcher et al. 2011). Yarning was an appropriate method in this case because many of the participants were already known to the researcher and therefore this method ensured that participants were more comfortable responding. Subjects were introduced in a deliberately open manner and were aimed at gaining information around the topics of what should be done with dogs in the community and the ways in which these suggestions could be delivered in a culturally appropriate manner ('good ways') (Appendix 11). There was no set way of asking questions and the delivery often differed with each participant according to the flow of the current conversation. Conversations were recorded in writing by hand. At the end of the yarn the transcription was read back to the participant for confirmation and validation. This process often engaged further yarning.

6.2.2 Participants

Prospective participants were informed of the research aims verbally and required to sign a consent form if they agreed to participate and have their responses recorded in writing by hand.

Ten community members were spoken with; 4 Aboriginal community members who own dogs (CD), 3 Aboriginal community members who did not own a dog

(including one Elder) (CND), and 3 non-Indigenous people who worked in the community (NI).

6.2.3 Analysis

Participant conversations were transcribed into Microsoft Word documents. Direct quotes were kept in the exact language of the participant and therefore should be interpreted accordingly. Each individual conversation was treated as a separate entity regardless of whether more than one participant was interviewed at the one time. Each participant's yarn was transferred into NVIVO version 9 (QSR International Pty Ltd) qualitative analysis tool. NVIVO is an analysis tool used to analyse, arrange and organise unstructured data such as yarns into categories and themes. In this study NVIVO was used to collate data and organise data. Each yarn was thoroughly examined and responses were collated into preliminary themes based on the content. Further examination of these themes resulted in the data of each theme being assigned to one of the following three categories: (i) issues identified, (ii) gaps in knowledge, and (iii) suggested strategies.

6.2.4 Development and implementation of knowledge translation strategies

The results of the community yarns were used by the researcher, AMW and EHO to collaboratively develop knowledge translation strategies assuming that this would improve capacity of dog owners (and other community members) to care for their dogs. The suggested strategies implemented and knowledge gaps perceived by the community yarns were used to develop KT content and strategies of delivery through a participatory action methodology. The PAR methodology has previously been described in chapter 3. In this study it was a cyclical process where the researcher, AMW and EHO worked collaboratively to plan KT strategies and implement and modify these according to their reflections. This process has previously been illustrated in Figure 6.1.

6.3 Results

6.3.1 Community yarns

Several themes were drawn from the community yarns that highlighted the perceived issues of dogs in the community and offered strategies for improvements (Table 6.1).

Table 6.1. Dog health issues identified and strategies suggested to improve dog health in the Yarrabah community

Issues identified	Strategies suggested
Dogs and disease	-Community days (dog care days): treatment of scabies, parasites and fleas -Dog scabies reporting by community members
Stray dogs/high population of dogs	-Dog registration -Improved fencing to contain dogs -Spaying programs -Implementing 1-2 dog restriction
Cheeky (aggressive) dogs	-Improved fencing to contain dogs -Dog obedience training
Lack of regular veterinary service	-Regular veterinarian service
Gaps in knowledge identified by participants	
Basic dog care	-Community days (dog care days) -Dog care promotional materials -School visits for educational awareness -Regular dog wash service
Routes of transmission of zoonoses	-Community days (dog care days) -KT materials about canine zoonoses
Diagnosis of disease in dogs	-Regular veterinarian service and/or consultation days

6.3.2 Dogs and disease

The potential of dogs transmitting zoonoses was recognised as an issue by all participants. Furthermore, many of the severely mangey dogs were rejected from households and roamed the streets with community members often unwilling to handle them. First-hand knowledge of canine zoonoses was also experienced by community members;

> *"My daughter had a growth on her head that caused a bald spot they said [doctor] it was from a dog. We don't even own a dog and she must've*

touched a dog and then touched her head and she had to do intense treatment." (CND2)

"One of my cousins he owned a lot of dogs and had dog worm in his heart and had to fly down to Brisbane." (CND1)

The strategies suggested to control zoonoses included a community day organised by the AMW with treatments for scabies, worms and fleas available for community use. Another participant suggested that community members should also report mangey dogs that may have scabies to the AMW for treatment or disposal;

"Get community members to report mangey dogs. The community has to take responsibility as well and report that dog." (CD1)

6.3.3 Stray dogs/high population of roaming dogs

The high population of roaming dogs was perceived as playing a role in dog health issues with some respondents believing that approximately half the dogs were strays. Implementing dog registration and improvements in household fencing were suggested by participants to establish ownership of dogs and also inhibit the ability of dogs to roam freely.

"Most of the dogs, probably half, aren't owned by anyone. Have a dog registration day and all the others should be killed" (CD1)

Respondents also acknowledged the role of puppies in the high numbers of stray or unwanted dogs and reflected on the benefits of the yearly spaying program.

"Lots of dogs aren't owned by anyone, they grow up from pups and leave their mother cause there's no food there, then they go to other places to eat… desexing was good, we enjoy it now, no pups to worry about." (CD3)

The high numbers of dogs per household was also perceived as an issue and suggestions to enforce a 1-2 dog restriction per household were made.

> *"Elders could have 2-3 dogs, after their partner goes they look to that [dog] for comfort, they need to be supported because they have a connection with that dog. But 2 dogs per house is sufficient, 3-5 is too many".* (CND3)

6.3.4 Cheeky (aggressive) dogs

Community members also commented on their fear for their own or their children's safety with aggressive dogs (referred to as "cheeky dogs" by community members) roaming the streets. Respondents reflected on experiences where family members or other community members had been attacked or bitten by dogs:

> *"They [pack of dogs] were all jumping on her and trying to drag her up the road and the gutter… if they had of kept going I think she would be dead."* (CD3)

An elder also expressed her concern for her own safety from free roaming cheeky dogs. All respondents that spoke about dog aggression suggested that improvements to household fencing and stopping dogs from roaming freely would be of benefit to community members. Dog behaviour classes were also suggested to improve aggressive dog behaviour.

> *"Main concern is safety. You can't walk down the road without a dog rushing you [running towards you aggressively]. I won't walk anywhere, I'd rather go in the car... fence the dogs in."* (CND3)

> *"When you have a pack of dogs, always keep them dogs locked up, it's up to the owner to look after them. It only takes one dog to kick it off, just imagine if a kid walks into that persons yard."* (CD3)

6.3.5 Lack of regular veterinary services

The community is 40 minutes away from a veterinary service over a large mountain range. Lack of transport and the low income status of many residents in the community were recognised as barriers inhibiting the ability of dog owners to receive veterinary treatment for their dogs.

> *"It's hard for people to take their dogs into town. There are some people who don't have money to do it because when you go to the vet you have to pay up front. Let us keep the dogs in the pound, let us check on them till payday comes and then the vet comes over...Biggest thing is to have a vet...We need a vet here 24/7... so when your dog's sick, straightaway take them there, straightaway...A lot of people love their dogs but people just don't know what's wrong with their dogs."* (CD3)

6.3.6 Knowledge translation strategies to improve capacity of community members to implement basic dog care

Respondents felt that many community members needed to be informed of basic dog care. Strategies suggested for knowledge translation of dog ownership and care included community days that dog owners could attend to discuss dog issues and be provided with information, such as pamphlets, that they can take home.

> *"…people need more information on basic dog care, they [dog owners] just know how to feed them and some don't even do that".* (CND3)

School visits were also suggested and promotional posters placed strategically at places of business such as the Post Office and community controlled health organisation.

> *"Go into the school, kids need to be educated about the dogs. All we know is you have a dog and feed it scraps...education in the school will be good because those kids they love dogs".* (CND2)

One respondent also reflected on a mobile dog wash service that had previously visited the community on a fortnightly basis.

> *"You should wash your dog, but we don't hardly wash dogs here. A dog wash would be good, I paid $25-$30 a fortnight, he would just drive passed and people would sing [yell] out and he would bath them in front of the house, the dog wash worked in this place."* (CD3)

6.3.7 Strategies for knowledge translation of routes of zoonotic transmission

Many respondents identified that more information was needed on the pathways of transmission of canine zoonoses and how these zoonoses are presented in both dog and humans. All respondents recognised the role of scabies in dogs. Furthermore, one respondent commented that he had let his dog lick his child's leg sores under the common belief that the saliva of the dog had antiseptic qualities. The researcher and other family members also reflected on this belief from their own childhood, it is unknown whether this is widely practiced by other community members.

> *"I gave a community member a lift home who thought that sleeping next to his dog had put him at risk of a dog heart worm crawling out of the dog and into his heart. People need to know about transfer, like not just through direct contact but also through the dirt and the environment."* (NI1)

> *"People don't need to know what mange mites look like, people will know straight away because of the sores on the dog. Everybody knows that when a dog with a good coat plays with a mangey dog they can get it too."* (CD3)

School visits, community dog days and the development of promotional materials about the transmission of canine zoonotic agents were all suggested by participants to improve the community knowledge and capacity to care for their dogs.

> *"The ranger [AMW] should put a day on… a dog day in each little suburb… with slides and a microscope… and other people from outside to come and talk about dogs, like a vet or something."* (CD2)

6.3.8 Summary of Interviews and planning strategies advised by the EHO and AMW

Community members who participated in the study perceived unhealthy dogs as an issue affecting human health mainly via zoonoses such as the dog scabies mite and dog attacks. However, for other zoonotic diseases, such as the dog heart worm, there was no clear understanding of the pathways and likelihood of transmission. This lack of information appears to have resulted in a fear of catching sickness from dogs to the point that it was a factor inhibiting dog ownership from participants who did not own dogs.

All respondents commented on the importance of community engagement in implementing any strategies for dog health improvements. The most resounding strategy was the development of a community dog day. Yarrabah community members were already familiar with community awareness days which were currently used by the health services to promote human health awareness. It was suggested by almost all respondents that a dog health awareness day would be beneficial in engaging with community members and dog owners.

Suggested strategies were discussed with the EHO and AMW who chose which actions to implement based on their priorities and current resources. The EHO had also previously suggested the development of a dog care day which were obviously supported and confirmed by discussions with community members. Subsequent discussions with the researcher, AMW and EHO resulted in the implementation of a 'Yarrabah dog care day'. The AMW and EHO felt that it was important because its establishment could serve as a platform for knowledge translation and dissemination of the other strategies, such as dog registration and desexing.

The results of the community interviews also provided motivation for the AMW and EHO because it showed that community members were interested in dog health

issues and had strategies to improve dog health and ownership that often corresponded with the EHO and AMW's ideas.

6.4 Outcomes: Knowledge translation strategies implemented in this study

This combination of community yarns and collaboration with the AMW and EHO resulted in the implementation of various KT strategies described in Figure 6.2. These strategies are described in further detail below.

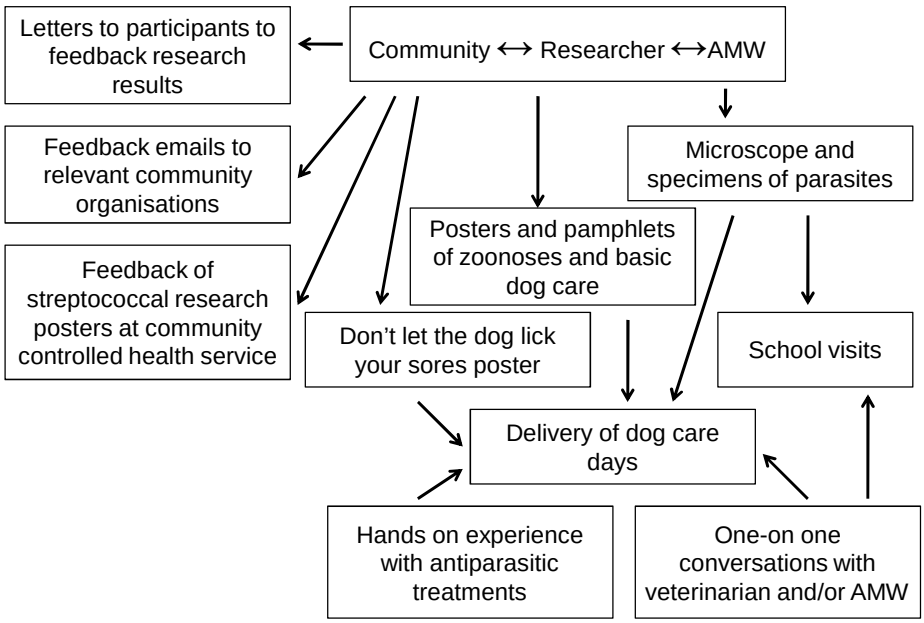

Figure 6.2. Knowledge translation strategies used in this study

As described in Figure 6.2, the KT that occurred between the community yarns, researcher and AMW collaborations were two-way and continual. From these interactions KT strategies and content could be implemented based on community knowledge and readiness.

6.4.1 Feedback of streptococcal research

Feedback of streptococcal research results were written in community language and delivered via individual letters sent to each of the participants' home, emails to relevant organisations and a poster at the community controlled health

organisation (Appendix 3). In all strategies, the content of the KT differed slightly to suit the reader and all reiterated a theme already promoted by the health service around the importance of keeping skin healthy.

The finding that some individuals within the community may allow dogs to lick their children's sores as a means of cleaning them combined with the association of SDSE and invasive disease may increase the risk of zoonotic transmissions in both directions. The belief that dogs can heal human sores by licking them is also shared by other cultures and communities (Benjamin et al. 1997). There have been documented cases of wound healing following dog licking (Verrier 1970; Benjamin et al. 1997; Williams 2005). However, since there have been no randomised control trials on dogs licking sores evidence of the beneficial effects remains low level. Furthermore, dog licking has been associated with cases of cellulitis, septicaemia, necrotising fasciitis, sinusitis and renal failure (Yu, Boike & Hladik 1995; Anderson, Jayawardene & Carmichael 2000; Schmulewitz et al. 2008), although streptococci were not isolated as the causative agent in any of these documented cases.

It is unknown how widely this is practiced within the community but subsequent conversations suggest that it is rare. The community controlled health service runs a healthy skin program in conjunction with the school twice a week. The teachers report children with sores to the Aboriginal health workers who clean and bandage the sores and discuss the importance of keeping skin clean. To further re-iterate this, another KT poster was developed to encourage children to not allow dogs to lick them and was displayed at the dog care days (Appendix 12). Education programs promoting healthy skin combined with promotional materials developed by the AMW are important in informing community members of the importance of keeping skin healthy.

6.4.2 Development of Yarrabah dog care days

The development of Yarrabah dog care day was extremely successful and its formation is important as a platform for other KT strategies as shown in Figure 6.2.

The dog care days were promoted by an initial visit to the Primary School in which a veterinarian and AMW (or EHO) were available to discuss cheeky (aggressive) dog behaviour and build awareness around dog care. The dog care days were also promoted through the use of posters and pamphlets which were placed strategically around the community and handed to community members. The local Indigenous radio station (Bumma Bippera Media) also announced the event during the week prior to the event day. The date of each dog care day was chosen by the EHO and AMW and was chosen according to the days that community members would be available to attend (based on pay week, rostered days off and other community events). Therefore the days were usually run on the first Saturday of each school holiday.

Approximately 30-50 dogs were treated at each Yarrabah dog care day with requests from community members for the continuation of these days. Therefore, during the research project 3 dog care days were held, reflected on and improved by the EHO, AMW and researcher (Figure 6.3). The days were initially held in only one location but in response to suggestions from community members resulted in a progressive service that moved between 3 locations in the one day, to ensure all community members had access to the service. Reflections also resulted in the provision of leashes to inhibit dog fights and promote the use of leashes when walking with dogs around the community.

Figure 6.3. Yarrabah dog care day at two separate locations within the community.

Throughout the research project the researcher continued to work with the AMW to build her capacity and experience in the facilitation of dog care days. This was to ensure the sustainability for the continued organisation of these days. An

instructions booklet was developed with the AMW describing procedures for organising a dog care day which included a time line of events and contact details of volunteers usually involved in the day. At the end of this research project the AMW was provided with a box of dog care day resources which included a large supply of posters, pamphlets, specimens of parasites (accompanied by organism descriptions), dissecting microscope and instructions for organising a dog care day. All promotional materials were also loaded onto the AMW's computer and backed up on a CD-Rom included in the resource box.

6.4.3 One-on-one conversations with veterinarians, animal management workers and other individuals.

Volunteer veterinarians and research veterinarians who were currently conducting research in the community were encouraged to attend the dog care days and prior school visit to share knowledge about dog health. The AMW and EHO were available to discuss other dog issues with owners and assist in the application of anti-parasitic treatments. An RSPCA representative also attended and provided donations of dog food and dog toys to be taken home by dog owners.

6.4.4 Hands on experience for dog owners in applying anti-parasitic treatments

Medications provided by the AMW at each dog care day were made available at no cost and included flea/tick spray (Permoxin Dermacare-Vet Pty Ltd) and mange treatment (Cydectin or Ivomec). Dog owners were encouraged to apply treatments to their own animals (Figure 6.4).

Figure 6.4. Hands on application of anti-parasitic treatments by dog owners and EHO.

6.4.5 Use of microscope and specimens

A dissecting microscope was used during the dog care days and at the primary school visits. Children (and often adults) were encouraged to view the specimens of roundworms, tapeworms, heartworms, ticks and fleas either under the microscope or directly in the specimen container. A one page description with dot points briefly summarising each organism and a large picture were available to complement the parasitic specimens. This was very successful in giving both adults and children an opportunity to view these organisms first-hand. During the dog care days many children opted to remove ticks from their own pets and view them under the microscope (Figure 6.5).

Figure 6.5: Children viewing a tick under the microscope at a dog care day.

6.4.6 Development of KT materials

The AMW was included in the development of all posters, pamphlets and dog care day resources. Pamphlets describing correct dog washing techniques, cydectin application, flea (permoxin) treatment application and dog nutrition were previously developed by the AMW and another researcher (Constable 2012).

Posters were developed to ensure they were visually appealing with short comical slogans. The use of health promotion materials that are localised (e.g., using community language), personalised (e.g., by use of images of community residents) and made more humorous have previously been shown to be successful in conveying health messages in Aboriginal communities (Massey et al. 2011). Both researcher and AMW agreed that short comical slogans would be accepted well in the Yarrabah community. The posters covered the issues of securing aggressive dogs, treating dogs for parasites and scabies, discouraging allowing dogs to lick sores, good diets for dogs and dog restrictions, some of which are available to view in Appendix 12.

6.5 Conclusion

During the yarning process the importance of community engagement was clearly expressed and the success of any strategies to improve dog health in the community was dependent on KT, community involvement and capacity building. The development of Yarrabah dog care days and their regular operation was extremely important in providing information to build the capacity of dog owners to care for their pets. Many dog owners that brought their animals for treatment were only seeking confirmation that they were doing an adequate job. However, other issues such as lack of, or damaged fences and no regular veterinary service made it difficult for community members to practice responsible pet ownership. In addition, the lack of fencing has also impeded the ability of the AMW to enforce registration and containment laws, which has a direct effect on the ability of the AMW to distinguish stray dogs from owned dogs.

In conclusion, the development of dog care days at the request of the community has been successful, with the EHO aiming for the continued operation of 4 dog care days per year. The collaborative development of KT materials and resources with the AMW has ensured inclusion of community language and capacity building of the processes required to organise this event. Whilst it is not expected that the dog health of the community will improve immediately from the instigation of these days, they function as a place for open dialogue between community dog owners, community animal workers and veterinarians in which further strategies can be implemented.

Further strategies and dog health improvements rely on the improvements in other areas of the community such as improved household fencing including gates and regular veterinary services, will ensure that dog owners are supported to practice healthy dog ownership.

This chapter has shown that members of the Yarrabah community are concerned about their dogs and are eager to improve the well being of their animals through building capacity of dog owners (and other community members) to practice healthy dog ownership.

This chapter has also demonstrated how KT can be used to transform research that is largely laboratory based science into useful outcomes for the participant community. Qualitative research methods allowed the two-way exchange of information and greatly enhanced the effectiveness of the KT process.

The next chapter concludes the thesis, drawing together the findings of both components of the study and offering recommendations for further study.

Chapter 7: General conclusions and recommendations

This study produced a number of outcomes related to it aims:

Aim 1: This study has shown that dogs carry species of β-haemolytic streptococci: *S. canis*, SD/SDSE, *S. agalactiae* and SESZ.

Aim 2: There were no significant relationships found between health and social parameters in dogs and the likelihood of isolating β-haemolytic streptococci.

Aim 3: Children in this study were found to be infected with one or more of two streptococcal species *S. pyogenes* and SDSE with one child sharing a homologous strain of SDSE with a dog from the same household. The genetic characterisation of strains of SD/SDSE from dogs and children revealed that, while many of dog derived strains belonged to a separate genetic cluster, strains have the ability to be shared among both human and animal hosts. The frequency at which this occurs is unknown and requires further study. The evidence of exchange of genetic information between *Streptococcus* spp. inhabiting dogs and humans also lends evidence to the notion that some streptococci move between dogs and humans in the community potentially acquiring adverse traits that may relate to host adaptation or potential virulence.

Aim 4: The use of a qualitative methodology has provided a means for knowledge translation of the research findings that are in context with the community. It also provided evidence of community concerns around safety, transmission of canine zoonoses and the health and welfare of dogs within this community.

The combined use of the two methodologies and the implementation of a KT component resulted in the ability to conduct a strongly scientific study in a culturally appropriate manner that has provided outcomes for the study community. The

implementation of KT into other scientific studies conducted in Aboriginal communities would also be beneficial and is recommended.

Although the significance of dogs as a source of streptococcal disease in humans cannot be obtained from the data in this study, interventions (and people residing in these communities) often cannot wait for evidence. In this study, simple actions to minimise transfer can still be implemented. Encouraging healthy skin protocols in humans and mange treatments in dogs is likely to be of benefit for several reasons, one of which could be to minimise streptococcal infection and disease in both people and dogs. Other studies investigating the effect of poor nutritional condition in animals and excretion of salmonella showed that animals of poor nutritional condition were more likely to excrete salmonella and in higher numbers (Hart, Bradshaw & Iveson 1984; Speare & Thomas 1988). This has not been shown for any *Streptococcus* spp. but it can be assumed that healthier dogs would have a reduced likelihood of possessing high numbers of streptococci than dogs in poorer condition. Therefore programs that reduce dog numbers and improve dog health are likely to have some benefit for community members.

Quantitative bacterial studies of dogs and humans in which bacterial concentrations can be assessed, by using quantitative PCR techniques for example, coupled with genetic markers would provide much greater insight into the routes of transmission and forces driving infectious diseases in the human-canine environment of Aboriginal communities.

Comparative studies investigating zoonoses in Aboriginal Australian communities are important. However, the ability to translate research and provide tangible outcomes to the study community that coincide with the community's priorities is equally important. The National Health and Research Medical Council (NHMRC) (2010), recently published ethical guidelines for conduct of research in Aboriginal and Torres Strait Islander communities which outlined the critical importance of community collaboration and knowledge translation in the research process.

The following recommendations are made for further research on canine zoonoses in Aboriginal communities:

The history of the study community and current health issues should be investigated prior to developing a research proposal so that the research project can include components that are relevant to the community;

The researcher should live in close proximity to the study community or be willing to relocate because the benefit of being in constant contact with community is crucial in engaging with community but also translating outcomes;

Establishment of early collaborations with relevant community organisations and the community controlled health service, while time consuming, will be beneficial in meeting deadlines in the long-term;

Research should include components that are beneficial to the community, build capacity of community members and deliver tangible outcomes for the community.

This study, like many other studies of zoonoses in Aboriginal communities has provided grounds for further studies on the role of dogs in the epidemiology of streptococcal infections and disease in Aboriginal communities. These results should not be ignored. However, their significance should not be over or underestimated. It seems likely that the continued sharing of environments by Aboriginal people and their animals will result in further two-way transfer of other micro-organisms and the evolution of novel strains which may or may not have pathological relevance in one or other host.

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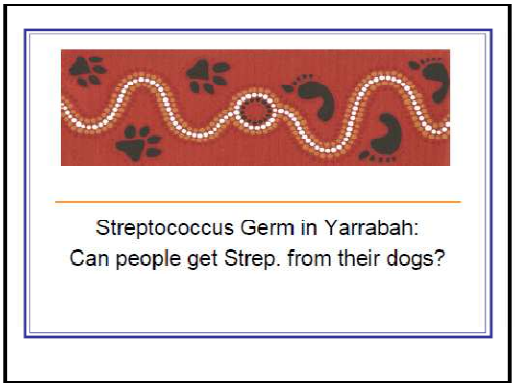
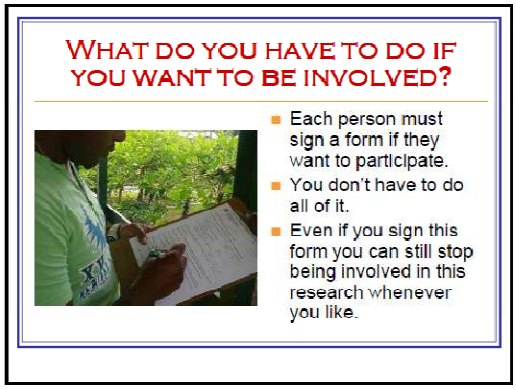
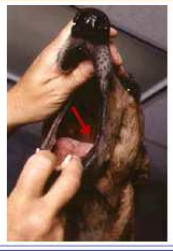
Appendices

Appendix 1: Picture book used to recruit participants into streptococcal research component.

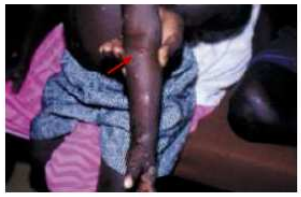




1. Let me swab your dog



Allow a swab to be wiped on your child's skin sore



Answer one page survey

- All questions are only for this research to find out why dogs might be carrying strep. I am not here to look down on you in any way.
- Your answers will NOT be given to ANYONE else or organisation behind your back.

Your information will be kept very private

No names or addresses will be on swabs or surveys. These codes will be kept private

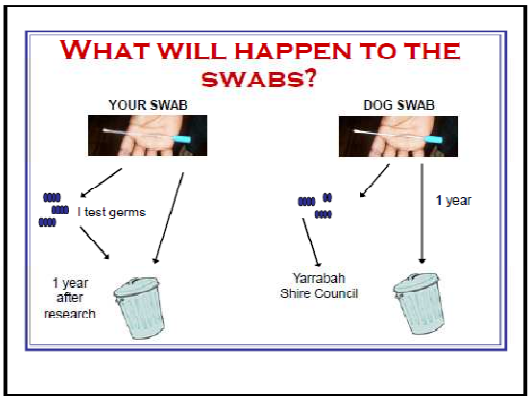
How will you get your results?

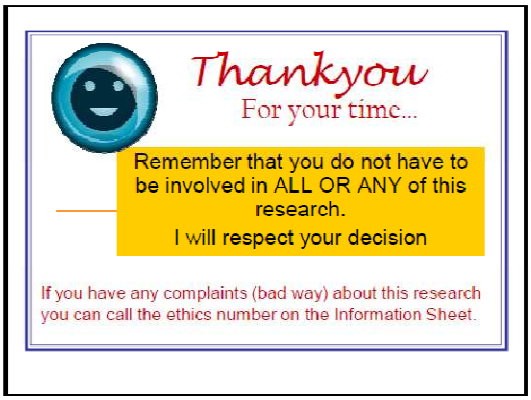
YOU WILL ONLY GET YOUR RESULT IF YOU NEED MEDICINE

you will receive a letter in the mail with your results on it. But you can call me anytime for your results too.

Final Results

- I am hoping to finish by the end of next year 2011.
- By this time, I will send you a letter telling you what I found out through the whole research, (No names will be mentioned).


WHAT WILL HAPPEN TO THE SWABS?
YOUR SWAB
DOG SWAB
I test germs
1 year
1 year after research
Yarrabah Shire Council


Thankyou
For your time...
Remember that you do not have to be involved in ALL OR ANY of this research.
I will respect your decision
If you have any complaints (bad way) about this research you can call the ethics number on the Information Sheet.

Appendix 2: Grading system used to measure body condition and skin condition. PBC developed by Nestle Purina.

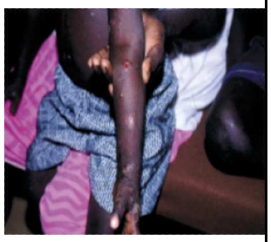
	Purina Body Condition (PBC)	Grade	Skin Condition
TOO THIN	Not applicable	**0**	No visible lesions
	-Ribs, lumbar vertebrae, pelvic bones and all bony prominences evident from a distance. -No discernable body fat -Obvious loss of muscle	**1**	Lesions on feet or slight balding on one body part.
	-Ribs. Lumbar vertebrae, pelvic bones and all bony prominences easily visible -No palpable body fat -minimal loss of muscle mass	**2**	¼ to ½ of the body affected by balding and/or lesions.
	-Ribs easily palpable, with no palpable fat covering -waist easily noted -abdomen tucked up when viewed from the side	**3**	½ to ¾ of the body affected by balding and/or lesions.
IDEAL	-Ribs palpable with minimal fat covering -Waist easily noted, viewed from above -Abdominal tuck evident	**4**	¾ to full body affected by balding and/or lesions but hair still visible in areas.
	-Ribs palpable without excess fat covering. -Waist observed behind ribs when viewed from above -Abdomen tucked	**5**	complete hair loss.
TOO HEAVY	-Ribs palpable with slight excess fat covering -waist is discernable viewed from above but is not prominent -Abdominal tuck apparent	**6**	Not applicable
	-Ribs palpable with difficulty -heavy fat cover -Noticeable fat deposits over lumbar area and base of tail -Waist absent or barely visible -Abdominal tuck may be present	**7**	Not applicable
	-Ribs not palpable under very heavy fat coverage, or palpable only with significant pressure -Heavy fat deposits over lumbar area and base of tail -Waist absent -No abdominal tuck (obvious distension may be present)	**8**	Not applicable
	-Massive fat deposits over thorax, spine and base of tail -Waist and abdominal tuck absent -Fat deposits on neck and limbs -Obvious abdominal distension	**9**	Not applicable

Appendix 3: Streptococcal research feedback poster.

Research results:
Dogs and Strep germ in Yarrabah

What did we do?

In May 2010, Layla Schrieber looked for different types of strep germ on 61 dogs and 6 people from Yarrabah.



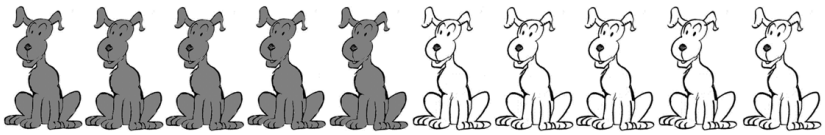
Strep skin sores can look like the picture above.

What is Strep?

"Strep" is a germ that can cause kidney, heart disease and skin sores or skin infections.
There are many different types of strep.
The main strep that causes disease in people is called Strep pyogenes.

What did we find?

We found that 50% of dogs had different types of strep. So if you had 10 dogs, 5 of them might have these streps (this is like saying that the grey dogs in the picture below would have strep).
Even though mangey/skinny and healthy/solid dogs have got strep, keeping dogs skin healthy and keeping your dogs inside the fence at your house and not having a lot of dogs in the one house will probably help to stop strep spreading in dogs.



Most **people** had strep pyogenes but NONE of the **dogs** had this type of strep that usually makes people sick. So that is good news. The dogs did have other types of strep that can sometimes infect a cut, an old burn or a diabetes ulcer and make a person sick.

What does this mean?

This means that sometimes dogs can catch strep from people and that maybe people and dogs can share some types of strep.
WE DON'T KNOW if this happens all the time or only sometimes.
WE DON'T KNOW if these streps are making people sick in Yarrabah.

Do Not be scared or worried about catching strep from your dog and YOU DO NOT HAVE TO KILL YOUR DOG

But if you have a skin sore, cut, burn or a diabetes ulcer you should keep it clean and covered just in case. Keep your dog healthy and don't let it run around with a big mob of feral dogs.

This research project was conducted by Layla Schrieber through the University of Sydney: email layla.schrieber@hotmail.com
Thankyou to all people involved in the study including:
-Yarrabah dog owners and community members
-Yarrabah Aboriginal Shire Council
-Stephen Canendo, Shanna Mossman, Alfred Gray Jnr and Thomas Gyemore.
-Gurriny Yealamucka Health Services Aboriginal Corporation;
Steve Isbister, Dr. Ashley Peake and Savannah Bulmer

Appendix 4: GenBank accession numbers of 16S rRNA sequences found in this study.

Isolated ID	Species	Lancefield group	GenBank accession number
A3	*S. canis*	G	JN176316
A7	*S. canis*	G	JN176317
B1	*S. canis*	G	JN176318
B9	*S. canis*	G	JN176319
B10	*S. canis*	G	JN176320
C1	*S. canis*	G	JN176321
C8	SDSE	C	JN176322
C10	*S. canis*	C	JN176323
D1	*S. canis*	C	JN176324
E6	*S. canis*	G	JN176325
E8	SDSE	C	JN176326
E9	*S. canis*	G	JN176327
F8	*S. canis*	G	JN176328
F9	SDSE	A/G	JN176329
F10	SDSE	C	JN176330
B3	SDSE	C	JN176331
B4	SDSE	C	JN176332
B5	*S. pyogenes*	A	JN176333
C2	*S. canis*	G	JN176334
C3	*S. canis*	G	JN176335
C4	*S. pyogenes*	A	JN176336
D2	*S. canis*	G	JN176337
D5	*S. canis*	G	JN176338
D6	*S. canis*	G	JN176339
F1	SDSE	C	JN176340
F3	SDSE	C	JN176341
F5	*S. canis*	G	JN176342
G1	SDSE	C	JN176343
G2	*S. pyogenes*	A	JN176344
G4	*S. pyogenes*	A	JN176345
B6	*S. canis*	G	JN176346
B7	*S. agalactiae*	B	JN176347
C5	SDSE	G	JN176348
C6	*S. canis*	G	JN176349
D8	*S. canis*	G	JN176350
E2	*S. canis*	C	JN176351
F6	*S. canis*	G	JN176352
F7	SESZ	C	JN176353
G5	*S. canis*	G	JN176354
G6	SDSE	C	JN176355
G7	*S. agalactiae*	B	JN176358

Appendix 5: 16S rRNA sequence alignment of *S. canis* producing Lancefield group C antigen and GenBank *S. canis* sequence (AJ413205.1). (*) along the bottom row indicate identical nucleotides and (.) represents nucleotides that are different. (-) represent missing data.

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S.canis_AJ413205.1          AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTAGAAC 60
S.canis_group_C_C10         ------------TGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTAGAAC 48
                                        ************************************************

S.canis_AJ413205.1          GCTGAGGACAGGTGCTTGCACTAGTCTAAGGAGTTGCGAACGGGTGAGTAACGCGTAGGT 120
S.canis_group_C_C10         GCTGAGGACTGGTGCTTGCACCGGTCCAAGGAGTTGCGAACGGGTGAGTAACGCGTAGGT 108
                            *********.**********.****.**********************************

S.canis_AJ413205.1          AACCTACCTGATAGCGGGGGATAACTATTGGAAACGATAGCTAATACCGCATAAAAGTGC 180
S.canis_group_C_C10         AACCTACCTGATAGCGGGGGATAACTATTGGAAACGATAGCTAATACCGCATAAAAGTGC 168
                            ************************************************************

S.canis_AJ413205.1          TTAACACATGTTAAGAACTTAAAAGGGGCAACTGCTCCACTATGAGATGGACCTGCGTTG 240
S.canis_group_C_C10         TTAACACATGTTAAGAACTTAAAAGGGGCAACTGCTCCACTATGAGATGGACCTGCGTTG 228
                            ************************************************************

S.canis_AJ413205.1          TATTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGGCGACGATACATAGCCGACCTGAGAG 300
S.canis_group_C_C10         TATTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGGCGACGATACATAGCCGACCTGAGAG 288
                            ************************************************************

S.canis_AJ413205.1          GGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGG 360
S.canis_group_C_C10         GGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGG 348
                            ************************************************************

S.canis_AJ413205.1          GAATCTTCGGCAATGGGGGGAACCCTGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTT 420
S.canis_group_C_C10         GAATCTTCGGCAATGGGGGGAACCCTGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTT 408
                            ************************************************************

S.canis_AJ413205.1          CGGATCGTAAAGCTCTGTTGTTAGAGAAGAACGGTAATGGGAGTGGAAAACCCATTATGT 480
S.canis_group_C_C10         CGGATCGTAAAGCTCTGTTGTTAGAGAAGAACGGTAATGGGAGTGGAAAACCCATTATGT 468
                            ************************************************************

S.canis_AJ413205.1          GACGGTAACTAACCAGAAAGGGACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG 540
S.canis_group_C_C10         GACGGTAACTAACCAGAAAGGGACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG 528
                            ************************************************************

S.canis_AJ413205.1          GTCCCGAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTCTTTAAGTCT 600
S.canis_group_C_C10         GTCCCGAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTCTTTAAGTCT 588
                            ************************************************************

S.canis_AJ413205.1          GAAGTTAAAGGCAGTGGCTCAACCATTGTACGCTTTGGAAACTGGAGAACTTGAGTGCAG 660
S.canis_group_C_C10         GAAGTTAAAGGCAGTGGCTCAACCATTGTACGCTTTGGAAACTGGAGAACTTGAGTGCAG 648
                            ************************************************************

S.canis_AJ413205.1          AAGGGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAACACCG 720
S.canis_group_C_C10         AAGGGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAACACCG 708
                            ************************************************************

S.canis_AJ413205.1          GTGGCGAAAGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCAA 780
S.canis_group_C_C10         GTGGCGAAAGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCAA 768
                            ************************************************************

S.canis_AJ413205.1          ACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAGGTGTTAGGCCCT 840
S.canis_group_C_C10         ACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAGGTGTTAGGCCCT 828
                            ************************************************************

S.canis_AJ413205.1          TTCCGGGGCTTAGTGCCGGAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCA 900
S.canis_group_C_C10         TTCCGGGGCTTAGTGCCGGAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCA 888
                            ************************************************************

S.canis_AJ413205.1          AGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAAT 960
S.canis_group_C_C10         AGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAAT 948
                            ************************************************************

S.canis_AJ413205.1          TCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCCGATGCCCGCTCTAGAGATAG 1020
S.canis_group_C_C10         TCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCCGATGCCCGCTCTAGAGATAG 1008
                            ************************************************************

S.canis_AJ413205.1          AGTTTTACTTCGGTACATCGGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGA 1080
S.canis_group_C_C10         AGTTTTACTTCGGTACATCGGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGA 1068
                            ************************************************************

S.canis_AJ413205.1          GATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTATTGTTAGTTGCCATCATTAAGTT 1140
S.canis_group_C_C10         GATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTATTGTTAGTTGCCATCATTAAGTT 1128
                            ************************************************************

S.canis_AJ413205.1          GGGCACTCTAGCGAGACTGCCGGTAATAAACCGGAGGAAGGTGGGGATGACGTCAAATCA 1200
S.canis_group_C_C10         GGGCACTCTAGCGAGACTGCCGGTAATAAACCGGAGGAAGGTGGGGATGACGTCAAATCA 1188
                            ************************************************************

S.canis_AJ413205.1          TCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGTTGGTACAACGAGTCGCAA 1260
S.canis_group_C_C10         TCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGTTGGTACAACGAGTCGCAA 1248
                            ************************************************************

S.canis_AJ413205.1          GCCGGTGACGGCAAGCTAATCTCTGAAAGCCAATCTCAGTTCGGATTGTAGGCTGCAACT 1320
S.canis_group_C_C10         GCCGGTGACGGCAAGCTAATCTCTGAAAGCCAATCTCAGTTCGGATTGTAGGCTGCAACT 1308
                            ************************************************************

S.canis_AJ413205.1          CGCCTACATGAAGTCGGAATCGCTAGTAATCGCGGATCAGCACGCCGCGGTGAATACGTT 1380
S.canis_group_C_C10         CGCCTACATGAAGTCGGAATCGCTAGTAATCGCGGATCAGCACGCCGCGG---------- 1358
                            ************************************************

S.canis_AJ413205.1          CCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTG 1440
S.canis_group_C_C10         ------------------------------------------------------------
S.canis_AJ413205.1          AGGTAACCTATTAGGAGCCAGCCGCCTAAGGTG 1473
S.canis_group_C_C10         -------------------------------
```

Appendix 6: SDSE MLST database.

SDSE allelic profiles and corresponding ST's. ST's found in Yarrabah SDSE isolates are shaded. New alleles indicated by asterisks (*).

ST	*gki*	*gtr*	*murI*	*mutS*	*recP*	*xpt*	*atoB*
1	10	6	6	6	12	13	8
2	5	4	4	1	2	15	2
3	5	3	4	1	6	2	1
4	2	2	4	1	8	7	2
5	2	2	4	1	12	12	7
6	1	3	1	1	1	1	4
7	1	1	1	1	1	1	3
8	1	1	1	1	1	1	4
9	1	1	1	1	1	1	2
10	10	4	7	7	12	13	8
11	11	3	4	1	2	7	5
12	4	4	5	2	17	6	2
13	10	5	6	6	12	13	9
14	10	4	7	6	12	13	8
15	3	3	2	2	9	8	2
16	4	4	1	2	17	1	2
17	4	4	1	2	17	6	2
18	4	2	4	1	8	7	2
19	3	8	4	1	8	7	2
20	3	3	2	8	9	6	6
21	3	8	2	2	9	8	2
22	3	3	2	8	1	11	6
23	3	3	4	1	3	1	1
24	3	2	1	5	15	4	3
25	3	2	1	5	7	4	3
26	3	2	1	1	7	10	2
27	2	2	4	1	13	12	7
28	3	3	4	2	16	14	2
29	3	2	4	2	7	1	3
30	3	2	4	1	7	10	2
31	3	2	4	1	8	7	2
32	3	2	4	1	4	10	5
33	3	8	2	8	9	6	6
34	3	7	4	1	14	15	10
35	3	7	4	1	14	15	2
36	4	4	1	2	17	6	3
37	3	2	1	1	4	1	3
38	1	1	1	1	1	1	1
39	1	1	1	1	1	21	4
40	1	1	1	4	1	1	4
41	1	1	1	9	1	1	1
42	1	1	4	1	1	1	4
43	2	2	4	1	1	1	2
44	2	2	4	2	3	7	1
45	2	2	4	10	8	19	2
47	3	2	1	1	20	1	3
48	3	2	1	2	10	4	2
49	3	2	3	1	5	5	2

50	3	2	3	1	5	18	2
51	3	2	4	1	11	1	2
52	3	2	4	1	11	3	5
53	3	2	4	2	3	7	1
54	3	2	4	2	7	1	12
55	3	2	4	2	20	1	3
56	3	2	4	5	7	4	3
57	3	2	8	9	1	16	2
58	3	3	1	2	7	4	3
59	3	3	2	2	9	1	2
60	3	3	2	5	9	6	3
61	3	3	4	1	4	16	2
62	3	3	4	1	9	3	2
63	3	3	4	1	11	3	5
64	3	3	4	2	4	20	2
65	3	3	4	2	9	8	2
66	3	3	4	2	9	22	2
67	3	3	4	2	9	22	7
68	3	4	1	3	4	9	2
69	3	9	1	1	4	1	11
70	3	10	4	1	5	10	2
71	4	3	5	2	17	6	2
72	4	4	1	2	18	6	2
73	4	4	10	2	17	6	2
74	6	2	4	1	8	3	1
75	6	2	9	1	8	3	1
76	6	3	5	1	8	3	2
77	7	2	4	2	7	1	3
78	8	3	4	1	6	2	1
79	9	3	4	1	2	3	5
80	12	2	4	2	5	7	2
81	3	2	4	1	5	7	2
82	3	2	4	2	5	10	2
83	3	10	4	13	8	7	2
84	6	3	9	1	8	3	2
85	6	3	9	11	22	3	2
86	2	2	4	2	3	7	17
87	3	3	9	1	8	3	2
88	3	3	4	1	5	2	2
89	13	3	5	1	9	26	2
90	3	3	4	1	5	7	1
91	2	12	4	2	4	4	2
92	13	3	14	1	22	26	14
93	13	3	5	1	8	26	2
94	3	7	13	1	14	15	10
95	2	2	5	2	3	7	13
96	2	2	4	12	3	7	1
97	4	3	1	8	8	26	2
98	2	2	5	2	3	7	1
99	2	2	5	2	3	7	2
100	4	2	4	2	9	22	16
101	2	2	4	15	3	7	1
102	6	3	5	1	9	30	2
103	2	2	4	2	3	7	2

104	5	3	5	1	6	2	1
105	3	3	9	14	8	28	2
106	3	3	9	1	8	7	2
107	3	3	4	1	8	7	2
108	4	3	1	8	25	26	2
109	2	4	4	16	24	29	2
110	3	3	4	1	11	24	2
111	3	3	4	2	9	25	2
112	2	2	4	2	3	7	7
113	3	3	4	1	16	15	2
114	3	10	4	2	5	10	2
115	3	2	1	2	5	10	2
116	2	2	4	2	3	7	14
117	15	2	4	2	3	7	1
118	3	2	4	2	7	23	7
119	4	3	4	1	21	3	2
120	2	2	12	2	3	7	7
121	2	3	4	2	9	22	2
122	16	2	4	1	3	7	15
123	16	2	4	2	3	7	1
124	3	2	4	1	4	1	2
125	3	10	4	1	14	15	10
126	13	3	15	1	9	26	2
127	3	2	1	5	7	33	3
128	3	2	1	1	4	1	11
129	2	2	4	1	5	7	2
130	8	3	4	5	6	2	1
131	3	4	1	2	17	6	2
132	3	3	2	2	7	8	2
133	4	4	1	2	17	1	11
134*	17*	14*	16*	7	12	13	9
135	2	3	4	2	16	3	18
136	19	2	4	2	9	1	2
137	4	2	1	2	17	6	2
138	3	15	1	2	7	32	3
139	18	2	4	1	5	7	2
140	2	2	4	2	3	10	1
141	3	2	2	8	1	8	19
142*	10	5	17*	6	12	13	9
143*	10	13*	16*	7	12	13	8
144	2	4	4	1	19	17	20
1001	3	3	4	1	26	15	2
1002	2	2	5	2	2	31	2

SDSE *gki* alleles and sequences. Shaded sequences are new alleles.

gki	Sequence (498 bp)
1	AACCTTAACTGGAAAGAAACTCAGGAAGTCGGTTCCATTATTGAAAAAGAATTAGGTATTCCTTTTGCCATTGATAATGATGCCAAT GTGGCTGCTCTTGGTGAGCGTTGGGTTGGAGCTGGCGAAAACAATCCTGATGTTGTTTTCATGACACTAGGAACTGGTGTCGGTGGG GGGATTATCGCCGATGGTAACCTGATTCATGGTGTTGCAGGAGCAGGTGGTGAAATCGGCCACATGATTGTTGAACCGGAAAATGGC TTTGCTTGTACTTGTGGCTCACACGGCTGTTTGGAAACAGTAGCCTCAGCAACAGGTGTTGTCAAGGTAGCACGTTTATTGGCAGAA GCTTATGAAGGAGATTCTACTATTAAAGCCGCCATTGATAATGGGGACAATGTCACCAGCAAAGATATTTTCATGGCGGCTGAAGCA GGGGATTCCTTTGCTGATTCTGTTGTGGAAAAGGTTGGTTACTACCTTGGTCTTGCGTCAGCA
2	AACCTTAACTGGAAAGAAACTCAGGAAGTCGGTTCCATTATTGAAAAAGAATTAGGTATTCCTTTTGCCATTGATAATGATGCCAAT GTGGCTGCTCTTGGTGAGCGTTGGGTTGGAGCTGGCGAAAACAATCCTGATGTTGTTTTCATGACACTAGGAACTGGTGTCGGTGGA GGGATTATCGCCGATGGTAACCTGATTCATGGTGTTGCAGGAGCAGGTGGTGAAATCGGCCACATGATTGTTGAACCGGAAAATGGC TTTGCTTGTACTTGTGGCTCACACGGCTGTTTGGAAACAGTAGCCTCAGCAACAGGTGTTGTCAAGGTAGCACGTTTATTGGCAGAA GCTTATGAAGGAGATTCTACTATTAAAGCCGCCATTGATAATGGGGACAATGTCACCAGCAAAGATATTTTCATGGCTGCTGAAGCA GGAGATTCCTTTGCAGATTCTGTTGTGGAAAAGGTTGGTTACTACCTTGGTCTTGCGTCAGCA
3	AACCTTAACTGGAAAGAAACTCAGGAAGTCGGTTCCATTATTGAAAAAGAATTAGGTATTCCTTTTGCCATTGATAATGATGCCAAT GTGGCTGCTCTTGGTGAGCGTTGGGTTGGAGCTGGCGAAAACAATCCTGATGTTGTTTTCATGACACTAGGAACTGGTGTCGGTGGG GGGATTATCGCCGATGGTAACCTGATTCATGGTGTTGCAGGAGCAGGTGGTGAAATCGGCCACATGATTGTTGAACCGGAAAATGGC TTTGCTTGTACTTGTGGCTCACACGGCTGTTTGGAAACAGTAGCCTCAGCAACAGGTGTTGTCAAGGTAGCACGTTTATTGGCAGAA GCTTATGAAGGAGATTCTACTATTAAAGCCGCCATTGATAATGGGGACAATGTCACCAGCAAAGATATTTTCATGGCCGCTGAAGCA GGAGATTCCTTTGCAGATTCTGTTGTGGAAAAGGTTGGTTACTACCTTGGTCTTGCGTCAGCA
4	AACCTTAACTGGAAAGAAACTCAGGAAGTCGGTTCCATTATTGAAAAAGAATTAGGTATTCCTTTTGCCATTGATAATGATGCCAAT GTGGCTGCTCTTGGTGAGCGTTGGGTTGGAGCTGGCGAAAACAATCCTGATGTTGTTTTCATGACACTAGGAACTGGTGTCGGTGGG GGGATTATCGCCGATGGTAACTTGATTCATGGTGTTGCAGGAGCAGGTGGTGAAATCGGCCACATGATTGTTGAGCCAGAAAATGGC TTTGCTTGTACTTGTGGCTCACACGGCTGTTTGGAAACAGTAGCTTCAGCAACAGGAGTTGTCAAAGTGGCACGTTTACTGGCAGAA GCTTATGAAGGAGATTCTACCATTAAAGCCGCCATTGATAATGGGGACAATGTCACCAGCAAAGATATTTTCATGGCCGCTGAAGCA GGAGATTCCTTTGCAGATTCTGTTGTGGAAAAGGTTGGTTACTACCTTGGTCTTGCGTCAGCA
5	AACCTTAACTGGAAAGAAACTCAGGAAGTCGGTTCCATTATTGAAAAAGAATTAGGTATTCCTTTTGCCATTGATAATGATGCCAAT GTGGCTGCTCTTGGTGAGCGTTGGGTTGGAGCTGGCGAAAACAATCCTGATGTTGTTTTCATGACACTAGGAACTGGTGTCGGTGGG GGGATTATCGCCGATGGTAACCTGATTCATGGTGTTGCAGGAGCAGGTGGTGAAATCGGCCACATGATTGTTGAACCGGAAGATGGC TTTGCTTGTACTTGTGGCTCACACGGCTGTTTGGAAACAGTAGCCTCAGCAACAGGTGTTGTCAAGGTAGCACGTTTATTGGCAGAA GCTTATGAAGGAGATTCTACTATTAAAGCCGCCATTGATAATGGGGACAATGTCACCAGCAAAGATATTTTCATGGCCGCTGAAGCA GGAGATTCCTTTGCAGATTCTGTTGTGGAAAAGGTTGGTTACTACCTTGGTCTTGCGTCAGCA
6	AACCTTAACTGGAAAGAAACTCAGGAAGTCGGTTCCATTATTGAAAAAGAATTAGGTATTCCTTTTGCCATTGATAATGATGCCAAT GTGGCTGCTCTTGGTGAGCGTTGGGTTGGAGCTGGCGAAAACAATCCTGATGTTGTTTTCATGACACTAGGAACTGGTGTCGGTGGG GGGATTATCGCCGATGGTAACCTGATTCATGGTGTTGCAGGAGCAGGTGGTGAAATCGGCCACATGATTGTTGAACCGGAAAATGGC TTTGCTTGTACTTGTGGCTCACACGGCTGTTTGGAAACAGTAGCCTCAGCAACAGGTGTTGTCAAGGTAGCACGTTTATTGGCAGAA GCTTATGAAGGAGATTCTACTATTAAAGCCGCCATTGATAATGGGGACAATGTCACCAGCAAAGATATTTTCATGGCCGCTGAAGCA GGAGATTCCTTTGCAGATTCTGTTGTGGAAAAGGTTGGTTACTACCTTGGCCTTGCTTCAGCA
7	AACCTTAACTGGAAAGAAAATCAGGAAGTCGGTTCCATTATTGAAAAAGAATTAGGTATTCCTTTTGCCATTGATAATGATGCCAAT GTGGCTGCTCTTGGTGAGCGTTGGGTTGGAGCTGGCGAAAACAATCCTGATGTTGTTTTCATGACACTAGGAACTGGTGTCGGTGGG GGGATTATCGCCGATGGTAACCTGATTCATGGTGTTGCAGGAGCAGGTGGTGAAATCGGCCACATGATTGTTGAACCGGAAAATGGC TTTGCTTGTACTTGTGGCTCACACGGCTGTTTGGAAACAGTAGCCTCAGCAACAGGTGTTGTCAAGGTAGCACGTTTATTGGCAGAA GCTTATGAAGGAGATTCTACTATTAAAGCCGCCATTGATAATGGGGACAATGTCACCAGCAAAGATATTTTCATGGCCGCTGAAGCA GGAGATTCCTTTGCAGATTCTGTTGTGGAAAAGGTTGGTTACTACCTTGGTCTTGCGTCAGCA
8	AACCTTAACTGGAAAGAAACTCAGGAAGTCGGTTCCATTATTGAAAAAGAATTAGGTATTCCTTTTGCCATTGATAATGATGCCAAT GTGGCTGCTCTTGGTGAGCGTTGGGTTGGAGCTGGCGAAAACAATCCTGATGTTGTTTTCATGACACTAGGAACTGGTGTCGGTGGG GGGATTATCGCCGATGGTAACCTGATTCATGGTGTTGCAGGAGCAGGTGGTGAAATCGGCCACATGATTGTTGAACCGGAAGATGGC TTTGCTTGTACTTGTGGCTCACACGGCTGTTTGGAAACAGTAGCCTCAGCAACAGGTGTTGTCAAGGTAGCACGTTTATTGGCAGAA GCTTATGAAGGAGATTCTACTATTAAAGCCGCCATTGATAATGGGGACAATGTCACCAGCAAAGATATTTTCATGGCTGCTGAAGCA GGAGATTCCTTTGCAGATTCTGTTGTGGAAAAGGTTGGTTACTACCTTGGTCTTGCGTCAGCA
9	AACCTTAACTGGAAAGAAACTCAGGAAGTCGGTTCCATTATTGAAAAAGAATTAGGTATTCCTTTTGCCATTGATAATGATGCCAAT GTGGCTGCTCTTGGTGAGCGTTGGGTTGGAGCTGGCGAAAACAATCCTGATGTTGTTTTCATGACACTAGGAACTGGTGTCGGTGGG GGGATTATCGCCGATGGTAATCTGATTCATGGTGTTGCAGGAGCAGGTGGTGAAATCGGCCACATGATTGTTGAACCGGAAAATGGC TTTGCTTGTACTTGTGGCTCACACGGCTGTTTGGAAACAGTAGCCTCAGCAACAGGTGTTGTCAAGGTAGCACGTTTATTGGCAGAA GCTTATGAAGGAGATTCTACTATTAAAGCCGCCATTGATAATGGGGACAATGTCACCAGCAAAGATATTTTCATGGCTGCTGAAGCA GGAGATTCCTTTGCAGATTCTGTTGTGGAAAAGGTTGGTTACTACCTTGGTCTTGCGTCAGCA
10	AACCTTAACTGGAAAGAAACTCAGGAAGTCGGTTCCATTATTGAAAAAGAATTAGGTATTCCTTTTTCCATCGACAATGATGCCAAT GTGGCTGCTCTTGGTGAGCGTTGGGTTGGAGCTGGCGAAAACAATCCTGATGTTGTTTTCATGACACTAGGAACTGGTGTTGGTGGG GGGATTATCGCCGATGGGAACCTGATTCATGGTGTTGCAGGAGCAGGTGGTGAAATCGGCCACATGATTGTTGAACCAGAAAATGGC TTTGCTTGTACTTGTGGCTCACACGGCTGTTTGGAAACAGTAGCCTCAGCAACAGGTGTTGTCAAGGTAGCACGTTTGTTGGCAGAA GCTTATGAAGGAGATTCTGCTATTAAAGCCGCCATTGATAATGGAGACAATGTCACCAGCAAAGATATTTTCATGGCCGCTGAAGCA GGGGATTCCTTTGCAGATTCTGTTGTGGAAAAGGTTGGTTACTATCTTGGACTTGCTTCAGCA
11	AACCTTAACTGGAAAGAAACTCAGGAAGTCGGTTCCATTATTGAAAAAGAATTAGGTATTCCTTTTGCCATTGATAATGATGCCAAT GTGGCTGCTCTTGGTGAGCGTTGGGTTGGAGCTGGCGAAAACAATCCTGATGTTGTTTTCATGACACTAGGAACTGGTGTCGGTGGG GGGATTATCGCCGATGGTAATCTGATTCATGGTGTTGCAGGAGCAGGTGGTGAAATCGGCCACATGATTGTTGAACCGGAAAATGGC TTTGCTTGTACTTGTGGCTCACACGGCTGTTTGGAAACAGTAGCCTCAGCAACAGGTGTTGTCAAGGTAGCACGTTTATTGGCAGAA GCTTATGAAGGAGATTCTACTATTAAAGCCGCCATTGATAATGGGGACAATGTCACCAGCAAAGATATTTTCATGGCCGCTGAAGCA GGAGATTCCTTTGCAGATTCTGTTGTGGAAAAGGTTGGTTACTACCTTGGTCTTGCGTCAGCA
12	AACCTTAATTGGAAAGAAACCCAAGAAGTCGGTTCCGTTGTTGAAAAAGAATTGGGCATTCCTTTTGCCATTGACAATGATGCCAAT GTGGCTGCCCTTGGTGAACGTTGGGTAGGTGCTGGTGAAAATAACCCAGATGTCGTCTTCATGACACTTGGAACAGGTGTCGGTGGA GGCGTTATTGCTGATGGTAACTTGATTCATGGTGTTGCAGGAGCAGGTGGTGAAATCGGCCACATGATTGTTGAGCCAGAAAATGGC

	TTTGCTTGTACTTGTGGCTCACACGGCTGTTTGGAAACAGTAGCCTCAGCAACAGGTGTTGTCAAGGTAGCACGTTTATTGGCAGAA
	GCTTATGAAGGAGATTCTACTATTAAAGCCGCCATTGATAATGGGGACAATGTCACCAGCAAAGATATTTTCATGGCTGCTGAAGCA
	GGAGATTCCTTTGCAGATTCTGTTGTGGAAAAGGTTGGTTACTACCTTGGTCTTGCGTCAGCA
13	AACCTTAATTGGAAAGAAACCCAAGAAGTCGGTTCCGTTGTTGAAAAAGAATTGGGCATTCCTTTTGCCATTGACAATGATGCCAAT
	GTGGCTGCCCTTGGTGAACGTTGGGTAGGTGCTGGTGAAAATAACCCAGATGTCGTCTTCATGACACTTGGAACAGGTGTCGGTGGA
	GGCGTTATTGCTGATGGTAACTTGATTCATGGTGTTGCAGGAGCAGGTGGTGAAATCGGCCACATGATTGTTGAGCCAGAAAATGGC
	TTTGCTTGTACTTGTGGCTCACACGGCTGTTTGGAAACAGTAGCTTCAGCAACAGGAGTTGTCAAAGTGGCACGTTTACTGGCAGAA
	GCCTACGAAGGGGATTCAGCCATCAAAGCAGCTATTGACAATGGTGAAGGTGTCACCAGCAAAGACATTTTCATGGCGGCTGAAGCA
	GGGGATTCCTTTGCTGATTCTGTTGTGGAAAAGGTTGGTTACTACCTTGGCCTTGCTTCAGCA
15	AACCTTAATTGGAAAGAAACCCAAGAAGTCGGTTCCGTTGTTGAAAAAGAATTGGGCATTCCTTTTGCCATTGACAATGATGCCAAT
	GTGGCTGCCCTTGGTGAACGTTGGGTAGGTGCTGGTGAAAATAACCCAGATGTCGTCTTCATGACACTTGGAACAGGTGTCGGTGGA
	GGCATTATTGCTGATGGTAACTTGATTCATGGTGTTGCAGGAGCAGGTGGTGAAATCGGCCACATGATTGTTGAGCCAGAAAATGGC
	TTTGCTTGTACTTGTGGCTCACACGGCTGTTTGGAAACAGTAGCTTCAGCAACAGGAGTTGTCAAAGTGGCACGTTTACTGGCAGAA
	GCTTATGAAGGGGATTCAGCCATCAAAGCAGCTATTGACAATGGTGAAGGTGTTACCAGCAAAGACATTTTCATGGCGGCTGAAGCA
	GGGGATTCCTTTGCTGATTCTGTTGTGGAAAAGGTTGGTTACTACCTTGGCCTTGCTTCAGCA
16	AACCTTAATTGGAAAGAAACCCAAGAAGTCGGTTCCGTTGTTGAAAAAGAATTGGGCATTCCTTTTGCCATTGACAATGATGCCAAT
	GTGGCTGCCCTTGGTGAACGTTGGGTAGGTGCTGGTGAAAATAACCCAGATGTCGTCTTCATGACACTTGGAACAGGTGTCGGTGGA
	GGCATTATTGCTGATGGTAACTTGATTCATGGTGTTGCAGGAGCAGGTGGTGAAATCGGCCACATGATTGTTGAGCCAGAAAATGGC
	TTTGCTTGTACTTGTGGCTCACACGGCTGTTTGGAAACAGTAGCTTCAGCAACAGGAGTTGTCAAAGTGGCACGTTTACTGGCAGAA
	GCCTACGAAGGGGATTCAGCCATCAAAGCAGCTATTGACAATGGTGAAGGTGTTACCAGCAAAGACATTTTCATGGCGGCTGAAGCA
	GGGGATTCCTTTGCTGATTCTGTTGTGGAAAAGGTTGGTTACTACCTTGGCCTTGCTTCAGCA
17*	AACCTTAACTGGAAAGAAACTCAGGAAGTCGGTTCCATTATTGAAAAAGAATTAGGTATTCCTTTTGCCATCGACAATGATGCCAAT
	GTGGCTGCTCTTGGTGAGCGTTGGGTTGGAGCTGGCGAAAACAATCCTGATGTTGTTTTCATGACACTAGGAACTGGTGTCGGTGGG
	GGGATTATCGCCGATGGTAACCTGATTCATGGTGTTGCAGGAGCAGGTGGTGAAATCGGCCACATGATTGTTGAACCAGAAAATGGC
	TTTGCTTGTACTTGTGGCTCACACGGCTGTTTGGAAACAGTAGCCTCAGCAACAGGTGTTGTCAAGGTAGCACGTTTGTTGGCAGAA
	GCTTATGAAGGAGATTCTGCTATTAAAGCCGCCATTGACAATGGAGACAATGTCACCAGCAAAGATATTTTCATGGCCGCTGAAGCA
	GGGGATTCCTTTGCAGATTCTGTTGTGGAAAAGGTTGGTTACTATCTTGGACTTGCTTCAGCA
18	AACCTTAACTGGAAAGAAACTCAGGAAGTCGGTTCCATTATTGAAAAAGAATTAGGTATTCCTTTTGCCATTGATAATGATGCCAAT
	GTGGCTGCTCTTGGTGAGCGTTGGGTTGGAGCTGGCGAAAACAATCCTGATGTTGTTTTCATGACACTAGGAACTGGTGTCGGTGGA
	GGGATTATCGCCGATGGTAACCTGATTCATGGTGTTGCAGGAGCAGGTGGTGAAATCGGCCACATGATTGTTGAACCGGAAAATGGC
	TTTGCTTGTACTTGTGGCTCACACGGCTGTTTGGAAACAGTAGCCTCAGCAACAGGTGTTGTCAAGGTAGCACGTTTATTGGCAGAA
	GCTTATGAAGGAGATTCTACTATTAAAGCCGCCATTGATAATGGGGACAATGTCACCAGCAAAGATATTTTCATGGCTGCTAAAGCA
	GGAGATTCCTTTGCAGATTCTGTTGTGGAAAAGGTTGGTTACTACCTTGGTCTTGCGTCAGCA
19	AACCTTAACTGGAAAGAAACTCAGGAAGTCGGTTCCATTATTGAAAAAGAATTAGGTATTCCTTTTGCCATTGATAATGATGCCAAT
	GTGGCTGCTCTTGGTGAGCGTTGGGTTGGAGCTGGCGAAAACAATCCTGATGTTGTTTTCATGACACTAGGAACTGGTGTCGGTGGG
	GGGATTATCGCCGATGGTAACCTGATTCATGGTGTTGCAGGAGCAGGTGGTGAAATCGGCCACATGATTGTTGAACCGGAAAATGGC
	TTTGCTTGTACTTGTGGCTCACACGGCTGTTTGGAAACAGTAGCCTCAGCAACAGGTGTTGTCAAGGTAGCACGTTTATTGGCAGAA
	GCTTATGAAGGAGATTCTACTATTAAAGCCGCCATTGATAATGGGGACAATGTCACCAGCAAAGATATTTTCATGGCTGCTGAAGCA
	GGAGATTCCTTTGCAGATTCTGTTGTGGAAAAGGTTGGTTACTACCTTGGTCTTGCGTCAGCA

SDSE *gtr* alleles and sequences. Shaded sequences are new alleles.

gtr	Sequence (450 bp)
1	ACTTTACTTCGTACCATGAACCTTCTTGAGGTTCCTACGAAAGGTCAGATAACGTTTGAAGGCATTGACATTACAGATAAAAGAA ATGACATTTTTACAATGCGTGAAAAAATGGGAATGGTATTTCAACAATTTAATCTTTTCCCTAATATGACCGTTTTAGACAATAT TACCTTGTCACCTATTAAAACGAAAGGCATTCCTAAAGTAGAAGCTGACAAAACAGCTTTGACACTGTTAGATAAAGTTGGATTG TCAGAAAAAGCCAATGCTTACCCATCTAGTCTATCTGGGGGACAGCAGCAGCGAATTGCGATTGCGCGTGGCCTAGCTATGGATC CAGACGTCTTACTATTTGACGAGCCTACTTCAGCCTTGGATCCCGAAATGGTGGGTGAGGTTTTGGCTGTTATGCAGGATTTGGC TAAATCTGGGATGACGATGGTCATT
2	ACTTTACTTCGTACCATGAACCTTCTTGAGGTTCCTACGAAAGGTCAGATAACGTTTGAAGGCATTGACATTACAGATAAAAGAA ATGACATTTTTACAATGCGTGAAAAAATGGGAATGGTATTTCAACAATTTAATCTTTTCCCTAATATGACCGTTTTAGACAATAT TACCTTGTCACCTATTAAAACGAAAGGCATTTCTAAAGTAGAAGCTGACAAAACAGCTTTGGCACTGTTAGATAAAGTTGGATTG TCAGAAAAAGCCAATGCTTACCCATCTAGTCTATCTGGGGGACAGCAGCAGCGAATTGCGATTGCGCGTGGCCTAGCTATGGATC CAGACGTCTTACTATTTGACGAGCCTACTTCAGCCTTGGATCCCGAAATGGTGGGTGAGGTTTTGGCTGTTATGCAGGATTTGGC TAAATCTGGGATGACGATGGTCATT
3	ACTTTACTTCGTACCATGAACCTTCTTGAGGTTCCTACGAAAGGTCAGATAACGTTTGAAGGCATTGACATTACAGATAAAAGAA ATGACATTTTTACAATGCGTGAAAAAATGGGAATGGTATTTCAACAATTTAATCTTTTCCCTAATATGACCGTTTTAGACAATAT TACCTTGTCACCTATTAAAACGAAAGGCATTCCTAAAGTAGAAGCTGACAAAACAGCTTTGGCACTGTTAGATAAAGTTGGATTG TCAGAAAAAGCCAATGCTTACCCATCTAGTCTATCTGGGGGACAGCAGCAGCGAATTGCGATTGCGCGTGGCCTAGCTATGGATC CAGACGTCTTACTATTTGACGAGCCTACTTCAGCCTTGGATCCCGAAATGGTGGGTGAGGTTTTGGCTGTTATGCAGGATTTGGC TAAATCTGGGATGACGATGGTCATT
4	ACTTTACTTCGTACCATGAACCTTCTTGAGGTTCCTACGAAAGGTCAGATAACATTTGAAGGCATTGACATTACAGATAAAAGAA ATGACATTTTTACAATGCGTGAAAAAATGGGAATGGTATTTCAACAATTTAATCTTTTCCCTAATATGACCGTTTTAGACAATAT TACCTTGTCACCTATTAAAACGAAAGGCATTCCTAAAGTAGAAGCTGACAAAACAGCTTTGGCACTGTTAGATAAAGTTGGATTG TCAGAAAAAGCCAATGCTTACCCATCTAGTCTATCTGGGGGACAGCAGCAGCGAATTGCGATTGCGCGTGGCCTAGCTATGGATC CAGACGTCTTACTATTTGACGAGCCTACTTCAGCCTTGGATCCCGAAATGGTGGGTGAGGTTTTGGCTGTTATGCAGGATTTGGC TAAATCTGGGATGACGATGGTCATT
5	ACTTTACTTCGCACCATGAACCTTCTTGAGGTTCCTACGAAAGGTCAGATAACGTTTGAAGGTATTGACATTACAGATAAAAGAA ATGACATTTTTACAATGCGTGAAAAAATGGGAATGGTATTTCAACAATTTAATCTTTTCCTTAATATGACCGTTTTAGACAATAT TACCTTGTCACCTATTAAAACGAAAGGCATTTCTAAAGCAGAAGCTGACAAAACGGCTTTGGCACTGTTAGATAAAGTTGGATTG TCAGAAAAAGCCAATGCTTACCCATCTAGTCTATCTGGGGGACAGCAGCAGCGAATTGCAATTGCGCGTGGCCTAGCCATGGATC CAGATGTCTTACTATTTGACGAGCCTACTTCAGCCTTGGATCCCGAAATGGTGGGTGAGGTTTTGGCTGTTATGCAGGATTTGGC TAAATCTGGGATGACGATGGTCATT
6	ACTTTACTTCGCACCATGAACCTTCTTGAGGTTCCTACGAAAGGTCAGATAACGTTTGAAGGCATTGACATTACAGATAAAAGAA ATGACATTTTTACAATGCGTGAAAAAATGGGAATGGTATTTCAACAATTTAATCTTTTCCTTAATATGACCGTTTTAGACAATAT TACCTTGTCACCTATTAAAACGAAAGGCATTTCTAAAGCAGAAGCTGACAAAACGGCTTTGGCACTGTTAGATAAAGTTGGATTG TCAGAAAAAGCCAATGCTTACCCATCTAGTCTATCTGGGGGACAGCAGCAGCGAATTGCAATTGCGCGTGGCCTAGCCATGGATC CAGATGTCTTACTATTTGACGAGCCTACTTCAGCCTTGGATCCCGAAATGGTGGGTGAGGTTTTGGCTGTTATGCAGGATTTGGC TAAATCTGGGATGACGATGGTCATT
7	ACTTTACTTCGTACCATGAACCTTCTTGAGGTTCCTACGAAAGGTCAGATAACATTTGAAGGCATTGACATTACAGATAAAAGAA ATGACATTTTTACAATGCGTGAAAAAATGGGAATGGTATTTCAACAATTTAATCTTTTCCCTAATATGACCGTTTTAGACAATAT TACCTTGTCACCTATTAAAACGAAAGGCATTTCTAAAGTAGAAGCTGACAAAACAGCTTTGGCACTGTTAGATAAAGTTGGATTG TCAGAAAAAGCCAATGCTTACCTATCTAGTCTATCTGGGGGACAGCAGCAGCGAATTGCGATTGCGCGTGGCCTAGCTATGGATC CAGACGTCTTACTATTTGACGAGCCTACTTCAGCCTTGGATCCCGAAATGGTGGGTGAGGTTTTGGCTGTTATGCAGGATTTGGC TAAATCTGGGATGACGATGGTCATT
8	ACTTTACTTCGTACCATGAACCTTCTTGAGGTTCCTACGAAAGGTCAGATAACGTTTGAAGGCATTGACATTACAGATAAAAAAA ATGACATTTTTACAATGCGTGAAAAAATGGGAATGGTATTTCAACAATTTAATCTTTTCCCTAATATGACCGTTTTAGACAATAT TACCTTGTCACCTATTAAAACGAAAGGCATTCCTAAAGTAGAAGCTGACAAAACAGCTTTGGCACTGTTAGATAAAGTTGGATTG TCAGAAAAAGCCAATGCTTACCCATCTAGTCTATCTGGGGGACAGCAGCAGCGAATTGCGATTGCGCGTGGCCTAGCTATGGATC CAGACGTCTTACTATTTGACGAGCCTACTTCAGCCTTGGATCCCGAAATGGTGGGTGAGGTTTTGGCTGTTATGCAGGATTTGGC TAAATCTGGGATGACGATGGTCATT
9	ACTTTACTTCGTACCATGAACCTTCTTGAGGTTCCTACGAAAGGTCAGATAACGTTTGAAGGCATTGACATTACAGATAAAAGAA ATGACATTTTTACAATGCGTGAAAAAATGGGAATGGTATTTCAACAATTTAATCTTTTCCCTAATATGACCGTTTTAGACAATAT TACCTTGTCACCTATTAAAACGAAAGGCATTTCTAAAGTAGAAGCTGACAAAACAGCTTTGGCACTGTTAGATAAAGTTGGATTG TCAGAAAAAGCCAATGCTTACCCATCTAGTCTATCTGGGGGACAGCAGCAGCGAATTGCGATTGCGCGTGGCCTAGCTATGGATC CAGACGTCTTACTATTTGACGAGCCTACTTCAGCCTTGGATCCCGAAATGGTGGGTGAGGTTTTGGCTGTTATGCAGGATTTGGC TAAATCTGGGATGACAATGGTCATT
10	ACTTTACTTCGTACCATGAACCTTCTTGAGGTTCCTACGAAAGGTCATATAACGTTTGAAGGCATTGACATTACAGATAAAAGAA ATGACATTTTTACAATGCGTGAAAAAATGGGAATGGTATTTCAACAATTTAATCTTTTCCCTAATATGACCGTTTTAGACAATAT TACCTTGTCACCTATTAAAACGAAAGGCATTCCTAAAGTAGAAGCTGACAAAACAGCTTTGGCACTGTTAGATAAAGTTGGATTG TCAGAAAAAGCCAATGCTTACCCATCTAGTCTATCTGGGGGACAGCAGCAGCGAATTGCGATTGCGCGTGGCCTAGCTATGGATC CAGACGTCTTACTATTTGACGAGCCTACTTCAGCCTTGGATCCCGAAATGGTGGGTGAGGTTTTGGCTGTTATGCAGGATTTGGC TAAATCTGGGATGACGATGGTCATT
11	ACCTTACTTCGAACCATGAATCTTTTGGAAGTACCAACTAAGGGACAGATTAGGTTTGAGGGGATTGATATTACCGATAAGAAGA ACGATATTTTCAGCATGCGTGAAAAAATGGGAATGGTTTTCCAGCAGTTTAACCTCTTTCCCAATATGACTATTTTAGAAAATAT CACTTTATCGCCAATCAAAACTAAGGGAATGGCTAAAGCAGAGGCTGACAAAACAGCCTTGAGCTTGTTGGACAAAGTTGGATTA TCAGAAAAAGCCAAGGCTTATCCTGCTAGTCTTTCTGGTGGGCAACAGCAGCGGATTGCGATTGCGCGTGGACTGGCTATGGATC CAGATGTTTTACTCTTTGATGAACCAACTTCAGCTCTAGACCCAGAAATGGTGGGTGAGGTCTTGGCTGTCATGCAAGATTTGGC TAAATCTGGGATGACTATGGTTATT
12	ACCTTACTTCGAACCATGAATCTTTTGGAAGTACCAACTAAGGGACAGATTAGGTTTGAGGGGATTGATATTACCGATAAGAAGA ATGATATTTTCAGCATGCGTGAAAAAATGGGAATGGTTTTCCAGCAGTTTAACCTCTTTCCCAATATGACTATTTTAGAAAATAT CACTTTATCGCCAATCAAAACTAAGGGAATGGCTAAAGCAGAGGCTGACAAAACAGCCTTGAGCTTGTTGGACAAAGTTGGATTA TCAGAAAAAGCCAAGGCTTATCCTGCTAGTCTTTCTGGTGGGCAACAGCAGCGGATTGCGATTGCGCGTGGACTGGCTATGGATC CAGATGTTTTACTCTTTGATGAACCAACTTCAGCTCTAGACCCAGAAATGGTGGGTGAGGTCTTGGCTGTCATGCAAGATTTGGC TAAATCTGGGATGACTATGGTTATT
13*	ACTTTACTTCGCACCATGAACCTTCTTGAGGTTCCTACGAAAGGTCAGATAACGTTTGAAGGCATTGACATTACAGATAAAAGAA

	ATGACATTTTTACAATGCGTGAAAAAATGGGAATGGTATTTCAACAATTTAATCTTTTCCCTAATATGACCGTTTTAGACAATAT TACCTTGTCACCTATTAAAACGAAAGGCATTTCTAAAGCAGAAGCTGACAAAACGGCTTTGGCACTGTTAGATAAAGTTGGATTG TCAGAAAAAGCCAATGCTTACCCATCTAGTCTATCTGGGGGACAGCAGCAGCGAATTGCAATTGCGCGTGGCCTAGCCATGGATC CAGATGTCTTACTATTTGACGAGCCTACTTCAGCCTTGGATCCCGAAATGGTGGGTGAGGTTTTGGCTGTTATGCAGGATTTGGC TAAATCTGGGATGACGATGGTCATT
14*	ACTTTACTTCGCACCATGAACCTTCTTGAGGTTCCTACGAAAGGTCAGATAACGTTTGAAGGCATTGACATTACAGATAAAAGAA ATGACATTTTTACAATGCGTGAAAAAATGGGAATGGTATTTCAACAATTTAATCTTTTCCCTAATATGACCGTTTTAGACAATAT TACCTTGTCACCTATTAAAACGAAAGGCATTTCTAAAGCAGAAGCTGACAAAATGGCTTTGGTACTGTTAGATAAAGTTGGATTG TCAGAAAAAGCCAAGGCTTACCCATCTAGTCTATCTGGGGGACAGCAGCAGCGAATTGCGATTGCGCGTGGCCTAGCCATGGATC CAGACGTCTTACTATTTGACGAGCCTACTTCAGCCTTGGATCCCGAAATGGTGGGTGAGGTTTTGGCTGTTATGCAGGATTTGGC TAAATCTGGGATGACGATGGTCATT
15	ACTTTACTTCGTACCATGAACCTTCTTGAGGTTCCTACGAAAGGTCAGATAACGTTTGAAGGCATTGACATTACAGATAAAAGAA ATGACATTTTTACAATGCGTGAAAAAATGGGAATGGTATTTCAACAATTTAATCTTTTCCCTAATATGACCGTTTTAGACAATAT TACCTTGTCACCTATTAAAACGAAAGGCATTTCTAAAGTAGAAGCTGACAAAACAGCTTTGGCACTGTTAGATAAAGTTGGATTG TCAGAAAAAGCCAATGCTTACCCATCTAGTCTATCTGGGGGACAGCAGCAGCGAATTGCGATTGCGCGTGGCCTAGCTATGAATC CAGACGTCTTACTATTTGACGAGCCTACTTCAGCCTTGGATCCCGAAATGGTGGGTGAGGTTTTGGCTGTTATGCAGGATTTGGC TAAATCTGGGATGACGATGGTCATT

SDSE *murI* alleles and sequences. Shaded sequences are new alleles.

murI	Sequence (438 bp)
1	GCTTGTAATACAGCAACAGCAGTAGCTTGGGAAGAGGTCAAAGAAGCTCTGGACATCCCTGTTTTAGGTGTGATTTTACCTGGAT CGAGTGCAGCAATAAAATCGACAACCAAAGGTCAGGTTGGTGTGATTGGAACACCGATGACAATAGCTTCGGACATTTACCGTCA AAAAATTCAATTACTAGCACCAACAGTAGAGGTGGCTAGCCTAGCTTGTCCTAAATTTGTCCCCATTGTCGAGTCTAATGAAATA CGTTCTAGCGTCGCTAAAAAAGTCGTCTATGAAAGTTTGACGCCCTTGGTGGGTAAAATTGACACGCTCGTTCTAGGCTGTACGC ATTACCCTCTGCTTCGTCCGATTATTCAAAACGTTATGGGGCCGTCGGTCAAGTTGATCGATAGCGGGGCAGAATGTGTTCGGGA TATTTCTGTTCTT
2	GCTTGTAATACAGCAACAGCAGTAGCTTGGGAAGAGGTCAAAGAAGCTCTGGACATCCCTGTTTTAGGTGTGATTTTACCTGGAT CAAGTGCAGCAATAAAATCGACAACCAAAGGTCAGGTTGGTGTGATTGGAACACCGATGACAATAGCTTCGGACATTTACCGTCA AAAAATTCAATTATTAGCACCAACAGTAGAGGTGATTAGCCTAGCTTGCCCTAAATTTGTCCCCATTGTCGAGTCTAATGAAATA CGTTCTAGCGTCGCTAAAAAAGTCGTCTATGAAAGTTTGACGCCCTTGGTGGGTAAAATTGACACGCTCGTTCTAGGCTGTACGC ATTATCCTCTGCTTCGTCCGATTATTCAAAACGTTATGGGGCCGTCGGTCAAGTTGATCGATAGCGGGGCAGAATGTGTTCGGGA TATTTCTGTTCTT
3	GCTTGTAATACAGCAACAGCAGTAGCTTGGGAAGAGGTCAAAGAAGCTCTGGACATCCCTGTTTTAGGTGTGATTTTACCTGGAT CGAGTGCAGCAATAAAATCGACAACCAAAGGTCAGGTTGGTGTGATTGGAACACCGATGACAATAGCTTCGGACATTTACCGTCA AAAAATTCAATTACTAGCACCAACAGTAGAGGTGGCTAGCCTAGCTTGTCCTAAATTTGTCCCCATTGTCGAGTCTAATGAAATA CGTTCTAGCGTCGCTAAAAAAGTCGTCTATGAAAGTTTGACGCCCTTGGTGGGTAAAATTGACACGCTCGTTCTAGGCTGTACGC ATTATCCTCTGCTTCGTCCGATTATTCAAAACGTTATGGGGCCGTCGGTCAAGTTGATCGATAGCGGGGCAGAATGTGTTCGGGA TATTTCTGTTCTT
4	GCTTGTAATACAGCAACAGCAGTAGCTTGGGAAGAGGTCAAAGAAGCTCTGGACATCCCTGTTTTAGGTGTGATTTTACCTGGAT CAAGTGCAGCAATAAAATCGACAACCAAAGGTCAGGTTGGTGTGATTGGAACACCGATGACAATAGCTTCGGACATTTACCGTCA AAAAATTCAATTACTAGCACCAACAGTAGAGGTGGCTAGCCTAGCTTGTCCTAAATTTGTCCCCATTGTCGAGTCTAATGAAATA CGTTCTAGCGTCGCTAAAAAAGTCGTCTATGAAAGTTTGACGCCCTTGGTGGGTAAAATTGACACGCTCGTTCTAGGCTGTACGC ATTACCCTCTGCTTCGTCCGATTATTCAAAACGTTATGGGGCCGTCGGTCAAGTTGATCGATAGCGGGGCAGAATGTGTTCGGGA TATTTCTGTTCTT
5	GCTTGTAATACAGCAACAGCAGTAGCTTGGGAAGAGGTCAAAGAAGCTTTGGACATCCCTGTTTTAGGTGTGATTTTACCTGGAT CTAGTGCAGCAATCAAATCGACAACCAAAGGTCAGGTTGGTGTGATTGGAACACCGATGACAATAGCTTCGGACATTTACCGTCA AAAAATTCAATTATTAGCACCAACAGTAGAGGTGATTAGCCTAGCTTGCCCTAAATTTGTCCCCATTGTCGAGTCTAATGAAATA CGTTCTAGCGTCGCTAAAAAAGTCGTCTATGAAAGTTTGACGCCCTTGGTGGGTAAAATTGACACGCTCGTTCTAGGCTGTACGC ATTATCCTCTGCTTCGTCCGATTATTCAAAACGTTATGGGGCCGTCGGTCAAGTTGATCGATAGCGGGGCAGAATGTGTTCGGGA TATTTCTGTTCTT
6	GCTTGTAATACAGCAACAGCAGTAGCTTGGGAAGAGGTCAAAGAAGCTTTGGACATCCCTGTTTTAGGTGTGATTTTACCTGGAT CTAGTGCAGCAATCAAATCGACAACCAAAGGTCAGGTTGGTGTGATTGGAACACCGATGACAATAGCTTCGGACATTTACCGTCA AAAAATTCAATTATTAGCACCAACAGTAGAGGTGACTAGCCTAGCTTGCCCTAAATTTGTCCCCATTGTCGAGTCTAATGAAATA CGTTCTAGCGTCGCTAAAAAAGTCGTCTATGAAAGTTTGACGCCCTTGGTGGGTAAAATTGACACGCTCGTTCTAGGCTGTACGC ATTATCCTCTGCTTCGTCCGATTATTCAAAACGTTATGGGGCCGTCGGTCAAGTTGATCGATAGCGGGGCAGAATGTGTTCGGGA TATTTCTGTTCTT
7	GCTTGTAATACAGCAACAGCAGTAGCTTGGGAAGAGGTCAAAGAAGCTTTGGACATTCCTGTTTTAGGTGTGATTTTACCTGGAT CGAGTGCAGCAATCAAATCGACAACCAAAGGTCAGGTTGGTGTGATTGGAACACCGATGACAATAGCTTCGGACATTTACCGTCA AAAAATTCAATTACTAGCACCAACAGTAGAGGTGACTAGCCTAGCTTGTCCTAAATTTGTTCCCATTGTCGAGTCTAATGAAATA CGTTCTAGCGTCGCTAAAAAAGTCGTCTATGAAAGTTTGACGCCCTTGGTGGGTAAAATTGACACGCTCGTTCTAGGCTGTACGC ATTATCCTCTGCTTCGTCCGATTATTCAAAACGTTATGGGGCCGTCGGTCAAGTTGATTGATAGCGGGGCAGAATGTGTTCGGGA TATTTCTGTTCTT
8	GCTTGTAATACAGCAACAGCAGTAGCTTGGGAAGAGGTCAAAGAAGCTCTGGACATCCCTGTTTTAGGTGTGATTTTACCTGGAT CAAGTGCAGCAATAAAATCGACAACCAAAGGTCAGGTTGGTGTGATTGGAACACCGATGACAATAGCTTCGGACATTTACCGTCA AAAAATTCAATTACTAGCACCAACAGTAGAGGTGGCTAGCCTAGCTTGTCCTAAATTTGTCCCCATTGTCGAGTCTAATGAAATA CGTTCTAGCGTCGCTAAAAAAGTCGTCTATGAAAGTTTGACGCCCTTGGTGGGTAAAATTGACACGCTCGTTCTAGGCTGTACGC ATTATCCTCTGCTTCGTCCGATTATTCAAAACGTTATGGGGCCGTCGGTCAAGTTGATCGATAGCGGGGCAGAATGTGTTCGGGA TATTTCTGTTCTT
9	GCTTGTAATACAGCAACAGCAGTAGCTTGGGAAGAGGTCAAAGAAGCTTTGGACATCCCTGTTTTAGGTGTGATTTTACCTGGAT CGAGTGCAGCAATAAAATCGACAACCAAAGGTCAGGTTGGTGTGATTGGAACACCGATGACAATAGCTTCGGACATTTACCGTCA AAAAATTCAATTATTAGCACCAACAGTAGAGGTGATTAGCCTAGCTTGCCCTAAATTTGTCCCCATTGTCGAGTCTAATGAAATA CGTTCTAGCGTCGCTAAAAAAGTCGTCTATGAAAGTTTGACGCCCTTGGTGGGTAAAATTGACACGCTCGTTCTAGGCTGTACGC ATTATCCTCTGCTTCGTCCGATTATTCAAAACGTTATGGGGCCGTCGGTCAAGTTGATCGATAGCGGGGCAGAATGTGTTCGGGA TATTTCTGTTCTT
10	GCTTGTAATACAGCCACAGCAGTAGCTTGGGAAGAGGTCAAAGAAGCTTTGGACATCCCTGTTTTAGGTGTGATTTTACCTGGAT CTAGTGCAGCAATCAAATCGACAACCAAAGGTCAGGTTGGTGTGATTGGAACACCGATGACAATAGCTTCGGACATTTACCGTCA AAAAATTCAATTATTAGCACCAACAGTAGAGGTGATTAGCCTAGCTTGCCCTAAATTTGTCCCCATTGTCGAGTCTAATGAAATA CGTTCTAGCGTCGCTAAAAAAGTCGTCTATGAAAGTTTGACGCCCTTGGTGGGTAAAATTGACACGCTCGTTCTAGGCTGTACGC ATTATCCTCTGCTTCGTCCGATTATTCAAAACGTTATGGGGCCGTCGGTCAAGTTGATCGATAGCGGGGCAGAATGTGTTCGGGA TATTTCTGTTCTT
11	GCATGCAATACCGCAACAGCGGTGGCTTGGGAAGAAGTAAAAGCAGCTTTAGATATTCCTGTTTTAGGGGTTGTCTTACCGGGGG CAAGCGCAGCTATTAAATCAACGACAAAAGGCCAGGTTGGGGTCATCGGAACCCCAATGACAGTGGCTTCAGACATTTATCGCAA AAAAATCCAGCTATTAGCACCATCTGTTCAAGTAAGGAGTCTTGCTTGCCCGAAGTTTGTACCGATTGTGGAATCAAATGAGATG TGTTCGAGTATAGCTAAAAAAATAGTTTATGACAGTCTAGCACCATTAGTCGGTAAAATAGATACCCTTGTACTAGGATGTACTC ACTATCCCTTGTTACGACCAATTATCCAAAATGTTATGGGGCCATCTGTTAAGCTGATTGACAGTGGAGCAGAATGCGTCCGAGA TATCTCTGTCTTA
12	GCTTGTAATACAGCAACAGCAGTAGCTTGGGAAGAGGTCAAAGAAGCTCTGGACATCCCTGTTTTAGGTGTGATTTTACCTGGAT CAAGTGCAGCAATAAAATCGACAACCAAAGGTCAGGTTGGTGTGATTGGAACACCGATGACAATAGCTTCGGACATTTACCGTCA AAAAATTCAATTACTAGCACCAACAGTAGAGGTGGCTAGCCTAGCTTGTCCTAAATTTGTCCCCATTGTCGAGTCTAATGAAATA CGTTCTAGCGTCGCTAAAAAAGTCGTCTATGAAAGTTTGACGCCCTTGGTGGGTAAAATTGACACGCTCGTTTTAGGCTGTACGC ATTACCCTCTGCTTCGTCCGATTATTCAAAACGTTATGGGGCCGTCGGTCAAGTTGATCGATAGCGGGGCAGAATGTGTTCGGGA TATTTCTGTTCTT

13	GCTTGTAATACAGCAACAGCAGTAGCTTGGGAAGAGGTCAAAGAAGCTCTGGACATCCCTGTTTTAGGTGTGATTTTACCTGGAT CAAGTGCAGCAATAAAATCGACAACCAAAGGTCAGGTTGGTGTGATTGGAACACCGATGACAATAGCTTCGGACATTTACCGTCA AAAAATTCAATTACTAGCACCAACAGTAGAGGTGGCTAGCCTAGCTTGTCCTAAATTTGTCCCCATTGTCGAGTCTAATGAAATA CGTTCTAGCGTCGCTAAAAAAGTCGTCTATGAAAGTTTGACGCCCTTGGTGGGTAAAATTGACACGCTCGTTTTAGGCTGTACGC ATTACCCTCTGCTTCGTCCGATTATTCAAAACGTTATGGGGCCGTCGGTCAAGTTGATCGATAGCGGGGCAGAATGTGTTCGGGA TATTTCTGTTCTT
14	GCTTGTAATACAGCAACAGCAGTAGCTTGGGAAGAGGTCAAAGAAGCTTTGGACATCCCTGTTTTAGGTGTGATTTTACCTGGAT CAAGTGCAGCAATCAAATCGACAACCAAAGGTCAGGTTGGTGTGATTGGAACACCGATGACAATAGCTTCGGACATTTACCGTCA AAAAATTCAATTATTAGCACCAACAGTAGAGGTGATTAGCCTAGCTTGCCCTAAATTTGTCCCCATTGTCGAGTCTAATGAAATA CGTTCTAGCGTCGCTAAAAAAGTCGTCTATGAAAGTTTGACGCCCTTGGTGGGTAAAATTGACACGCTCGTTCTAGGCTGTACGC ATTATCCTCTGCTTCGTCCGATTATTCAAAATGTTATGGGGCCGTCGGTCAAGTTGATCGATAGCGGGGCAGAATGTGTTCGGGA TATTTCTGTTCTT
15	GCATGCAATACCGCAACAGCGGTGGCTTGGGAAGAAGTAAAAGCAGCTTTAGATATTCCTGTTTTAGGAGTTGTCTTACCGGGGG CAAGCGCAGCTATTAAATCAACGACAAAAGGCCAGGTTGGGGTCATCGGAACCCCAATGACAGTGGCTTCAGACATTTATCGCAA AAAAATCCAGCTATTAGCACCATCTATTCAAGTAAGGAGTCTTGCTTGCCCGAAGTTTGTACCGATTGTGGAATCAAATGAGATG TGTTCGAGTATAGCTAAAAAAAATAGTTTATGACAGTCTAGCACCATTAGTCGGTAAAATAGATACCCTTGTACTAGGATGTACTC ACTATCCCTTGTTACGACCAATTATCCAAAATGTTATGGGGCCATCTGTTAAGCTGATTGACAGTGGAGCAGAATGCGTCCGAGA TATCTCTGTCTTA
16*	GCTTGTAATACAGCAACAGCAGTAGCTTGGGAAGAGGTCAAAGAAGCTTTGGACATCCCTGTTTTAGGTGTGATTTTACCTGGAT CTAGTGCAGCAATCAAATCGACAACCAAAGGTCAGGTTGGTGTGATTGGAACACCGATGACAATAGCTTCGGACATTTACCGTCA AAAAATTCAATTATTAGCACCAACAGTAGAGGTGACTAGCCTAGCTTGTCCTAAATTTGTCCCCATTGTCGAGTCTAATGAAATA CGTTCTAGCGTCGCTAAAAAAGTCGTCTATGAAAGTTTGACGCCCTTGGTGGGTAAAATTGACACGCTCGTTCTAGGCTGTACGC ATTATCCTCTGCTTCGTCCGATTATTCAAAACGTTATGGGGCCGTCGGTCAAGTTGATCGATAGCGGGGCAGAATGTGTTCGGGA TATTTCTGTTCTT
17*	GCTTGTAATACAGCAACAGCAGTAGCTTGGGAAGAGGTCAAAGAAGCTTTGGACATCCCTGTTTTAGGTGTGATTTTACCTGGAT CTAGTGCAGCAATCAAATCGACAACCAAAGGTCAGGTTGGTGTGATTGGAACACCGATGACAATAGTTTCGGACATTTACCGTCA AAAAATTCAATTATTAGCACCAACAGTAGAGGTGACTAGCCTAGCTTGCCCTAAATTTGTCCCCATTGTCGAGTCTAATGAAATA CGTTCTAGCGTCGCTAAAAAAGTCGTCTATGAAAGTTTGACGCCCTTGGTGGGTAAAATTGACACGCTCGTTCTAGGCTGTACGC ATTATCCTCTGCTTCGTCCGATTATTCAAAACGTTATGGGGCCGTCGGTCAAGTTGATCGATAGCGGGGCAGAATGTGTTCGGGA TATTTCTGTTCTT

SDSE *mutS* alleles and sequences.

mutS	Sequence (405 bp)
1	ACCTATATTAATCGTTTACAGAAACTGGCTAAAACTTTGGCAACGGTGGATGTTTTGCAAAGTTTAGCAGCCGTTGCCGAAACCA ATCATTATATCCGGCCGCAGTTCAATGATAATCATGTGATTAAAATTCAAGAAGGTCGTCACGCGGTTGTTGAAAAGGTTATGGG AGTGCAGGAATACATTCCCAATAGTATCTCTTTTAACCAAGAGACAAGCATTCAATTGATTACAGGTCCAAATATGAGCGGTAAG TCGACTTATATGAGACAGCTGGCCTTAACGGTTATCATGGCCCAGATGGGCTCATTTGTAGCTGCTGACCATGTTGATTTGCCTC TATTTGATGCGATTTTTACGCGTATTGGGGCTGCTGATGATTTGATTTCTGGGCAATCAACCTTT
2	ACCTATATTAATCGTTTACAGAAACTGGCTAAAACTTTGGCAACGGTGGATGTTTTGCAAAGTTTAGCAGCCGTTGCCGAAACCA ATCATTATATCCGGCCGCAGTTCAATGATAATCATGTGATTAAAATTCAAGAAGGTCGTCACGCGGTTGTTGAAAAGGTTATGGG AGTGCAGGAATACATTCCCAATAGTATCTCTTTTAACCAAGAGACAAGCATTCAATTGATTACAGGTCCAAATATGAGCGGTAAG TCGACTTATATGAGACAGCTGGCCTTAACGGTTATCATGGCTCAGATGGGCTCATTTGTAGCTGCTGACCATGTTGATTTGCCTC TATTTGATGCGATTTTTACGCGTATTGGGGCTGCTGATGATTTGATTTCTGGGCAATCAACCTTT
3	ACCTATATTAATCGTTTACAGAAACTGGCTAAAACTTTGGCAACGGTGGATGTTTTGCAAAGTTTAGCAGTCGTTGCTGAGACCA ATCATTATATCCGACCGCAGTTCAATGATAATCATGTGATTACAATTCAAGAAGGTCGTCACGCGGTTGTTGAAAAGGTCATGGG AGTGCAGGAATACATTCCCAATAGTATCTCTTTTGACCAACAGACCAGTATTCAGCTGATTACAGGTCCAAATATGAGTGGTAAG TCGACTTATATGAGACAGCTGGCCTTAACGGTTATCATGGCCCAGATGGGTTCATTTGTGGCTGCTGACCATGTTGATTTACCTT TATTTGATGCGATTTTTACGCGTATTGGGGCTGCTGATGATTTGATTTCTGGGCAATCAACCTTT
4	ACCTATATTAATCGTTTACAGAAACTGGCTAAAACTTTGGCAACGGTGGATGTTTTGCAAAGTTTAGCAGCCGTTGCCGAAACCA ATCATTATATCCGGCCGCAGTTCAATGATAATCATGTGATTAAAATTCAAGAAGGTCGTCACGCGGTTGTTGAAAAGGTTATGGG AGTGCAGGAATACATTCCCAATAGTATCTCTTTTAACCAAGAGACAAGTATTCAATTGATTACAGGTCCAAATATGAGCGGTAAG TCGACTTATATGAGACAGCTGGCCTTAACGGTTATCATGGCCCAGATGGGCTCATTTGTAGCTGCTGACCATGTTGATTTGCCTC TATTTGATGCGATTTTTACGCGTATTGGGGCTGCTGATGATTTGATTTCTGGGCAATCAACCTTT
5	ACCTATATTAATCGTTTACAGAAACTGGCTAAAACTTTGGCAACGGTGGATGTTTTGCAAAGTTTAGCAGCCGTTGCCGAAACCA ATCATTATATCCGGCCGCAGTTCAATGATAATCATGTGATTAAAATTCAAGAAGGTCGTCACGCGGTTGTTGAAAAGGTTATGGG AGTGCAGGAGTACATTCCCAATAGTATCTCTTTTAATCAAGAGACAAGCATTCAATTGATTACAGGTCCAAATATGAGCGGTAAG TCGACTTATATGAGACAGCTGGCCTTAACGGTTATCATGGCCCAGATGGGCTCATTTGTAGCTGCTGACCATGTTGATTTGCCTC TATTTGATGCGATTTTTACGCGTATTGGGGCTGCTGATGATTTGATTTCTGGGCAATCAACCTTT
6	ACCTATATTAATCGTTTACAGAAACTGGCTAAAACTTTGGCAACGGTGGATGTTTTGCAAAGTTTGGCAGTCGTTGCCGAAACCA ATCATTATATCCGGCCGCAGTTCAATGATAATCATGTGATTACAATTCAAGAAGGTCGTCACGCGGTTGTTGAAAAGGTTATGGG AGTGCAGGAATACATTCCCAATAGTATCTCTTTTAACCAAGAGACAAGCATTCAATTGATTACAGGTCCAAATATGAGCGGTAAG TCTACTTATATGAGACAGCTGGCCTTAACGGTTATCATGGCCCAGATGGGCTCATTTGTGGCTGCTGACCATGTTGATTTGCCTC TATTTGATGCGATTTTTACGCGTATTGGGGCTGCTGATGATTTGATTTCTGGGCAATCAACCTTT
7	ACCTATATTAATCGTTTACAGAAACTGGCTAAAACTTTGGCAACGGTGGATGTTTTGCAAAGTTTAGCAGTCGTTGCTGAAACCA ATCATTATATCCGGCCGCAGTTCAATGATAATCATGTGATTACAATTCAAGAAGGTCGTCACGCGGTTGTTGAAAAGGTTATGGG AGTGCAGGAATACATTCCCAATAGTATCTCTTTTAACCAAGAGACAAGCATTCAATTGATTACAGGTCCAAATATGAGCGGTAAG TCGACTTATATGAGACAGCTGGCCTTAACGGTTATCATGGCCCAGATGGGCTCATTTGTAGCTGCTAACCATGTTGATTTGCCTC TATTTGATGCGATTTTTACGCGTATTGGGGCTGCTGATGATTTGATTTCTGGACAATCAACCTTT
8	ACCTATATTAATCGTTTACAGAAACTGGCTAAAACTTTGGCAACGGTGGATGTTTTGCAAAGTTTAGCAGCCGTTGCCGAAACCA ATCATTATATCCGGCCGCAGTTCAATGATAATCATGTGATTAAAATTCAAGAAGGTCGTCACGCGGTTGTTGAAAAGGTTATGGG AGTGCAGGAATACATTCCCAATAGTATCTCTTTTAACCAAGAGACAAGCATTCAATTGATTACAGGTCCAAATATGAGCGGTAAG TCGACTTATATGAGACAGCTGGCCTTAACGGTTATTATGGCCCAGATGGGCTCATTTGTAGCTGCTGACCATGTTGATTTGCCTC TATTTGATGCGATTTTTACGCGTATTGGGGCTGCTGATGATTTGATTTCTGGGCAATCAACCTTT
9	ACCTATATTAATCGTTTACAGAAACTGGCTAAAACTTTGGCAACGGTGGATGTTTTGCAAAGTTTAGCAGCCGTTGCCGAAACCA ATCATTATATCCGGCCGCAGTTCAATGATAATCATGTGATTAAAATTCAAGAAGGTCGTCACGCGGTTGTTGAAAAGGTTATGGG AGTGCAGGAATACATTCCCAATAGTATCTCTTTTAACCAAGAGACAAGCATTCAATTGATTACAGGTCCAAATATGAGCGGTAAG TCGACTTATATGAGACAGCTGGCCTTAACGGTTATCATGGCCCAGCTGGGCTCATTTGTAGCTGCTGACCATGTTGATTTGCCTC TATTTGATGCGATTTTTACGCGTATTGGGGCTGCTGATGATTTGATTTCTGGGCAATCAACCTTT
10	ACCTATATTAATCGTTTACAGAAACTGGCTAAAACTTTGGCAACGGTGGATGTTTTGCAAAGTTTAGCAGCCGTTGCCGAAACCA ATCATTATATCCGGCCGCAGTTCAATGATAATCATGTGATTAAAATTCAAGAAGGTCGTCACGCGGTTGTTGAAAAGGTTATGGG AGTGCAGGAATACATTCCCAATAGTATCTCTTTTAACCAAGAGACAAGCATTCAATTGATTACAGGTCCAAATATGAGCGGTAAG TCGACTTATATGAGACAGCTGGCCTTAACGGTTATCATGGCCCAGATGGGCTCATTTGTAGCTGCTGACTATGTTGATTTGCCTC TATTTGATGCGATTTTTACGCGTATTGGGGCTGCTGATGATTTGATTTCTGGGCAATCAACCTTT
11	ACCTATATTAATCGTTTACAGAAACTGGCTAAAACTTTGGCAACGGTGGATGTTTTGCAAAGTTTAGCAGCCGTTGCCGAAACCA ATCATTATATCCGGCCGCAGTTCAATGATAATCATGTGATTAAAATTCAAGAAGGTCGTCACGCGGTTGTTGAAAAGGTTATGGG AGTGCAGGAATACATTCCCAATAGTATCTCTTTTAACCAAGAGACAAGCATTCAATTGATTACAGGTCCAAATATGAGCGGTAAG TCGACTTATATGAGACAGCTGGCCTTAACGGTTATCATGGCCCAGATGGGTTCATTTGTAGCTGCTGACCATGTTGATTTGCCTC TATTTGATGCGATTTTTACGCGTATTGGGGCTGCTGATGATTTGATTTCTGGGCAATCAACCTTT
12	ACCTATATTAATCGTTTACAGAAACTGGCTAAAACTTTGGCAACGGTGGATGTTTTGCAAAGTTTAGCAGCCGTTGCCGAAACCA ATCATTATATCCGGCCGCAGTTCAATGATAATCATGTGATTAAAATTCAAGAAGGTCGTCACGCGGTTGTTGAAAAGGTTATGGG AGTGCAGGAATACATTCCCAATAGTATCTCTTTTAACCAAGAGACAAGCATTCAATTGATTACAGGTCCAAATATGAGCGGTAAG TCGACTTATATGAGACAGCTGGCCTTAACGGTTATCATGGCTCAGATGGGCTCATTTGTGGCTGCTGACCATGTTGATTTGCCTC TATTTGATGCGATTTTTACGCGTATTGGGGCTGCTGATGATTTGATTTCTGGGCAATCAACCTTT
13	ACCTATATTAATCGTTTACAGAAACTGGCTAAAACTTTGGCAACGGTGGATGTTTTGCAAAGTTTAGCAGCCGTTGCCGAAACCA ATCATTATATCCGGCCGCAGTTCAATGATAATCATGTGATTAAAATTCAAGAAGGTCGTCACGCGGTTGTTGAAAAGGTTATGGG AGTGCAGGAATACATTCCCAATAGCATCTCTTTTAACCAAGAGACAAGCATTCAATTGATTACAGGTCCAAATATGAGCGGTAAG TCGACTTATATGAGACAGCTGGCCTTAACGGTTATCATGGCCCAGATGGGCTCATTTGTAGCTGCTGACCATGTTGATTTGCCTC TATTTGATGCGATTTTTACGCGTATTGGGGCTGCTGATGATTTGATTTCTGGGCAATCAACCTTT
14	ACCTATATTAATCGTTTACAGAAACTGGCTAAAATTTTGGCAACGGTAGATGTTTTGCAAAGTTTAGCAGTCGTTGCTGAAACCA ATCATTATATCCGGCCGCAGTTCAATGATAATCATGTGATTACAATTCAAGAAGGTCGTCACGCGGTTGTTGAAAAGGTTATGGG AGTGCAGGAATACATTCCCAATAGTATCTCTTTTGACCAACAGACCAGTATTCAGCTGATTACAGGTCCAAATATGAGTGGTAAG TCGACTTATATGAGACAGCTTGCCTTAACGGTTATCATGGCCCAGATGGGTTCATTTGTGGCTGCTGACCATGTTGATTTACCTT TATTTGATGCGATTTTTACGCGTATTGGGGCTGCTGATGATTTGATTTCTGGGCAATCAACCTTT
15	ACCTATATTAATCGTTTACAGAAACTGGCTAAAACTTTGGCAACGGTGGATGTTTTGCAAAGTTTAGCAGCCGTTTCCGAAACCA ATCATTATATCCGGCCGCAGTTCAATGATAATCATGTGATTAAAATTCAAGAAGGTCGTCACGCGGTTGTTGAAAAGGTTATGGG AGTGCAGGAATACATTCCCAATAGTATCTCTTTTAACCAAGAGACAAGCATTCAATTGATTACAGGTCCAAATATGAGCGGTAAG

	TCGACTTATATGAGACAGCTGGCCTTAACGGTTATCATGGCTCAGATGGGCTCATTTGTAGCTGCTGACCATGTTGATTTGCCTC
	TATTTGATGCGATTTTTACGCGTATTGGGGCTGCTGATGATTTGATTTCTGGGCAATCAACCTTT
16	ACCTATATTAATCGTTTACAGAAACTGGCTAAAACTTTGGCAACGGTGGATGTTTTGCAAAGTTTAGCAGCCGTTGCCGAAACCA
	ATCATTATATCCGACCGCAGTTCAATGATAATCATGTGATTAAAATTCAAGAAGGTCGTCACGCGGTTGTTGAAAAGGTTATGGG
	AGTGCAGGAATACATTCCCAATAGTATCTCTTTTAACCAAGAGACAAGCATTCAATTGATTACAGGTCCAAATATGAGCGGTAAG
	TCGACTTATATGAGACAGCTGGCCTTAACGGTTATCATGGCCCAGATGGGCTCATTTGTAGCTGCTGACCATGTTGATTTGCCTC
	TATTTGATGCGATTTTTACGCGTATTGGGGCTGCTGATGATTTGATTTCTGGGCAATCAACCTTT

SDSE *recP* alleles and sequences.

recP	Sequence (459 bp)
1	CCTATGGCCTACGTTCTTTGGAATCACTTCATGAACATTAATCCAAAAACAAGCCGTAATTGGTCAAACAGAGACCGTTTTATCCTA TCAGCGGGACACGGAAGTGCTATGCTTTATAGCTTGTTACACTTAGCTGGTTATGATTTATCTGTAGAGGATTTGAAAAACTTCCGT CAATGGGGTTCTAAAACACCAGGTCACCCAGAAGTTAATCATACCGATGGTGTGGAAGCAACTACAGGACCTCTTGGTCAAGGGATC GCAAATGCCGTTGGTATGGCCATGGCAGAAGCTCATCTAGCAGCTAAATTTAACAAACCAGGCTTTGACATCGTTGATCACTACACC TTTGCTTTGAATGGTGACGGTGACCTTATGGAAGGGGTCAGCCAAGAAGCAGCAAGTATGGCAGGACATTTAAAACTTGGGAAATTG GTCTTGCTATATGATTCAAACGAC
2	CCTATGGCCTATGTTCTTTGGAATCACTTCATGAACATCAATCCCAAAACAAGCCGTAATTGGTCAAACAGAGACCGTTTTATCCTA TCAGCAGGTCATGGAAGTGCCATGCTTTATAGCTTGTTACACTTAGCTGGTTATGATTTATCTGTAGAAGATTTAAAGAACTTCCGT CAATGGGGTTCTAAAACACCAGGTCACCCAGAAGTGAACCACACAGACGGTGTCGAAGCAACCACAGGACCTCTTGGTCAAGGGATC GCAAATGCCGTTGGGATGGCCATGGCAGAAGCTCATCTAGCAGCTAAATTTAACAAACCAGGCTTTGACATCGTTGATCACTACACA TTTGCTTTGAATGGTGACGGTGACCTTATGGAAGGGGTCAGCCAAGAAGCAGCAAGTATGGCAGGACATTTAAAACTTGGGAAATTG GTCTTGCTATATGATTCAAACGAC
3	CCTATGGCCTACGTTCTTTGGAATCACTTCATGAACATTAATCCAAAAACAAGCCGTAATTGGTCAAACAGAGACCGTTTTATCCTA TCAGCGGGACACGGAAGTGCTATGCTTTATAGCTTGTTACACTTAGCTGGTTATGATTTATCTGTAGAGGATTTGAAAAACTTCCGT CAATGGGGTTCTAAAACACCAGGTCACCCAGAAGTTAATCATACCGATGGTGTGGAAGCAACTACAGGACCTCTTGGTCAAGGGATC GCAAATGCTGTTGGTATGGCCATGGCAGAAGCTCATCTAGCAGCTAAATTTAACAAACCAGGCTTTGACATCGTTGATCATTACACA TTTGCTTTGAATGGTGACGGTGACCTTATGGAAGGGGTCAGCCAAGAATCAGCAAGTATGGCAGGACATTTAAAACTTGGGAAATTG GTCTTGCTATATGATTCAAACGAC
4	CCTATGGCCTACGTTCTTTGGAATCACTTCATGAACATTAATCCAAAAACAAGCCGTAATTGGTCAAACAGAGACCGTTTTATCCTA TCAGCGGGACACGGAAGTGCTATGCTTTATAGCTTGTTACACTTAGCTGGTTATGATTTATCTGTAGAGGATTTGAAAAACTTCCGT CAATGGGGTTCTAAAACACCAGGTCACCCAGAAGTTAATCATACCGATGGTGTGGAAGCAACTACAGGACCTCTTGGTCAAGGGATC GCAAATGCTGTTGGTATGGCCATGGCAGAAGCTCATCTAGCAGCTAAATTTAACAAACCAGGCTTTGACATCGTTGATCATTACACA TTTGCTTTGAATGGTGACGGTGACCTTATGGAAGGTGTCAGTCAAGAAGCAGCAAGTATGGCAGGACACTTAAAACTTGGCAAATTG GTCTTGCTTTACGATTCAAACGAT
5	CCTATGGCCTACGTTCTTTGGAATCACTTCATGAACATTAATCCAAAAACAAGCCGTAATTGGTCAAACAGAGACCGTTTTATCCTA TCAGCGGGACACGGAAGTGCTATGCTTTATAGCTTGTTACACTTAGCTGGTTATGATTTATCTGTAGAGGATTTGAAAAACTTCCGT CAATGGGGTTCTAAAACACCAGGTCACCCAGAAGTTAATCATACCGATGGTGTGGAAGCAACTACAGGACCTCTTGGTCAAGGGATC GCAAATGCTGTTGGTATGGCCATGGCAGAAGCTCATCTAGCAGCTAAATTTAACAAACCAGGCTTTGACATCGTTGATCATTACACA TTTGCTTTGAATGGTGACGGTGACCTTATGGAAGGTGTCAGTCAAGAAGCAGCAAGTATGGCAGGACACTTAAAACTTGGTAAATTG GTCTTGCTTTACGATTCAAACGAT
6	CCTATGGCCTATGTTCTTTGGAATCACTTCATGAACATCAATCCCAAAACAAGCCGTAATTGGTCAAACAGAGACCGTTTTATCCTA TCAGCAGGTCATGGAAGTGCCATGCTTTATAGCTTGTTACACTTAGCTGGTTATGATTTATCTGTAGAAGATTTAAAGAACTTCCGT CAATGGGGTTCTAAAACACCAGGTCACCCAGAAGTGAACCACACAGACGGTGTCGAAGCAACCACAGGACCTCTTGGTCAAGGGATC GCAAATGCCGTTGGGATGGCCATGGCAGAAGCTCATCTAGCAGCTAAATTTAACAAACCAGGCTTTGACATCGTTGATCACTACACA TTTGCTTTGAATGGTGACGGTGACCTTATGGAAGGTGTCAGTCAAGAAGCAGCAAGTATGGCAGGACACTTAAAACTTGGCAAATTG GTCTTGCTTTACGATTCAAACGAT
7	CCTATGGCCTATGTTCTTTGGAATCACTTCATGAACATCAATCCCAAAACAAGCCGTAATTGGTCAAACAGAGACCGTTTTATCCTA TCAGCAGGTCATGGAAGTGCCATGCTTTATAGCTTGTTACACTTAGCTGGTTATGATTTATCTGTAGAAGATTTAAAGAACTTCCGT CAATGGGGTTCTAAAACACCAGGTCACCCAGAAGTGAACCACACAGACGGTGTCGAAGCAACCACAGGACCTCTTGGTCAAGGGATC GCAAATGCCGTTGGTATGGCCATGGCAGAAGCTCATCTAGCAGCTAAATTTAACAAACCAGGCTTTGACATCGTTGATCACTACACC TTTGCTTTGAATGGTGACGGTGACCTTATGGAAGGTGTCAGTCAAGAAGCAGCAAGTATGGCAGGACACTTAAAACTTGGCAAATTG GTCTTGCTTTACGATTCAAACGAT
8	CCTATGGCCTATGTTCTTTGGAATCACTTCATGAACATCAATCCCAAAACAAGCCGTAATTGGTCAAACAGAGACCGTTTTATCCTA TCAGCGGGTCATGGAAGTGCCATGCTTTATAGCTTGTTACACTTAGCTGGTTATGATTTATCTGTAGAAGATTTAAAGAACTTCCGT CAATGGGGTTCTAAAACACCAGGTCACCCAGAAGTGAACCACACAGACGGTGTCGAAGCAACCACAGGACCTCTTGGTCAAGGGATC GCAAATGCCGTTGGTATGGCCATGGCAGAAGCTCATCTAGCAGCTAAATTTAACAAACCAGGCTTTGACATCGTTGATCACTACACC TTTGCTTTGAATGGTGACGGTGACCTTATGGAAGGGGTCAGCCAAGAAGCAGCAAGTATGGCAGGACATTTAAAACTTGGGAAATTG GTCTTGCTATATGATTCAAACGAC
9	CCTATGGCCTATGTTCTTTGGAATCACTTCATGAACATCAATCCCAAAACAAGCCGTAATTGGTCAAACAGAGACCGTTTTATCCTA TCAGCAGGTCATGGAAGTGCCATGCTTTATAGCTTGTTACACTTAGCTGGTTATGATTTATCTGTAGAAGATTTAAAGAACTTCCGT CAATGGGGTTCTAAAACACCAGGTCACCCAGAAGTGAACCACACAGACGGTGTCGAAGCAACCACAGGACCTCTTGGTCAAGGGATC GCAAATGCCGTTGGTATGGCCATGGCAGAAGCTCATCTAGCAGCTAAATTTAACAAACCAGGCTTTGACATCGTTGATCACTACACC TTTGCTTTGAATGGTGACGGTGACCTTATGGAAGGGGTCAGCCAAGAAGCAGCAAGTATGGCAGGACATTTAAAACTTGGGAAATTG GTCTTGCTATATGATTCAAACGAC
10	CCTATGGCCTACGTTCTTTGGAATCACTTCATGAACATTAATCCAAAAACAAGCCGTAATTGGTCAAACAGAGACCGTTTTATCCTA TCAGCGGGACACGGAAGTGCTATGCTTTATAGCTTGTTACACTTAGCTGGTTATGATTTATCTGTAGAGGATTTGAAAAACTTCCGT CAATGGGGTTCTAAAACACCAGGTCACCCAGAAGTTAATCATACCGATGGTGTGGAAGCAACTACAGGACCTCTTGGTCAAGGGATC GCAAATGCTGTTGGTATGGCCATGGCAGAAGCTCATCTAGCAGCTAAATTTAACAAACCAGGCTTTGACATCGTTGATCATTACACA TTTGCGTTGAATGGTGACGGTGACCTTATGGAAGGTGTCAGTCAAGAAGCAGCAAGTATGGCAGGACACTTAAAACTTGGCAAATTG GTCTTGCTTTACGATTCAAACGAT
11	CCTATGGCCTACGTTCTTTGGAATCACTTCATGAACATTAATCCAAAAACAAGCCGTAATTGGTCAAACAGAGACCGTTTTATCCTA TCAGCGGGACACGGAAGTGCTATGCTTTATAGCTTGTTACACTTAGCTGGTTATGATTTATCTGTAGAGGATTTGAAAAACTTCCGT CAATGGGGTTCTAAAACACCAGGTCACCCAGAAGTTAATCATACCGATGGTGTGGAAGCAACTACAGGACCTCTTGGTCAAGGGATC GCAAATGCTGTTGGTATGGCCATGGCAGAAGCTCATCTAGCAGCTAAATTTAACAAACCAGGCTTTGACATCGTTGATCATTACACA TTTGCTTTGAATGGTGACGGTGACCTTATGGAAGGGGTCAGTCAAGAAGCAGCAAGTATGGCAGGACACTTAAAACTTGGCAAATTG GTCTTGCTTTACGATTCAAACGAT
12	CCTATGGCCTACGTTCTTTGGAATCACTTCATGAACATTAATCCAAAAACAAGCCGTAATTGGTCAAACAGAGACCGTTTTATCCTA TCAGCGGGACACGGAAGTGCTATGCTTTATAGCTTGTTACACTTAGCTGGTTATGATTTATCTGTAGAAGATTTAAAGAACTTCCGT CAATGGGGTTCTAAAACACCAGGTCACCCAGAAGTTAATCATACCGATGGTGTGGAAGCAACTACAGGACCTCTTGGTCAAGGGATT GCGAATGCTGTTGGTATGGCCATGGCAGAAGCTCATCTAGCAGCTAAATTTAACAAACCAGGATTTGACATCGTTGATCACTACACA TTTGCTTTGAATGGTGACGGTGACCTTATGGAAGGTGTCAGTCAAGAAGCAGCAAGTATGGCAGGACACTTAAAACTTGGCAAATTG GTCTTGCTTTACGATTCAAACGAT
13	CCTATGGCCTATGTTCTTTGGAATCACTTCATGAACATTAATCCAAAAACAAGCCGTAATTGGTCAAACAGAGACCGTTTTATCCTA

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	GTCTTGCTTTACGATTCAAACGAT
14	CCTATGGCCTACGTTCTTTGGAATCACTTCATGAACATTAATCCAAAAACAAGCCGTAATTGGTCAAACAGAGACCGTTTTATCCTA
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	GCAAATGCCGTTGGTATGGCCATGGCAGAAGCTCACCTAGCAGCTAAATTTAACAAACCAGGCTTTGACATCGTTGATCACTACACA
	TTTGCTTTGAATGGAGACGGTGACCTTATGGAAGGGGTCAGCCAAGAAGCAGCAAGTATGGCAGGACATTTAAAACTTGGGAAATTG
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15	CCTATGGCCTATGTTCTTTGGAATCACTTCATGAACATCAATCCCAAAACAAGCCGTAATTGGTCAAACAGAGACCGTTTTATCCTA
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17	CCTATGGCCTATGTTCTTTGGAATCACTTCATGAACATTAATCCAAAAACAAGCCGTAATTGGTCAAACAGAGACCGTTTTATCCTA
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20	CCTATGGCCTATGTTCTTTGGAATCACTTCATGAACATCAATCCCAAAACAAGCCGTAATTGGTCAAACAGAGACCGTTTTATCCTA
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	GTCCTTCTTTATGATTCAAATGAT
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	GCAAATGCCGTTGGTATGGCCATGGCAGAAGCTCATCTAGCAGCTAAATTTAACAAACCAGGCTTTGACATCGTTGATCACTACACA
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	GCAAATGCCGTTGGTATGGCCATGGCAGAAGCTCATCTAGCAGCTAAATTTAACAAACCAGGCTTTGACATCGTTGATCACTACACA
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GTCTTGCTATATGATTCAAACGAC

SDSE *xpt* alleles and sequences.

xpt	Sequence (450 bp)
1	GGAGAGAATATTCTAAAGGTAGATAATTTTTTAACTCATCAAGTTGATTACCGGTTGATGAAAGCAATTGGTAAAATGTTTGCTCAAA AATATGCTGAGGCTGGCATTACAAAAGTGGTTACAATCGAAGCTTCAGGTATTGCACCAGCTGTATACGCTGCAGAAGCAATGGATGT TCCTATGATTTTTGCGAAAAAACATAAAAACATTACCATGACAGAAGGCATTTTGACAGCAGAAGTTTATTCTTTCACGAAACAAGTG ACGAGCACGGTGTCTATCGCTGGTAAATTCCTATCTAAAGAAGACAAGGTTTTGATTATTGATGACTTTTTAGCTAATGGTCAGGCAG CCAAAGGCTTGATCGAGATTATTGGTCAAGCAGGGGCACAAGTCGTCGGCGTTGGTATTGTGATTGAGAAATCTTTCCAAGATGGTCG TCGATTGATT
2	GGAGAGAATATTCTAAAGGTAGATAATTTTTTAACTCATCAAGTTGATTACCGGTTGATGAAAGCAATTGGTAAAGTGTTTGCTCAAA AATATGCTGAGACTGGCATTACAAAAGTGGTTACAATCGAAGCTTCAGGTATTGCACCAGCCGTATACGCTGCAGAAGCAATGGATGT TCCTATGATTTTTGCAAAAAAACATAAAAACATTACCATGACAGAAGGCATTTTGACAGCAGAAGTTTATTCTTTCACGAAACAAGTG ACGAGCACGGTGTCTATCGCTGGTAAATTACTATCTAAAGAAGACAAGGTTTTGATTATTGATGACTTTTTAGCTAATGGTCAGGCAG CCAAAGGCTTGATCGAGATTATTGGTCAAGCAGGGGCACAAGTCGTCGGCGTTGGTATTGTGATTGAGAAATCTTTCCAAGATGGTCG TCGATTGATT
3	GGAGAGAATATTTTAAAGGTAGATAATTTTTTAACCCACCAAGTTGATTATCGATTGATGAAGGCAATCGGTAAGGTATTTGCTCAAA AATATGCTGAGGCAGGCATTACAAAAGTGGTTACAATCGAAGCTTCAGGTATTGCACCAGCCGTATACGCTGCAGAAGCAATGGATGT TCCTATGATTTTTGCAAAAAAACATAAAAACATTACCATGACAGAAGGCATTTTGACAGCAGAAGTTTATTCTTTCACGAAACAAGTG ACGAGCACGGTGTCTATCGCTGGTAAATTCCTATCTAAAGAAGACAAGGTTTTGATTATTGATGACTTTTTAGCTAATGGTCAGGCAG CCAAAGGCTTGATCGAGATTATTGGTCAAGCAGGGGCACAAGTCGTCGGCGTTGGTATTGTGATTGAGAAATCTTTCCAAGATGGTCG TCGATTGATT
4	GGAGAGAATATTCTAAAGGTAGATAATTTTTTAACTCATCAAGTTGATTACCGGTTGATGAAAGCAATTGGTAAAGTGTTTGCTCAAA AATATGCTGAGGCTGGCATTACAAAAGTGGTTACAATCGAAGCTTCAGGTATTGCACCAGCCGTATACGCTGCAGAAGCAATGGATGT TCCTATGATTTTTGCGAAAAAACATAAAAACATTACCATGACAGAAGGCATTTTGACAGCAGAAGTTTATTCTTTCACTAAACAAGTG ACGAGCACGGTGTCTATCGCTGGTAAATTCCTATCTAAAGAAGACAAGGTTTTGATTATTGATGACTTTTTAGCTAATGGTCAGGCAG CCAAAGGCTTGATTGAGATTATTGGTCAAGCAGGGGCACAAGTCGTCGGCGTTGGTATTGTGATTGAGAAATCTTTCCAAGATGGTCG TCGATTGATT
5	GGAGAGAATATTCTAAAGGTAGATAATTTTTTAACTCATCAAGTTGATTACCGGTTGATGAAAACAATTGGTAAAGTGTTTGCTCAAA AATATGCTGAGGCTGGCATTACAAAAGTGGTTACAATCGAAGCTTCAGGTATTGCACCAGCCGTATACGCTGCAGAAGCAATGGATGT TCCTATGATTTTTGCGAAAAAACATAAAAACATTACCATGACAGAAGGCATTTTGACAGCAGAAGTTTATTCTTTCACTAAACAAGTG ACGAGCACGGTGTCTATCGCTGGTAAATTCCTATCTAAAGAAGACAAGGTTTTGATTATTGATGACTTTTTAGCTAATGGTCAGGCAG CCAAAGGCTTGATCGAGATTATTGGTCAAGCAGGGGCACAAGTCGTCGGCGTTGGTATTGTGATTGAGAAATCTTTCCAAGATGGTCG TCGATTGATT
6	GGAGAGAATATTCTAAAGGTAGATAATTTTTTAACTCATCAAGTTGATTACCGGTTGATGAAAGCAATTGGTAAAGTGTTTGCTCAAA AATATGCTGAGGCTGGCATTACAAAAGTGGTTACAATCGAAGCTTCAGGTATTGCACCAGCTGTATACGCTGCAGAAGCAATGGATGT TCCTATGATTTTTGCGAAAAAACATAAAAACATTACCATGACAGAAGGCATTTTGACAGCAGAAGTTTATTCTTTCACGAAACAAGTG ACGAGCACGGTGTCTATCGCTGGTAAATTCCTATCTAAAGAAGATAAGGTTTTGATTATTGATGACTTTTTAGCCAATGGTCAGGCAG CAAAGGGTTTGATTGAGATTATTGGTCAAGCAGGGGCACAAGTCGTCGGCGTTGGTATTGTGATTGAGAAATCTTTCCAAGATGGTCG TCGATTGATT
7	GGAGAGAATATTCTAAAGGTAGATAATTTTTTAACTCATCAAGTTGATTACCGGTTGATGAAAGCAATTGGTAAAATGTTTGCTCAAA AATATGCTGAGGCTGGCATTACAAAAGTGGTTACAATCGAAGCTTCAGGTATTGCACCAGCTGTATACGCTGCAGAAGCAATGGATGT TCCTATGATTTTTGCGAAAAAACATAAAAACATTACCATGACAGAAGGCATTTTGACAGCAGAAGTTTATTCTTTCACGAAACAAGTG ACGAGCACGGTGTCTATCGCTGGTAAATTCCTATCTAAAGAAGATAAGGTTTTGATTATTGATGACTTTTTAGCCAATGGTCAGGCAG CAAAGGGTTTGATTGAGATTATTGGTCAAGCAGGGGCACAAGTCGTCGGCGTTGGTATTGTGATTGAGAAATCTTTCCAAGATGGTCG TCGATTGATT
8	GGAGAGAATATTCTAAAGGTAGATAATTTTTTAACTCATCAAGTTGATTACCGGTTGATGAAAGCAATTGGTAAAGTGTTTGCTCAAA AATATGCTGAGACTGGCATTACAAAAGTGGTTACAATCGAAGCTTCAGGTATTGCACCAGCCGTATACGCTGCAGAAGCAATGGATGT TCCTATGATTTTTGCGAAAAAACATAAAAACATTACCATGACAGAAGGCATTTTGACAGCAGAAGTTTATTCTTTCACGAAACAAGTG ACGAGCACGGTGTCTATCGCTGGTAAATTCCTATCTAAAGAAGATAAGGTTTTGATTATTGATGACTTTTTAGCCAATGGTCAGGCAG CAAAGGGTTTGATTGAGATTATTGGTCAAGCAGGGGCACAAGTCGTTGGCGTTGGTATTGTGATTGAGAAATCTTTCCAAGATGGTCG TCGATTGATT
9	GGAGAGAATATTCTAAAGGTAGATAATTTTTTAACTCATCAAGTTGATTACCGGTTGATGAAAGCAATTGGTAAAATGTTTGCTCAAA AATATGCTGAGGCTGGCATTACAAAAGTGGTTACAATCGAAGCTTCAGGTATTGCACCAGCTGTATACGCTGCAGAAGCAATGGATGT TCCTATGATTTTTGCGAAAAAACATAAAAACATTACCATGACAGAAGGCATTTTGACAGCAGAAGTTTATTCTTTCACTAAACAAGTG ACGAGCACGGTGTCTATCGCTGGTAAATTCCTATCTAAAGAAGACAAGGTTTTGATTATTGATGACTTTTTAGCTAATGGTCAGGCAG CCAAAGGCTTGATCGAGATTATTGGTCAAGCAGGGGCACAAGTCGTCGGCGTTGGTATTGTGATTGAGAAATCTTTCCAAGATGGTCG TCGATTGATT
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11	GGAGAGAATATTCTAAAGGTAGATAATTTTTTAACTCATCAAGTTGATTACCGGTTGATGAAAGCAATTGGTAAAGTGTTTGCTCAAA AATATGCTGAGACTGGCATTACAAAAGTGGTTACAATCGAAGCTTCAGGTATTGCACCAGCCGTATACGCTGCAGAAGCAATGGATGT TCCTATGATTTTTGCGAAAAAACATAAAAACATTACCATGACAGAAGGCATTTTGACAGCAGAAGTTTATTCTTTCACGAAACAAGTG ACGATCACGGTGTCTATCGCTGGTAAATTCCTATCTAAAGAAGATAAGGTTTTGATTATTGATGACTTTTTAGCCAATGGTCAGGCAG CAAAGGGTTTGATTGAGATTATTGGTCAAGCAGGGGCACAAGTCGTTGGCGTTGGTATTGTGATTGAGAAATCTTTCCAAGATGGTCG TCGATTGATT
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SDSE *atoB* alleles and sequences.

atoB	Sequence (434 bp)
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	TTCCTCCCTAAAGAAGGAACCGTTACTGCTGGGAATGCCTCAGGAATAAACGATGGTGCAGCCTTATTGATGTTAATGACTGAAGAA
	AAAGCTCTTGAATTAGGGTTAACACCTTTAGTCACTATTGAAAGCTATGCTAGTGCAGGTGTTGCACCTGAACTGATGGGAACTGGA
	CCAATTCCTGCCACTCAAAAAGCCCTTAAGAAAGCTGGATTAACAATTTCTGATTTGGACTTAGTGGAGTCCAATGAAGCGTTTGC
11	TTTGCTTTAGAAAGCCAAGAAAAAGCTGCTAGGGCATTAGAAAATCATCGTTTTGCTGACGAAATTGTCCCTGTCTCTGTGCCTCAA
	CGACGCAAAGATCCTTTAATTGTTACGACGGATGAATACCCAAAAGTTGACACAAGCCTTGAAAAATTACAGCAATTACGTCCTGCT
	TTCCTCCCTAAAGAAGGAACCGTTACTGCTGGAAATGCCTCAGGAATAAATGATGGTGCAGCCTTATTGATGTTAATGACTGAAGAA
	AAAGCTCTTGAATTAGGGTTAACACCTTTAGTCACTATTGAAAGCTATGCTAGTGCAGGTGTTGCACCTGAACTGATGGGAACTGGA
	CCAATTCCTGCCACTCAAAAAGCCCTTAAGAAAGCTGGATTAACAATTTCTGACTTGGACTTAGTGGAGTCCAATGAAGCATTTGC
12	TTTGCTTTAGAAAGCCAAGAAAAAGCTGCTAGGGCATTAGAAAATCATCGTTTTGCTGACGAAATTGTCCCTGTCTCTGTGCCTCAA
	CGACGCAAAGATCCTTTAATTGTTACGACGGATGAATACCCAAAAGTTGACACAAGCCTTGAAAAATTACAGCAATTACGTCCTGCT
	TTCCTCCCTAAAGAAGGAACCGTTACTGCTGGGAATGCCTCAGGAATAAACGATGGTGCAGCCTTATTGATGTTAATGACTGAAGAA
	AAAGCTCTTGAATTAGGGTTAACACCTTTAGTCACTATTGAAAGCTATGCTAGTGCAGGTGTTGCACCTGAACTGATGGGAACTGGA
	CCAATTCCTGCCACTCAAAAAGCCCTTAAGAAAGCTGGATTAACAATTTCTGATTTGGACTTAGTGGAGTCCAATGAAGCATTTGC
13	TTTGCTTTAGAAAGCCAAGAAAAAGCTGCTAGGGCATTAGAAAATCATCGTTTTGCTGACGAAATTGTCCCTGTCTCTGTGCCTCAA
	CGACGCAAAGATCCTTTAATTGTTACGACGGATGAATACCCAAAAGTTGACACAAGCCTTGAAAAATTACAGCAATTACGTCCTGCT
	TTCCTCCCTAAAGAAGGAACCGTTACTGCTGGAAATGCCTCTGGAATAAATGATGGTGCAGCCTTATTGATGTTAATGACTGAAGAA
	AAAGCTCTTGAATTAGGGTTAACACCTTTAGCCACTATTGAAAGCTATGCTAGTGCAGGTGTTGCACCTGAACTGATGGGAACTGGA
	CCAATTCCTGCCACTCAAAAAGCCCTTAAGAAAGCTGGATTAACAATTTCTGACTTGGACTTAGTGGAGTCCAATGAAGCATTTGC
14	TTTGCTTTAGAAAGCCAAGAAAAAGCTGCTAGGGCATTAGAAAATCATCGTTTTGCTGACGAAATTGTCCCTGTCTCTGTGCCTCAA
	CGACGCAAAGATCCTTTAATTGTTACGACGGATGAATACCCAAAAGTTGACACAAGCCTTGAAAAATTACAGCAATTACGTCCTGCT
	TTCCTCCCTAAAGAAGGAACCGTTACTGCTGGGAATGCCTCAGGAATAAACGATGGTGCAGCCTTATTGATGTTAATGACTGAAGAA
	AAAGCTCTTGAATTAGGGTTAACACCTTTAGTCACTATTGAAAGCTATGCTAGTGCAGGTGTTGCACCTGAACTGATGGGAACTGGA
	CCAATTCCTGCCACTCAAAAAGCCCTTAAGAAAGCTGGATTAACAATTTCTGATTTGGACTTAGTGGAGTCCAATGAAGCATTTGC
15	TTTGCAGCTGATAGCCAGCGTAAAGCCCAACTTGCCATAGAAAAAGGTCGTTTCAAAGAAGAGATTGCACCTGTCACTATTCCTCAG
	CGTAAAGGTGAACCTTTACTCGTTGATCAAGATGAATACCCTAAATTTGGAACGACAGTGGATAAGTTAGCAAAGTTACGCCCTGCT
	TTTATCAAAGATGAGGAGACAGTAACTGCTGGTAATGCTTCAGGAATCAATGATGGAGCAGCAGCAATTTTATTGATGAGTAAAGAA

	AAAGCTGAAGAATTAGGGCTCCCTATTTTAGCTAAAATCACTAGTTATGCAAGTGCAGGTGTAGACCCAAGTATTATGGGCTGCGGA
	CCAATACCTGCTACGAAAAAGGCTCTTGCAAAGGCTCAGCTGACAATTGATGACATTGATTTGATTGAAGCAAACGAAGCTTTTGC
16	TTTGCTTTAGAAAGCCAAGAAAAAGCGCTAAGTGCATTAGAAAATCATCGTTTTGCTGACGAAATTGTCCCTGTCTCTGTGCCTCAG
	CGCAAAGGTGATCCTTTAATTGTTGACGAGGATGAATACCCAAAAGTTGACACAAGCCTTGAAAAATTAGCACAATTACGTCCTGCT
	TTCCTCCCTAAAGAAGGAACCGTTACTGCTGGAAATGCCTCAGGAATAAATGATGGTGCAGCCTTATTGATGTTAATGACTGAAGAA
	AAAGCTCTTGAATTAGGGTTAACACCTTTAGTCACTATTGAAAGCTATGCTAGTGCAGGTGTTGACCTGAGACTGATGGGAACTGGA
	CCAATTCCTGCCACTCAAAAAGCCCTTAAGAAAGCTGGATTAACAATTTCTGACTTGGACTTGAGTGGAGCCAATGAAGCATTTGC
17	TTTGCTTTAGAAAGCCAAGAAAAAGCGCTACAGGCATTAGAAAATCATCGTTTTGCTGACGAAATTGTCCCTGTCTCTGTGCCTCAG
	CGCAAAGGTGATCCTTTAATTGTTGACGAGGATGAATACCCAAAAGTTGACACAAGCCTTGAAAAATTAGCACAATTACGTCCTGCT
	TTCCTCCCTAAAGAAGGAACCATTACTGCTGGAAATGCCTCAGGAATAAATGATGGTGCAGCCTTATTGATGTTAATGACTGAAGAA
	AAAGCTCTTGAATTAGGGTTAACACCTTTAGTCACTATTGAAAGCTATGCTAGTGCAGGTGTTGACCTGAGACTGATGGGAACTGGA
	CCAATTCCTGCCACTCAAAAAGCCCTTAAGAAAGCTGGATTAACAATTTCTGACTTGGACTTGAGTGGAGCCAATGAAGCATTTGC
18	TTTGCTTTAGAAAGCCAAGAAAAAGCTGCTAAGGCATTAGAAAATCATCGTTTTGCTGACGAAATTGTCCCTGTCTCTGTGCCTCAA
	CGACGCAAAGATCCTTTAATTCTTACGACGGATGAATACCCAAAAGTTGACACAAGCCTTGAAAAATTACAGCAATTACGTCCTGCT
	TTCCTCCCTAAAGAAGGAACCGTTACTGCTGGAAATGCCTCAGGAATAAATGATGGTGCAGCCTTATTGATGTTAATGACTGAAGAA
	AAAGCTCTTGAATTAGGGTTAACACCTTTAGTCACTATTGAAAGCTATGCTAGTGCAGGTGTTGCACCTGAACTGATGGGAACTGGA
	CCAATTCCTGCCACTCAAAAAGCCCTTAAGAAAGCTGGATTAACAATTTCTGACTTGGACTTAGTGGAGTCCAATGAAGCATTTGC
19	TTTGCTTTAGAAAGCCAAGAAAAAGCTGCTAAGGCATTAGAAAATCATCGTTTTGCTGACGAAATTGTCCCTGTCTCTGTGCCTCAA
	CGACGCAAAGATCCTTTAATTGTTACGACGGATGAATACCCAAAAGTTGACACAAGCCTTGAAAAATTACAGCAATTACGTCCTGCT
	TTCCTCCCTAAAGAAGGAACCGTTACTGCTGGAAATGCCTCAGGAATAAATGATGGTGCAGCCTTATTGATGTTAATGACTGAAGAA
	AAAGCTCTTGAATTAGGATTAACACCTTTAGTCACTATTGAAAGCTATGCTAGTGCAGGTGTTGCACCTGAACTGATGGGAACTGGA
	CCAATTCCTGCCACTCAAAAAGCCCTTAAGAAAGCTGGATTAACAATTTCTGACTTGGACTTAGTGGAGTCCAATGAAGCATTTGC
20	TTTGCTTTAGAAAGCCAAGAAAAAGCTGCTAAAGCATTAGAAAATCATCGTTTTGCTGACGAAATTGTCCCTGTCTCTGTACCTCAA
	CGACGCAAAGACCCTTTAATTGTTACAACAGATGAATACCCAAAAGGTGACACAAGCCTTGAAAAATTACAGCAATTACGTCCTGCC
	TTCCTCCCTAAAGAAGGAACCGTTACTGCTGGGAATGCTTCAGGAATAAACGATGGTGCAGCCTTATTGATGTTAATGACTGAAGAA
	AAAGCTCTTGAATTAGGGTTAACACCTTTAGTCACTATTGAAAGCTATGCTAGTGCAGGTGTTGCACCTGAACTGATGGGAACTGGA
	CCAATTCCTGCCACTCAAAAAGCCCTTAAGAAAGCTGGATTAACAATTTCTGATTTGGACTTAGTGGAGTCCAATGAAGCATTTGC

Appendix 7: *S.canis* MLST Database

***S.canis* allelic profiles and corresponding st's.**

gki	*gtr*	*murI*	*mutS*	*recP*	*xpt*	*atoB*	st	Reference
1	1	1	1	1	nd	1	Sc1[1]	Ahmad et al, 2009
1	1	1	1	1	1	1	1	R. Towers[2]
2	2	2	2	2	2	2	2	Ahmad et al, 2009
3	3	3	3	1	3	3	3	"
4	2	4	4	3	4	4	4	"
1	1	1	1	4	1	1	5	This study
4	2	4	4	5	4	4	6	"
5	4	5	3	4	5	5	7	"
6	5	6	3	4	6	1	8	"
3	6	3	3	1	3	3	9	R. Towers
4	6	3	4	1	3	3	10	"
4	2	4	4	6	4	4	11	"

Sc1-Sc4, first published in Ahmad et al, 2009, have been designated ST1-ST4 with the exception that Sc1, which is incomplete, has been replaced with ST1 provided by Dr Rebecca Towers (unpublished). ST1 is identical to Sc1 with respect to the other 6 loci but could be a possible single locus variant of Sc1.[2] Unpublished MLST data kindly provided by Dr Rebecca Towers of Menzies School of Health Research from isolates of canine origin collected in the Northern Territory.

S. canis *gki* alleles and sequences and corresponding GenBank accession number. Shaded alleles are from this study.

gki	Sequence (498 bp)	Acc#
1	AACCTTAATTGGAAGGAAACTCAAGAAGTTGGTTCCGTTATTGAAAAAGAATTAGGTATTCCTTTTGCCATTGACAATGATGCTAATG TTGCTGCTCTTGGTGAACGTTGGGTAGGTGCTGGTGAAAATAACCCAGATGTGGTCTTCATGACACTTGGAACAGGTGTCGGTGGAGG CATTATTGCTGATGGTAACCTGATTCATGGTGTTGCAGGAGCAGGTGGTGAAATCGGCCATATGATTGTTGAGCCAGAAAATGGTTTT GCCTGTACTTGTGGGTCACATGGTTGTCTAGAAACAGTGGCTTCAGCAACAGGAGTTGTCAAGGTGGCACGCTTATTGGCAGAAGCCT ACGAAGGAGATTCAGCTATCAAAGCAGCCATTGACAATGGCGAAGGTGTTACCAGTAAAGATATTTTCATGGCAGCTGAAGCAGGGGA TTCCTTTGCTGATTCTGTTGTGGAAAAGGTTGGTTACTACCTTGGTCTTGCGTCAGCA	EU768807
2	AACCTTAATTGGAAGGAAACTCAAGAAGTTGGTTCTGTTATTGAAAAAGAATTAGGTATTCCTTTTGCCATTGACAATGATGCTAATG TTGCTGCTCTTGGTGAACGTTGGGTAGGTGCTGGTGAAAATAACCCAGATGTGGTCTTCATGACACTTGGAACAGGTGTCGGTGGAGG CATTATTGCTGATGGTAACCTGATTCATGGTGTTGCAGGAGCAGGTGGTGAAATCGGCCACATGATTGTTGAGCCAGAAAATGGTTTT GCCTGTACTTGTGGGTCACATGGCTGTCTAGAAACAGTGGCTTCAGCAACAGGAGTTGTCAAGGTGGCACGCTTATTGGCAGAAGCCT ACGAAGGAGATTCAGCTATCAAAGCAGCCATTGACAATGGCGAAGGTGTTACCAGTAAAGATATTTTCATAGCAGCTGAAGCAGGGGA TTCCTTTGCTGATTCTGTTGTGGAAAAGGTTGGTTATTACCTTGGTCTTGCGTCAGCA	EU768805
3	AACCTTAATTGGAAGGAAACTCAAGAAGTTGGTTCCGTTATTGAAAAAGAATTAGGTATTCCTTTTGCCATTGACAATGATGCTAATG TTGCTGCTCTTGGTGAACGTTGGGTAGGTGCTGGTGAAAATAACCCAGATGTGGTCTTCATGACACTTGGAACAGGTGTCGGTGGAGG CATTATTGCTGATGGTAACCTGATTCATGGTGTTGCAGGAGCAGGTGGTGAAATCGGCCACATGATTGTTGAGCCAGAAAATGGTTTT GCCTGTACTTGTGGGTCACATGGTTGTCTAGAAACAGTGGCTTCAGCAACAGGAGTTGTCAAGGTGGCACGCTTATTGGCAGAAGCCT ACGAAGGAGATTCAGCTATCAAAGCAGCCATTGACAATGGCGAAGGTGTTACCAGTAAAGATATTTTCATGGCAGCTGAAGCAGGGGA TTCCTTTGCTGATTCTGTTGTGGAAAAGGTTGGTTACTACCTTGGTCTTGCGTCAGCA	EU768804
4	AACCTTAATTGGAAGGAAACTCAAGAAGTTGGTTCCGTTATTGAAAAAGAATTAGGTATTCCTTTTGCCATTGACAATGATGCTAATG TTGCTGCTCTTGGTGAACGTTGGGTAGGTGCTGGTGAAAATAACCCAGATGTGGTTTTCATGACACTTGGAACAGGTGTCGGTGGAGG CATTATTGCTGATGGTAACCTGATTCATGGTGTTGCAGGAGCAGGTGGTGAAATCGGCCACATGATTGTTGAGCCAGAAAATGGTTTT GCCTGTACTTGTGGGTCACATGGTTGTCTAGAAACAGTGGCTTCAGCAACAGGAGTTGTCAAGGTGGCACGCTTATTGGCAGAAGCCT ACGAAGGAGATTCAGCTATCAAAGCAGCCATTGACAATGGCGAAGGTGTTACCAGTAAAGATATTTTCATGGCAGCTGAAGCAGGGGA TTCCTTTGCTGATTCTGTTGTGGAAAAGGTTGGTTACTACCTTGGTCTTGCGTCAGCA	FJ238467
5	AACCTTAATTGGAAGGAAACTCAAGAAGTTGGTTCCGTTATTGAAAAAGAATTAGGTATTCCTTTTGCCATTGACAATGATGCTAATG TTGCTGCTCTTGGTGAACGTTGGGTAGGTGCTGGTGAAAATAACCCAGATGTGGTCTTCATGACACTTGGAACAGGTGTCGGCGGAGG CATTATTGCTGATGGTAACCTGATTCATGGTGTTGCAGGAGCAGGTGGTGAAATCGGTCACATGATTGTTGAGCCAGAAAATGGTTTT GCCTGTACTTGTGGGTCACATGGCTGTCTAGAAACAGTGGCTTCAGCAACAGGAGTTGTCAAGGTGGCACGCTTATTGGCAGAAGCCT ACGAAGGAGATTCTGCTATCAAAGCAGCCATTGACAATGGCGAAGGCGTTACCAGTAAAGATATTTTCATGGCAGCTGAAGCAGGGGA TTCCTTTGCTGATTCTGTTGTGGAAAAGGTTGGTTACTACCTTGGTCTTGCGTCAGCA	This study (C10)
6	AACCTTAATTGGAAGGAAACTCAAGAAGTTGGTTCCGTTATTGAAAAAGAATTAGGTATTCCTTTTGCCATTGACAATGATGCTAATG TTGCTGCTCTTGGTGAGCGTTGGGTTGGAGCTGGCGAAAACAATCCTGATGTTGTTTTCATGACACTAGGAACTGGTGTCGGTGGGGG GATTATCGCCGATGGTAACCTGATTCATGGTGTTGCAGGAGCAGGTGGTGAAATCGGCCACATGATTGTTGAACCAGAAAATGGCTTT GCTTGTACTTGTGGCTCATACGGCTGTTTGGAAACAGTAGCCTCAGCAACAGGGGTTGTCAAGGTATCACGTTTATTGGCAGAAGCTT ATGAAGGAGATTCTGCTATTAAAGCCGCCATTGATAATGGAGACAATGTCACCAGCAAAGATATTTTCATGGCCGCTGAAGCAGGGGA TTCCTTTGCAGATTCTGTTGTGGAAAAGGTTGGTTACTATCTTGGTCTTGCTTCAGCA	This study (F8)

***S. canis gtr* alleles and sequences and corresponding GenBank accession numbers.** Shaded alleles are from this study.

gtr	Sequence (450 bp)	Acc#
1	ACCTTGCTTCGAACCATGAATCTTTTGGAAGTACCGACCAAGGGACAGATTAGTTTTGAAGGGGTTGATATTACCGATAAGAAG AATGATATTTTTAGCATGCGCGAAAAAATGGGAATGGTTTTCCAGCAGTTTAACCTCTTTTCCAATATGACTGTTTTAGAAAAT ATCACCTTATCGCCAATCAAAACCAAGGGAATGGCTAAAGCAGAGGCTGACAAAACAGCCTTGAACTTGTTGGACAAAGTTGGA TTATCAGAAAAAGCCAAGGCTTATCCTGCTAGTCTTTCTGGTGGGCAGCAGCAGCGGATTGCCATTGCGCGTGGACTAGCTATG GATCCAGATGTTTTACTCTTTGATGAACCAACTTCAGCCCTTGATCCTGAAATGGTGGGCGAGGTCTTAGCCGTTATGCAGGAT TTGGCTAAATCTGGGATGACCATGGTTATT	FJ238468
2	ACCTTGCTTCGAACCATGAATCTTTTGGAAGTACCGACCAAGGGACAGATTAGTTTTGAAGGGGTTGATATTACCGATAAGAAG AATGATATTTTTAGCATGCGCGAAAAAATGGGAATGGTTTTCCAGCAGTTTAACCTCTTTTCCAATATGACTGTTTTAGAAAAT ATCACCTTATCGCCAATCAAAACCAAGGGAATGGCTAAAGCAGAGGCTGACAAAACAGCCTTGAACTTGTTGGACAAAGTTGGA TTATCAGAAAAAGCCAAGGCTTATCCTGCTAGCCTTTCTGGTGGGCAGCAGCAGCGGATTGCCATTGCGCGTGGACTAGCTATG GATCCAGATGTTTTACTCTTTGATGAACCAACTTCAGCCCTTGATCCTGAAATGGTGGGCGAGGTCTTAGCCGTTATGCAGGAT TTGGCTAAATCTGGGATGACCATGGTTATT	EU768802
3	ACCTTGCTTCGAACCATGAATCTTTTGGAAGTACCGACCAAGGGACAGATTAGTTTTGAAGGGGTTGATATTACTGATAAGAAG AATGATATTTTTAGCATGCGCGAAAAAATGGGAATGGTTTTCCAGCAGTTTAACCTCTTTTCCAATATGACTGTTTTAGAAAAT ATCACCTTATCGCCAATCAAAACCAAGGGAATGGCTAAAGCAGAGGCTGACAAAACAGCCTTGAACTTGTTGGACAAAGTTGGA TTATCAGAAAAAGCCAAGGCTTATCCTGCTAGCCTTTCTGGTGGGCAGCAGCAGCGGATTGCCATTGCGCGTGGACTAGCTATG GATCCAGATGTTTTACTCTTTGATGAACCAACTTCAGCCCTTGATCCTGAAATGGTGGGCGAGGTCTTAGCAGTTATGCAGGAT TTGGCTAAATCTGGGATGACCATGGTTATT	EU999155
4	ACCTTGCTTCGAACCATGAATCTTTTGGAAGTACCGACCAAGGGACAGATTAGTTTTGAAGGGGTTGATATTACCGATAAGAAG AATGATATTTTTAGCATGCGCGAAAAAATGGGAATGGTTTTCCAGCAGTTTAACCTCTTTTCCAATATGACTGTTTTAGAAAAT ATCACTTTATCGCCAATCAAAACCAAGGGAATGGCTAAAGCAGAGGCTGACAAAACAGCCTTGAACTTGTTGGACAAAGTTGGA TTATCAGAAAAAGCCAAGGCTTATCCTGCTAGTCTTTCTGGTGGGCAGCAGCAGCGGATTGCCATTGCGCGTGGACTAGCTATG GATCCAGATGTTTTACTCTTTGATGAACCAACTTCAGCCCTTGATCCTGAAATGGTGGGCGAGGTCTTAGCCGTTATGCAGGAT TTGGCTAAATCTGGGATGACCATGGTTATT	This study (C10)
5	ACCTTGCTTCGAACCATGAATCTTTTGGAAGTACCGACCAAGGGACAGATTAGTTTTGAAGGGGTTGATATTACCGATAAGAAG AATGATATTTTTAGCATGCGCGAAAAAATGGGAATGGTTTTCCAGCAGTTTAACCTCTTTTCCAATATGACTGTTTTAGAAAAT ATCACCTTATCGCCAATCAAAACCAAGGGAATGACTAAAGCAGAGGCTGACAAAACAGCCTTGAACTTGTTGGACAAAGTTGGA TTATCAGAAAAAGCCAAGGCTTATCCTGCTAGCCTTTCTGGTGGGCAGCAGCAGCGGATTGCCATTGCGCGTGGACTAGCTATG GATCCAGATGTTTTACTCTTTGATGAACCAACTTCAGCCCTTGATCCTGAAATGGTGGGCGAGGTCTTAGCCGTTATGCAGGAT TTGGCTAAATCTGGGATGACCATGGTTATT	This study (F8)
6	ACCTTGCTTCGAACCATGAATCTTTTGGAAGTACCGACCAAGGGACAGATTAGTTTTGAAGGGGTTGATATTACCGATAAGAAG AATGATATTTTTAGCATGCGCGAAAAAATGGGAATGGTTTTCCAGCAGTTTAACCTCTTTTCCAATATGACTGTTTTAGAAAAT ATCACCTTATCGCCAATCAAAACCAAGGGAATGGCTAAAGCAGAGGCTGACAAAACAGCCTTGAACTTGTTGGACAAAGTTGGA TTATCAGAAAAAGCCAAGGCTTATCCTGCTAGCCTTTCTGGTGGGCAGCAGCAGCGGATTGCCATTGCGCGTGGACTAGCTATG GATCCAGATGTTTTACTCTTTGATGAACCAACTTCAGCCCTTGATCCTGAAATGGTGGGCGAGGTCTTAGCAGTTATGCAGGAT TTGGCTAAATCTGGGATGACCATGGTTATT	R. Towers (G102)

***S. canis murI* alleles and sequences and corresponding GenBank accession numbers.** Shaded alleles are from this study.

murI	Sequence (438 bp)	Acc#
1	GCATGTAATACCGCAACAGCAGTAGCTTGGGAAGAGGTAAAAGCAGCTCTAGATATTCCTGTTTTAGGCGTTGTTCTGCCG GGGGCAAGCGCAGCTATTAAGTCAACGACAAAAGGTCAGGTTGGGGTTATCGGTACTCCTATGACCATAACATCTGACATT TACCGTCAAAAAATCCAATTATTAGCACCATCTGTGACAGTCACAAGCTTAGCCTGTCCTAAATTTGCACCTATTGTTGAG TCAAATGAAATTCATTCTAGTGTGGCTAAAAAAGTGGTTTACAAAAGCTTGGCTCCTTTAGTTGGGAAGGTAGATACTTTA GTACTAGGGTGTACTCACTATCCCTTGTTACGACCGATTATTCAAAATGTTATGGGACCGTCTGTTAAGTTGATTGACAGT GGAGCAGAGTGTGTTCGAGATATTTCTGTTTTG	EU999157
2	GCATGTAATACCGCAACAGCAGTAGCTTGGGAAGAGGTAAAAGCAGTTCTAGATATTCCTGTTTTAGGCGTTGTTCTGCCG GGGGCAAGCGCAGCTATTAAGTCAACGACAAAAGGTCAGGTTGGAGTTATCGGTACTCCTATGACCATAACATCTGACATT TACCGTCAAAAAATCCAATTATTAGCACCATCTGTGACAGTCACAAGCTTAGCCTGTCCTAAATTTGCACCTATTGTTGAG TCAAATGAAATTCATTCTAGTGTGGCTAAAAAAGTGGTTTACAAAAGCTTGGCTCCTTTAGTTGGGAAGGTAGATACTTTA GTACTAGGGTGTACTCACTATCCCTTGTTACGACCGATTATCCAAAATGTTATGGGACCGTCTGTTAAGTTGATTGATAGC GGGGCAGAATGTGTTCGAGATATTTCTGTTTTG	FJ151278
3	GCATGTAATACCGCAACAGCAGTAGCTTGGGAAGAGGTAAAAGCAGTTCTAGATATTCCTGTTTTAGGCGTTGTTCTGCCG GGGGCAAGCGCAGCTATTAAGTCAACGACAAAAGGTCAGGTTGGGGTTATCGGTACTCCTATGACCATAACATCTGACATT TACCGTCAAAAAATCCAATTATTAGCACCATCTGTGACAGTCACAAGCTTAGCCTGTCCTAAATTTGCACCTATTGTTGAG TCAAATGAAATTCATTCTAGTGTGGCTAAAAAAGTGGTTTACAAAAGCTTGGCTCCTTTAGTTGGGAAGGTAGATACTTTA GTACTAGGGTGTACTCACTATCCCTTGTTACGACCGATTATCCAAAATGTTATGGGACCGTCTGTTAAGTTGATTGACAGT GGAGCAGAGTGTGTTCGAGATATTTCTGTTTTG	FJ238469
4	GCATGTAATACCGCAACAGCAGTAGCTTGGGAAGAGGTAAAAGCAGTTCTAGATATTCCTGTTTTAGGCGTTGTTCTGCCG GGGGCAAGCGCAGCTATTAAGTCAACGACAAAAGGTCAGGTTGGGGTTATCGGTACTCCTATGACCATAACATCTGACATT TACCGTCAAAAAATCCAATTATTAGCACCATCTGTGACAGTCACAAGCTTAGCCTGTCCTAAATTTGCACCTATTGTTGAG TCAAATGAAATTCATTCTAGTGTGGCTAAAAAAGTGGTTTACAAAAGCTTGGCTCCTTTAGTTGGGAAGGTAGATACTTTA GTACTAGGGTGTACTCACTATCCCTTGTTACGACCGATTATCCAAAATGTTATGGGACCGTCTGTTAAGTTGATTGACAGT GGAGCAGAATGTGTTCGAGATATTTCTGTTTTG	FJ151277
5	GCATGTAATACCGCAACAGCAGTAGCTTGGGAAGAGGTAAAAGCAGTTCTAGATATTCCTGTTTTAGGCGTTGTTCTGCCG GGGGCAAGCGCAGCTATTAAGTCAACGACAAAAGGTCAGGTTGGGGTTATCGGTACTCCTATGACCATAACATCTGACATT TACCGTCAAAAAATCCAATTATTAGCACCATCTGTGACAGTCACAAGCTTAGCCTGTCCTAAATTTGCACCTATTGTTGAG TCAAATGAAATTCATTCTAGTGTGGCTAAAAAAGTGGTTTACAAAAGCTTGGCTCCTTTAGTTGGGAAGGTAGATACTTTA GTACTAGGGTGTACTCACTATCCCTTGTTACGACCGATTATCCAAAATGTTATGGGACCGTCTGTTAAGTTGATTGACAGT GGAGCAGAATGTGTTCGGGATATTTCTGTTTTG	This study (C10)
6	GCATGTAATACCGCAACAGCAGTAGCTTGGGAAGAGGTAAAAGCAGTTCTGGATATTCCTGTTTTAGGCGTTGTTCTGCCG GGGGCAAGCGCAGCTATTAAGTCAACGACAAAAGGTCAGGTTGGGGTTATCGGTACTCCTATGACCATAACATCTGACATT TACCGTCAAAAAATCCAATTATTAGCACCATCTGTGACAGTCACAAGCTTAGCCTGTCCTAAATTTGCACCTATTGTTGAG TCAAATGAAATTCATTCTAGTGTGGCTAAAAAAGTGGTTTACAAAAGCTTGGCTCCTTTAGTTGGGAAGGTAGATACTTTA GTACTAGGGTGTACTCACTATCCCTTGTTACGACCGATTATCCAAAATGTTATGGGACCGTCTGTTAAGTTGATTGACAGT GGAGCAGAGTGTGTTCGAGATATTTCTGTTTTG	This study (F8)

***S. canis mutS* alleles and sequences and corresponding GenBank accession numbers.**

mutS	Sequence (405 bp)	Acc#
1	ACCTATATTAATCGCTTGCAGAAGCTTGCCAAAACCTTGGCAACGGTTGATGTCTTGCAAAGTTTAGCAGTTGTTGCTG AGACGAACCATTACAGTCGGCCTAGTTTCAATGATGACCATGTGATTAAGATTCAAGATGGGCGTCATGCTGTCGTTGA AAAAGTTATGGGAGTGCAAGAGTACATTCCAAATAGTATTTCCTTTGATCAAAAAACAAGTATCCAATTGATTACGGGA CCAAATATGAGTGGTAAGTCTACTTATATGCGACAGCTTGCCTTAACGGTTATCATGGCACAGATGGGCTCGTTTGTGG CTGCTGACCATGTTGATTTACCTTTATTTGATGCTATTTTCACACGTATTGGAGCTGCTGATGATTTGATTTCTGGACA GTCAACCTTT	FJ238471
2	ACCTATATTAATCGCTTGCAGAAGCTTGCCAAAACCTTGGCAACGGTTGATGTCTTGCAAAGTTTAGCAGTTGTTGCTG AGACAAACCATTACAGTCGGCCTAGTTTCAATGATGACCATGTGATTAAGATTCAAGATGGACGTCATGCTGTTGTTGA AAAAGTTATGGGAGTGCAAGAATACATTCCAAATAGTATTTCCTTTGATCAAAAAACAAGTATCCAATTGATTACGGGA CCAAATATGAGTGGTAAGTCTACTTATATGCGGCAGCTTGCCTTAACGGTCATCATGGCACAGATGGGCTCGTTTGTGG CTGCTGACCATGTCGATTTGCCTTTATTTGATGCTATTTTCACACGTATTGGAGCTGCTGATGACTTGATTTCGGGACA GTCAACCTTT	AJ413210
3	ACCTATATTAATCGCTTGCAGAAGCTTGCCAAAACCTTGGCAACGGTTGATGTCTTGCAAAGTTTAGCAGTTGTTGCTG AGACGAACCATTACAGTCGGCCTAGTTTCAATGATGACCATGTGATTAAGATTCAAGATGGGCGTCATGCTGTTGTTGA AAAAGTTATGGGAGTGCAAGAATACATTCCAAATAGTATTTCCTTTGATCAAAAAACAAGTATCCAATTGATTACGGGA CCAAATATGAGTGGTAAGTCTACTTATATGCGGCAGCTTGCCTTAACGGTTATCATGGCACAGATGGGCTCGTTTGTGG CTGCTGACCATGTCGATTTACCTTTATTTGATGCTATTTTCACACGTATTGGAGCTGCTGATGACTTGATTTCTGGACA ATCAACCTTT	AJ413208
4	ACCTATATTAATCGCTTGCAGAAGCTTGCCAAAACCTTGGCAACGGTTGATGTCTTGCAAAGTTTAGCAGTTGTTGCTG AGACGAACCATTACAGTCGGCCTAGTTTCAATGATGACCATGTGATTAAGATTCAAGATGGGCGTCATGCTGTTGTTGA AAAAGTTATGGGAGTGCAAGAATACATTCCAAATAGTATTTCCTTTGATCAAAAAACAAGTATCCAATTGATTACGGGA CCAAATATGAGTGGTAAGTCTACTTATATGCGGCAGCTTGCCTTAACGGTTATCATGGCACAGATGGGCTCGTTTGTGG CTGCTGACCATGTCGATTTGCCTTTGTTTGATGCTATTTTCACACGTATTGGAGCTGCTGATGATTTGATTTCTGGACA ATCAACCTTT	AJ413209

***S. canis recP* alleles and sequences and corresponding GenBank accession numbers.** Shaded alleles are from this study.

recP	Sequence (459 bp)	Acc#
1	CCTATGGCCTATGTTTTATGGAATCACTTCATGAACATTAATCCGAAAACAGGCCGTAATTGGTCAAATAGAGACCGTT TTATCCTATCAGCAGGTCACGGAAGTGCCATGCTTTATAGCTTACTACACTTGGCAGGATATGACTTGTCAGTTGATGA TTTGAAAAATTTCCGTCAATGGGGCTCTAAAACACCGGGCCACCCTGAGGTGAACCACACAGATGGTGTTGAAGCAACC ACAGGACCTCTTGGTCAAGGGATTGCGAATGCCGTTGGTATGGCGATGGCAGAAGCTCACCTAGCAGCTAAATTTAACA AACCAGACTTTGATATTGTTGACCATTACACTTTTGCTTTGAATGGTGACGGTGACCTTATGGAAGGGGTCAGTCAAGA AGCAGCAAGTTTGGCAGGGCATTTAAAACTTGGCAAATTGGTCTTGCTTTATGATTCAAATGAT	EU283342
2	CCTATGGCCTATGTTTTATGGAATCACTTCATGAACATTAATCCGAAAACAGGCCGTAATTGGTCAAATAGAGACCGTT TTATCCTATCAGCAGGTCACGGAAGTGCCATGCTTTATAGCTTACTACACTTGGCAGGATATGACTTGTCAGTTGATGA TTTGAAAAATTTCCGTCAATGGGGCTCTAAAACACCGGGCCACCCTGAAGTGAGCCACACAGATGGTGTTGAAGCAACT ACAGGACCTCTTGGTCAAGGGATTGCGAATGCCGTTGGTATGGCGATGGCAGAAGCTCACCTAGCAGCTAAATTTAACA AACCAGACTTTGACATTGTTGACCATTACACTTTTGCTTTGAATGGTGACGGTGACCTTATGGAAGGGGTCAGTCAAGA AGCAGCAAGTTTGGCAGGGCATTTAAAACTTGGCAAATTGGTCTTGCTTTATGATTCAAATGAT	FJ238477
3	CCTATGGCCTATGTTTTATGGAATCGCTTCATGAACATTAATCCGAAAACAGGCCGTAATTGGTCAAATAGAGACCGTT TTATCCTATCAGCAGGTCACGGAAGTGCCATGCTTTATAGCTTACTACACTTGGCAGGATATGACTTGTCAGTTGATGA TTTGAAAAATTTCCGTCAATGGGACTCTAAAACACCGGGCCACCCTGAAGTGAGCCACACAGATGGTGTTGAAGCAACT ACAGGACCTCTTGGTCAAGGGATTGCGAATGCCGTTGGTATGGCGATGGCAGAAGCTCACCTAGCAGCTAAATTTAACA AACCAGACTTTGATATTGTTGACCATTACACTTTTGCTTTGAATGGTGACGGTGACCTTATGGAAGGGGTCAGTCAAGA AGCAGCAAGTTTGGCAGGGCATTTAAAACTTGGCAAATTGGTCTTGCTTTATGATTCAAATGAT	FJ238476
4	CCTATGGCCTATGTTTTATGGAATCACTTCATGAACATTAATCCGAAAACAGGCCGTAATTGGTCAAATAGAGACCGTT TTATCCTATCAGCAGGTCACGGAAGTGCCATGCTTTATAGCTTACTACACTTGGCAGGATATGACTTGTCAGTTGATGA TTTGAAAAATTTCCGTCAATGGGGCTCTAAAACACCGGGCCACCCTGAGGTGAACCACACAGATGGTGTTGAAGCAACC ACAGGACCTCTTGGTCAAGGGATTGCGAATGCCGTTGGTATGGCGATGGCAGAAGCTCACCTAGCAGCTAAATTTAACA AACCAGACTTTGACATTGTTGACCATTACACTTTTGCTTTGAATGGTGACGGTGACCTTATGGAAGGGGTCAGTCAAGA AGCAGCAAGTTTGGCAGGGCATTTAAAACTTGGCAAATTGGTCTTGCTTTATGATTCAAATGAT	This study (C10)
5	CCTATGGCCTATGTTTTATGGAATCGCTTCATGAACATTAATCCGAAAACAGGCCGTAATTGGTCAAATAGAGACCGTT TTATCCTATCAGCAGGTCACGGAAGTGCCATGCTTTATAGCTTACTACACTTGGCAGGATATGACTTGTCAGTTGATGA TTTGAAAAATTTCCGTCAATGGGACTCTAAAACACCGGGCCACCCTGAAGTGAGCCACACAGATGGTGTTGAAGCAACC ACAGGACCTCTTGGTCAAGGGATTGCGAATGCCGTTGGTATGGCGATGGCAGAAGCTCACCTAGCAGCTAAATTTAACA AACCAGACTTTGATATTGTTGACCATTACACTTTTGCTTTGAATGGTGACGGTGACCTTATGGAAGGGGTCAGTCAAGA AGCAGCAAGTTTGGCAGGGCATTTAAAACTTGGCAAATTGGTCTTGCTTTATGATTCAAATGAT	This study (A7)
6	CCTATGGCCTATGTTTTATGGAATCGCTTCATGAACATTAATCCGAAAACAGGCCGTAATTGGTCAAATAGAGACCGTT TTATCCTATCAGCAGGTCACGGAAGTGCCATGCTTTATAGCTTACTACACTTGGCAGGATATGACTTGTCAGTTGATGA TTTGAAAAATTTCCGTCAATGGGGCTCTAAAACACCGGGCCACCCTGAAGTGAGCCACACAGATGGTGTTGAAGCAACC ACAGGACCTCTTGGTCAAGGGATTGCGAATGCCGTTGGTATGGCGATGGCAGAAGCTCACCTAGCAGCTAAATTTAACA AACCAGACTTTGATATTGTTGACCATTACACTTTTGCTTTGAATGGTGACGGTGACCTTATGGAAGGGGTCAGTCAAGA AGCAGCAAGTTTGGCAGGGCATTTAAAACTTGGCAAATTGGTCTTGCTTTATGATTCAAATGAT	R. Towers (G109)

***S. canis xpt* alleles and sequences and corresponding GenBank accession numbers.** Shaded alleles are from this study.

xpt	Sequence (450 bp)	Acc#
1	GGAGAGAATATTTTGAAAGTGGATAATTTTTTAACACACCAAGTCGATTACCGTTTAATGAAAGAAATCGGCAAGGT TTTTGCTCAGAAATATACTGATGCTGGAATTACAAAAGTGGTTACTATTGAGGCATCAGGTATTACGCCAGCAGTCT ATGCAGCAGAAGCTTTAGAAGTTCCGATGATTTTTGCTAAAAAGCATAAAAATATTACCATGACAGAAGGTATTTTG ACAGCAGAAGTTTATTCTTTCACTAAACAGGTGACAAGTACTGTTTCAATTGCGGGCAAGTTTCTTTCTGAAGAAGA TAAGGTTTTGATTATCGATGACTTTTTAGCCAATGGTCAAGCAGCAAAGGGCTTGATTGAAATTATTGGTCAAGCAG GTGCGCAGGTGGTTGGAGTTGGGATCGTTATTGAAAAATCTTTCCAAAATGGTCGTCAATTAATT	R. Towers (G103)
2	GGAGAGAATATTTTGAAAGTGGATAATTTTTTAACACACCAAGTCGATTACCGTTTAATGAAAGAAATCGGCAAGGT TTTTGCTCAGAAATATACTGATGCTGGAATTACAAAAGTGGTTACTATTGAGGCATCAGGTATTGCGCCAGCAGTCT ATGCAGCAGAAGCTTTAGAAGTTCCGATGATTTTTGCTAAAAAGCATAAAAATATTACCATGACAGAAGGTATTTTG ACAGCAGAAATTTATTCTTTCACTAAACAGGTGACAAGTACTGTTTCAATTGCGGGCAAGTTTCTTTCTGAAGAAGA TAAGGTTTTGATTATCGATGACTTTTTAGCCAATGGTCAAGCAGCAAAGGGCTTGATTGAAATTATTGGTCAAGCAG GTGCGCAGGTGGTTGGAGTTGGGATCGTTATTGAAAAATCTTTCCAAAATGGTCGTCAATTAATT	FJ238483
3	GGAGAGAATATTTTGAAAGTGGATAATTTTTTAACACACCAAGTCGATTACCGTTTAATGAAAGAAATCGGCAAGGT TTTTGCTCAGAAATATACTGATGCTGGAATTACAAAAGTGGTTACTATTGAGGCATCAGGTATTGCGCCAGCAGTTT ATGCAGCAGAAGCTTTAGAAGTTCCGATGATTTTTGCTAAAAAGCATAAAAATATTACCATGACAGAAGGTATTTTG ACAGCAGAAGTTTATTCTTTCACTAAACAGGTGACAAGTACTGTTTCAATTGCGGGCAAGTTTCTTTCTGAAGAAGA TAAGGTTTTGATTATCGATGACTTTTTAGCCAATGGTCAAGCAGCAAAGGGCTTGATTGAAATTATTGGTCAAGCAG GTGCGCAGGTGGTTGGAGTTGGGATCGTTATTGAAAAATCTTTCCAAAATGGTCGTCAATTAATT	FJ238486
4	GGAGAGAATATTTTGAAAGTGGATAATTTTTTAACACACCAAGTCGATTACCGTTTAATGAAAGAAATCGGCAAGGT TTTTGCTCAGAAATATACTGATGCTGGAATTACAAAAGTGGTTACTATTGAGGCATCAGGTATTGCGCCAGCAGTCT ATGCAGCAGAAGCTTTAGAAGTTCCGATGATTTTTGCTAAAAAGCATAAAAATATTACCATGACAGAAGGTATTTTG ACAGCAGAAGTTTATTCTTTCACTAAACAGGTGACAAGTACTGTTTCAATTGCGGGCAAGTTTCTTTCTGAAGAAGA TAAGGTTTTGATTATCGATGACTTTTTAGCCAATGGTCAAGCAGCAAAGGGCTTGATTGAAATGATTGGTCAAGCAG GTGCGCAGGTGGTTGGAGTTGGGATCGTTATTGAAAAATCTTTCCAAAATGGTCGTCAATTAATT	FJ238482
5	GGAGAGAATATTTTGAAAGTGGATAATTTTTTAACACACCAAGTCGATTACCGTTTAATGAAAGAAATCGGCAAGGT TTTTGCTCAGAAATATACTGATGCTGGAATTACAAAAGTGGTTACTATTGAGGCATCAGGTATTGCGCCAGCAGTCT ATGCAGCAGAAGCTTTAGAAGTTCCGATGATTTTTGCTAAAAAGCATAAAAATATTACCATGACAGAAGGTATTTTG ACAGCAGAAATTTATTCTTTCACTAAACAGGTGACAAGTACTGTTTCAATTGCGGGCAAGTTCCTTTCTGAAGAAGA TAAGGTTTTGATTATCGATGACTTTTTAGCCAATGGTCAAGCAGCAAAGGGCTTGATTGAAATTATTGGTCAAGCAG GTGCGCAGGTGGTTGGAGTTGGGATCGTTATTGAAAAATCTTTCCAAAATGGTCGTCAATTAATT	This study (C10)
6	GGAGAGAATATTTTGAAAGTGGATAATTTTTTAACACACCAAGTCGATTACCGTTTAATGAAAGAAATCGGCAAGGT TTTTGCTCAGAAATATACTGATGCTGGAATTACAAAAGTGGTTACTATTGAGGCATCAGGTATTGCGCCAGCAGTCT ATGCAGCAGAAGCTTTAGAAGTTCCGATGATTTTTGCTAAAAAGCATAAAAATATTACCATGACAGAAGGTATTTTG ACAGCAGAAGTTTATTCTTTCACTAAACAGGTGACAAGTACTGTTTCAATTGCGGGCAAGTTTCTTTCTGAAGAAGA TAAGGTTTTGATTATCGATGACTTTTTAGCCAATGGTCAAGCAGCAAAGGGCTTGATTGAAATTATTGGTCAAGCAG GTGCGCAGGTGGTTGGAGTTGGGATCGTTATTGAAAAATCTTTCCAAAATGGTCGTCAATTAATT	This study (F8)

***S. canis atoB* alleles and sequences and corresponding GenBank accession numbers.** Shaded alleles are from this study.

atoB	Sequence (434 bp)	Acc#
1	TTTGCCCTTCACAGCCAAGAAAAAGCAGCTAAAGCCCTTGAAAGCAATCGTTTCGCTGAGGAAATCGTTCCTGTCTCAGTG CCACAACGTCACCATGACCCTTTAATCGTATCTGTTGATGAATACCCAAAAACCAATACTAGTCTTGAAAAACTTCAATCC TTGCGCCCTGCTTTTCTTCCTGAAAAAGGAACTGTAACTGCCGGTAATGCCTCTGGCATTAACGACGGTGCTGCCTTACTG ATGTTGATGACCGAAGAAAAGGCCGTGGAGCTTGGCTTAACCCCTCTGGTCACAATTGAAAGTTATGCTAGTGCAGGAGTT GCCCCTGAACTAATGGGAACTGGACCTATTCCTGCTACCAAAAAAGCGCTAAAAAAAGCTAGCTTGACTATTTCTGATTTG GATTTAGTGGAATCCAACGAAGCATTTGC	FJ263616
2	TTTGCCCTTCACAGCCAAGAAAAAGCAGCTAAAGCCCTTGAAAGCAATCGTTTCGCTGAGGAAATCGTTCCTGTCTCAGTG CCACAACGTCACCATGACCCTTTAATCGTATCTGTTGATGAATACCCAAAAACCAATACTAGTCTTGAAAAACTTCAATCC TTGCGCCCTGCTTTTCTTCCTGAAAAAGGAACTGTAACTGCCGGTAATGCCTCTGGCATTAACGATGGTGCTGCCTTACTG ATGTTGATGACCGAAGAAAAGGCCGTGGAGCTTGGCTTAACTCCTCTGGTCACAATTGAAAGTTATGCTAGTGCAGGGGTT GCCCCTGAACTAATGGGAACTGGACCTATTCCTGCTACCAAAAAAGCGCTAAAAAAAGCTAGCTTGACTATTTCTGATTTG GATTTAGTGGAATCCAACGAAGCATTTGC	FJ268647
3	TTTGCCCTTCACAGCCAAGAAAAAGCAGCTAAAGCCCTTGAAAGCAATCGTTTCGCTGAGGAAATCGTTCCTGTCTCAGTG CCACAACGTCACCATGACCCTTTAATCGTATCTGTTGATGAATACCCAAAAACCAATACTAGTCTTGAAAAACTTCAATCC TTGCGCCCTGCTTTTCTTCCTGAAAAAGGAACTGTAACTGCCGGTAATGCCTCTGGCATTAACGATGGTGCTGCCTTACTG ATGTTGATGACCGAAGAAAAGGCCGTGGAGCTTGGCTTAACCCCTCTGGTCACAATTGAAAGTTATGCTAGTGCAGGGGTT GCCCCTGAACTAATGGGAACTGGACCTATTCCTGCTACCAAAAAAGCGCTAAAAAAAGCTAGCTTGACTATTTCTGATTTG GATTTAGTGGAATCCAACGAAGCATTTGC	FJ268654
4	TTTGCCCTTCACAGCCAAGAAAAAGCAGCTAAAGCCCTTGAAAGCAATCGTTTCGCTGAGGAAATCGTTCCTGTCTCAGTG CCACAACGTCACCATGACCCTTTAATCGTATCTGTTGATGAATACCCAAAAACCAATACTAGTCTTGAAAAACTTCAATCC TTGCGCCCTGCTTTTCTTCCTGAAAAAGGAACTGCTGGTAACGCTTCTGGCATTAACGACGGTGCTGCCTTACTG ATGTTGATGACCGAAGAAAAGGCCGTGGAGCTTGGCTTAACCCCTCTGGTCACAATTGAAAGTTATGCTAGTGCAGGGGTT GCCCCTGAACTAATGGGAACTGGACCTATTCCTGCTACCAAAAAAGCGCTAAAAAAAGCTAGCTTGACTATTTCTGATTTG GATTTAGTGGAATCCAACGAAGCATTTGC	FJ263615
5	TTTGCCCTTCACAGCCAAGAAAAAGCAGCTAAAGCCCTTGAAAGCAATCGTTTCGCTGAGGAAATCGTTCCTGTCTCAGTG CCACAACGTCACCATGACCCTTTAATCGTATCTGTTGATGAATACCCAAAAACCAATACTAGTCTTGAAAAACTTCAATCC TTGCGCCCTGCTTTTCTTCCTGAAAAAGGAACTGTAACTGCCGGTAATGCCTCTGGCATTAACGACGGTGCTGCCTTACTG ATGTTGATGACCGAAGAAAAGGCCGTGGAGCTTGGCTTAACCCCTCTGGTCACAATTGAAAGTTATGCTAGTGCAGGAGTT GCCCCTGAACTAATGGGAACTGGACCTATTCCTGCTACCAAAAAAGCGCTAAAAAAAGGCTAGCTTGACTATTTCTGATTTG GATTTAGTGGAATCCAACGAAGCATTTGC	This study (C10)

Appendix 8: MLST and *emm* typing of SDSE isolates previously collected from Northern Territory Aboriginal communities used in this study.

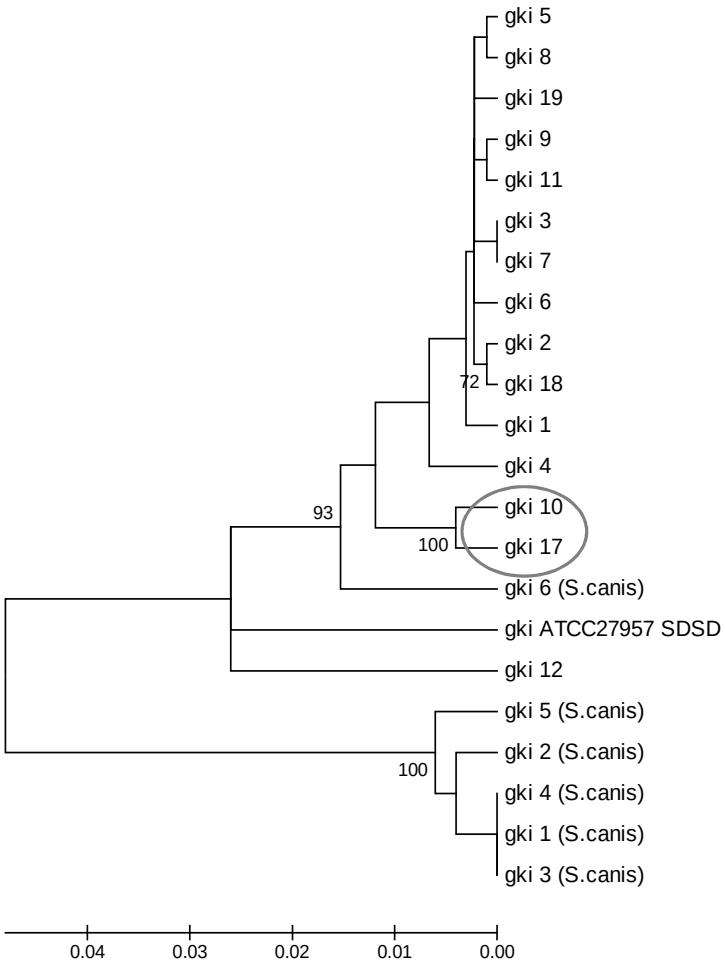
Isolate	Group	Host	*emm*ST	*gki*	*gtr*	*murI*	*mutS*	*recP*	*xpt*	*atoB*	ST
dogD14	L	dog	stL1929.0	10	6	6	6	12	13	8	1
G108	G	dog	stL1929.0	10	6	6	6	12	13	8	1
4599	C	human	stC839.2	5	3	4	1	6	2	1	3
7177	C	human	stG653.0	5	3	4	1	6	2	1	3
3881	G	human	stG6792.0	2	2	4	1	8	7	2	4
4567	G	human	stC36.0	2	2	4	1	8	7	2	4
3872	G	human	stG480.0	1	1	1	1	1	1	4	8
4703	G	human	stG480.3	1	1	1	1	1	1	4	8
G107	G	possum	stG643.0	4	4	5	2	17	6	2	12
4770	G	human	stG10.0	3	3	2	2	9	8	2	15
NS542*	G	human	stG652.0	3	3	2	2	9	8	2	15
3874	G	human	stC74a.0	4	4	1	2	17	6	2	17
4572	G	human	stG2078.0	4	4	1	2	17	6	2	17
4760	G	human	stC6979.0	4	4	1	2	17	6	2	17
5121	G	human	stG166b.0	3	2	1	5	7	4	3	25
4566	C	human	stC6746.0	2	2	4	1	13	12	7	27
4775	G	human	stG6.0	3	2	4	2	7	1	3	29
G121	G	human	stC74a	3	2	4	2	7	1	3	29
3396*	G	human	stG480	1	1	1	1	1	21	4	39
GGS10b	G	human	stG6	2	2	4	2	3	7	1	44
GGS24	G	human	stG6	2	2	4	2	3	7	1	44
NS752*	G	human	stG6.0	2	2	4	2	3	7	1	44
4785	G	human	stC1400.4	3	3	4	2	9	22	2	66
5155	G	human	stC1400.3	3	3	4	2	9	22	2	66
GGS120	G	human	stG4831	6	2	4	1	8	3	1	74
3864	G	human	stG4831.0	6	2	9	1	8	3	1	75
NS1121	G	human	stG4831	6	2	9	1	8	3	1	75
G111	L	dog	-	17	14	16	7	12	13	9	134
5041	C	human	stC2sk.1	2	3	4	2	16	3	18	135
7181	C	human	stC2sk.1	2	3	4	2	16	3	18	135
4708	G	human	stC-NSRT2.0	19	2	4	2	9	1	2	136
4186	G	human	stC6979.0	4	2	1	2	17	6	2	137
4096	G	human	stC1400.0	3	3	4	2	9	22	2	44
4054	G	human	stG652.0	3	15	1	2	7	32	3	138
4034	C	human	stC6979.0	18	2	4	1	5	7	2	139
4026	G	human	stC6979.0	2	2	4	2	3	10	1	140
3997	G	human	stC5344.1	3	3	4	1	26	15	2	1001
3936	C	human	stG643.0	2	2	5	2	2	31	2	1002
3712	C	human	stGrobn.0	3	2	2	8	1	8	19	141

*hospital isolates

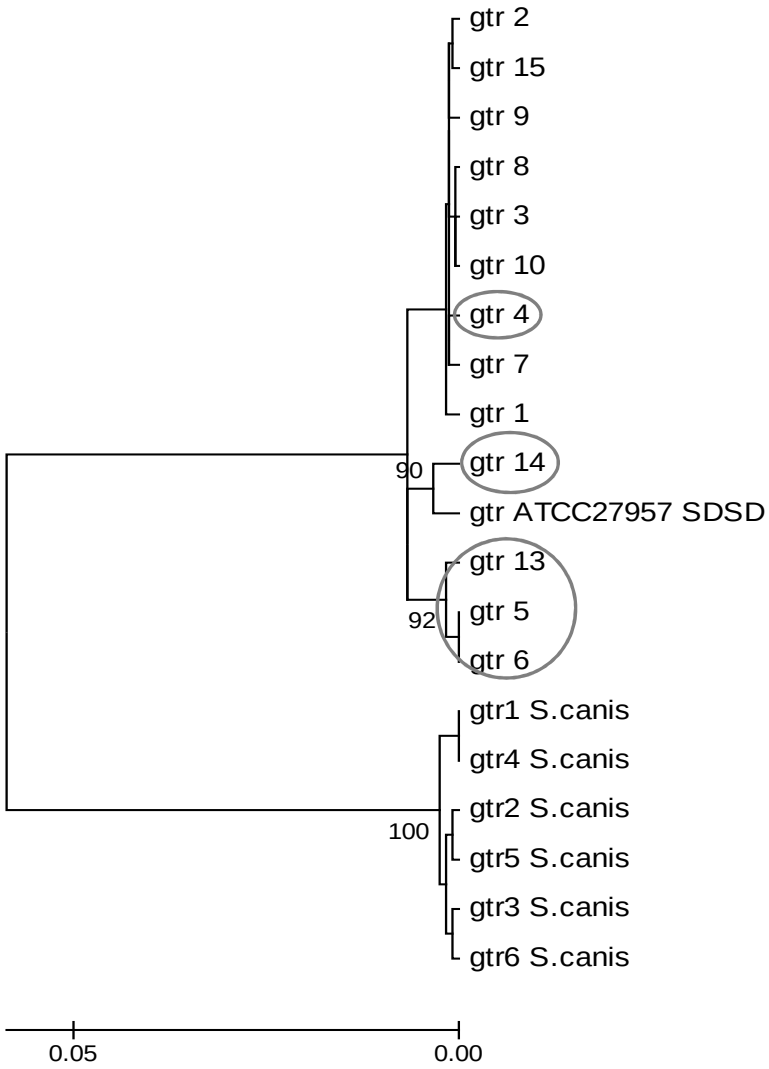
Appendix 9. Probe regions used to design primers to detect virulence genes. Shaded region illustrates primers.

Virulence Gene	Probe
sfbI	CCTCTAGCGGGTGAGTCTGGTGAGACGGAGTATATTACCGAAGTCTATGGAAATCAACAGAACCC
cpa	ACAGTTACAGATGGAAAAGCTACCTTCACATTAAAGGATGGAGAATCATTGATTGTTGAGGGGCTACCTTC
speG	AAAGGGGATAAGGTCGATGTTTTTGGTCTCCCTTATAATTTTTCCCCACCTTATGTAGATAATATTTATGGTGGTATTGTAAAACATTCGAATCAAGGAAATAAATCATTACAGTTTGTAGGAATTTTAAATCAAGATGGGAAAGAAACTTATTTGCCCTCTGAGGCTGTTCGCATAAAAAAGAAACAGTTTACTTTACAGGAATTTGATTTTAAAATAAGAAAATTTCTAATGGAAAAATACAATATCTATGATTCGGAATCGCGTTATACATCGGGGAGCCTTTTCCTTGCTACT
SfbX	AAAACTTGCTCCTCATGATAGCCACACAACCCCTCAAACTTCAGATGATACAGCAGCCCCTCACTTGCCT
sdn	AAAAAAGAGTAACCGTATCTGATGTAGTTTATAACCCATTAGATGGCTATAAACGTTCAACAGGCGCTTA
sdaB	ATGTTGTTCTAAATGATGGCGCAAGCAAGTACCTAAACGAAGCATTAGCTTGGACATTCAATGACAGTCC
prtF2	GGATTTCAGATGGACAAGTGAAAGATTTCTACCTGATGCCAGGAAAATATACATTTGTCGAAACCGCAGC

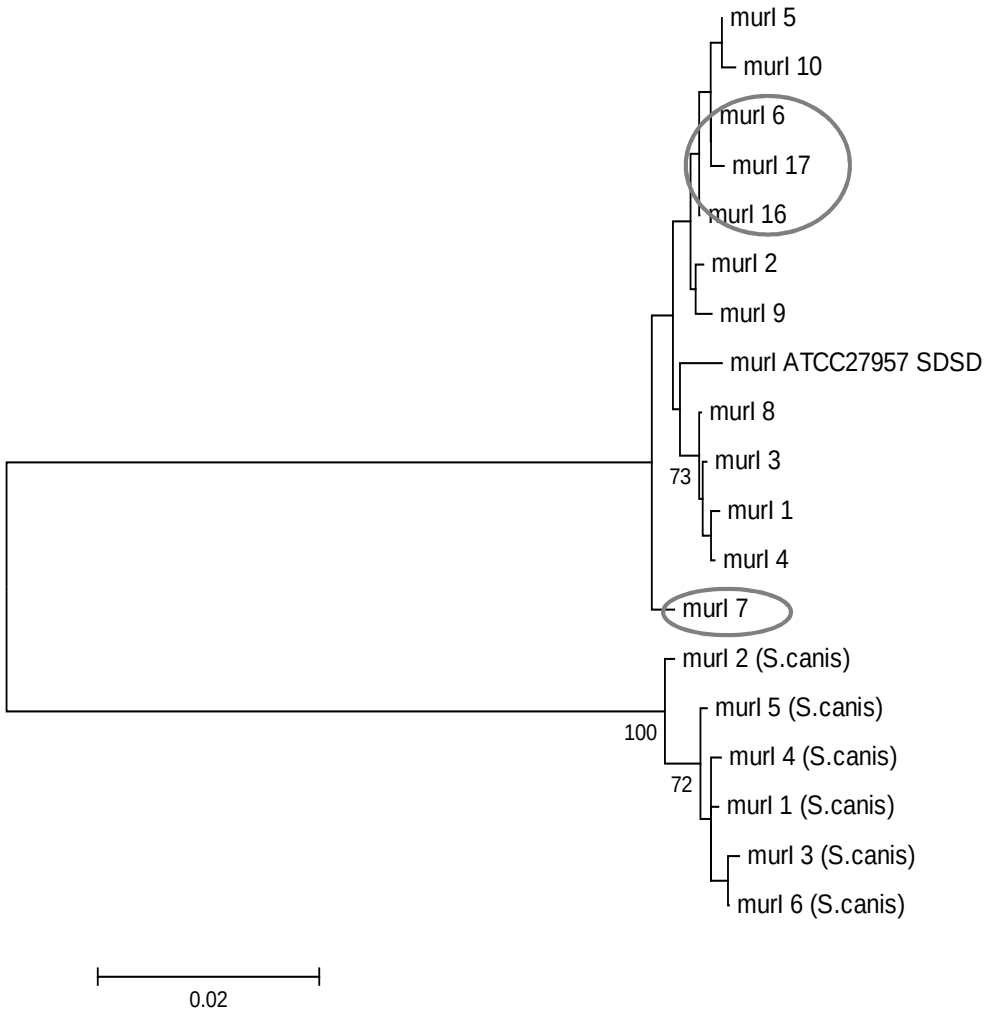
Appendix 10: SDSE MLST Housekeeping loci



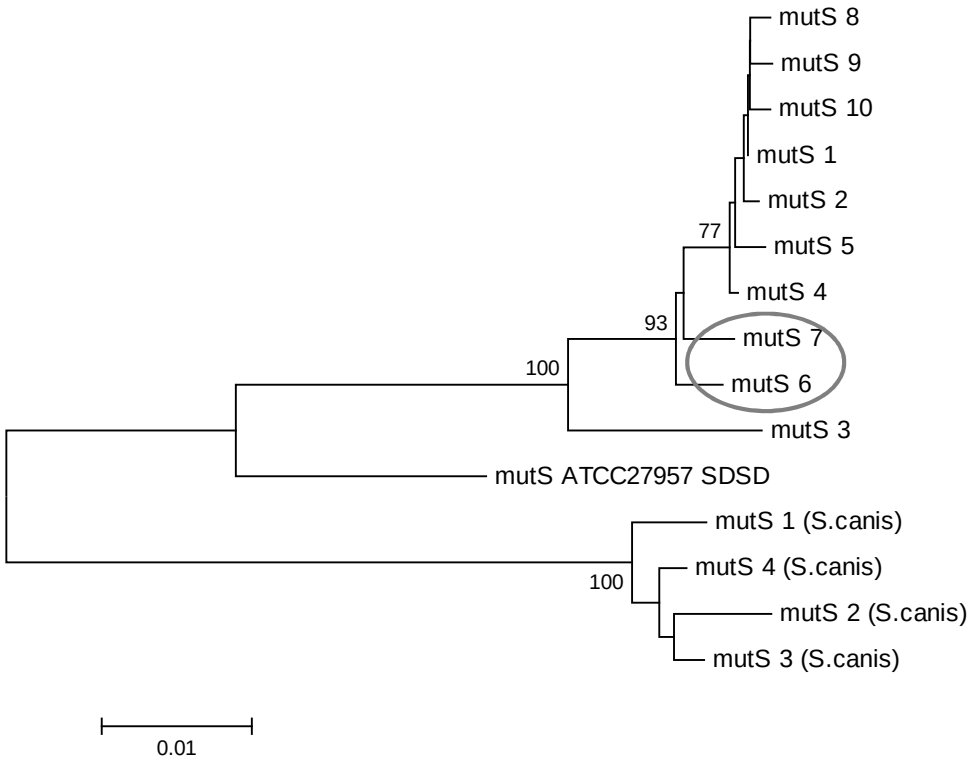
Minimum Evolutionary tree of *gki* alleles of SDSE, *S. canis* and an SDSD. Circled region illustrates alleles found in dogs. Bootstrap values less than 70% are not shown.



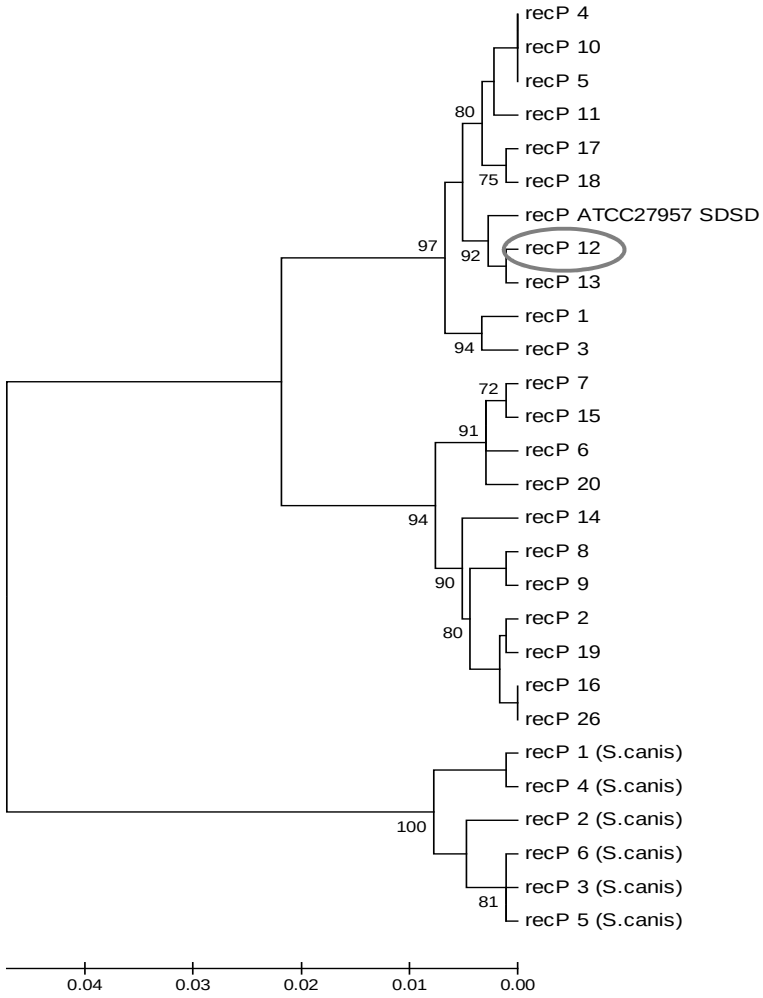
Minimum Evolutionary tree of *gtr* alleles of SDSE, *S. canis* and an SDSD. Circled areas illustrate alleles found in dogs. Bootstrap values less than 70% are not shown.



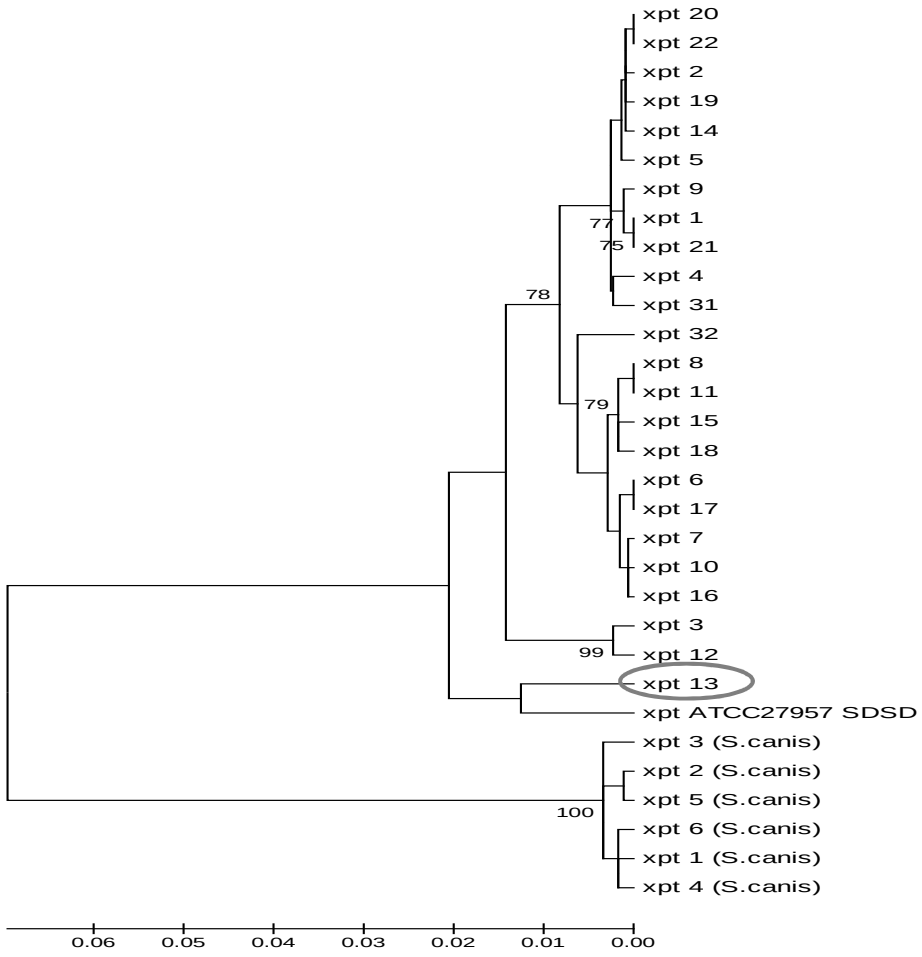
Minimum Evolutionary tree of *murI* alleles of SDSE, *S. canis* and an SDSD. Circled regions illustrate alleles found in dogs. Bootstrap values less than 70% are not shown.



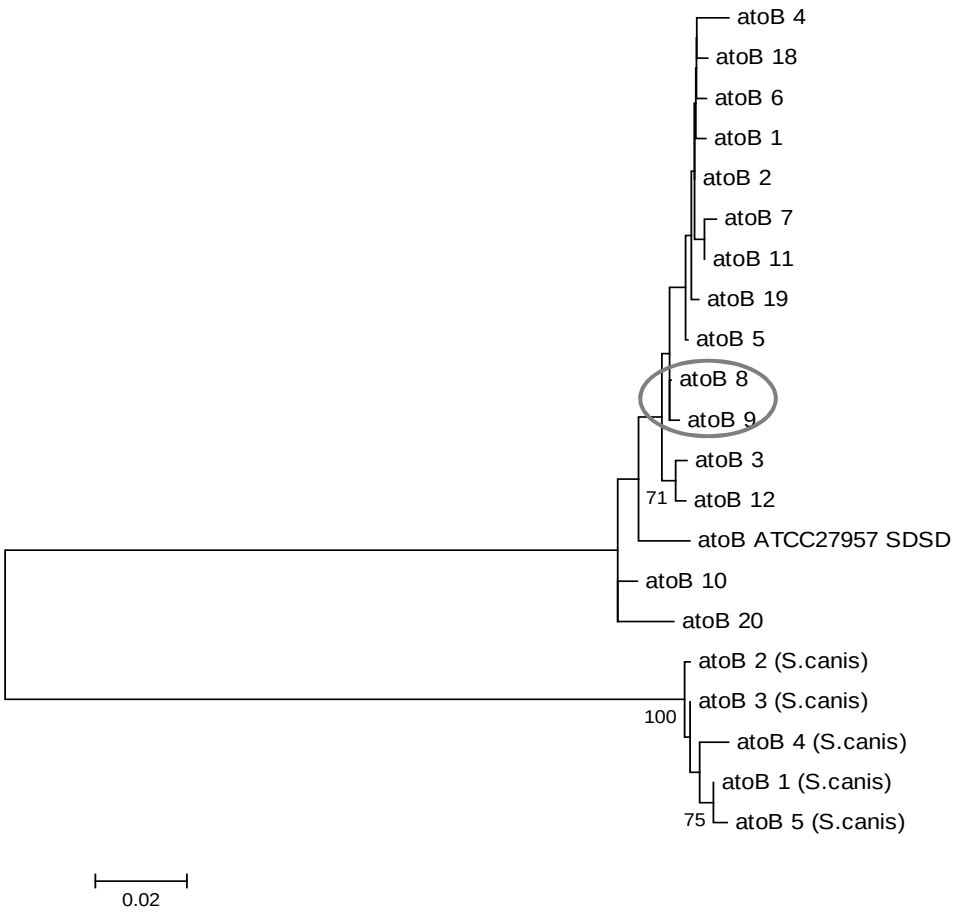
Minimum Evolutionary tree of *mutS* alleles of SDSE, *S. canis* and an SDSD. Circled region illustrates alleles found in dogs. Bootstrap values less than 70% are not shown.



Minimum Evolutionary tree of *recP* alleles of SDSE, *S. canis* and an SDSD. Circled area illustrates allele found in dogs. Bootstrap values less than 70% are not shown.



Minimum Evolutionary tree of *xpt* alleles of SDSE, *S. canis* and an SDSD. Circled region illustrates allele found in dogs. Bootstrap values less than 70% are not shown.



Minimum Evolutionary tree of *atoB* alleles of SDSE, *S. canis* and an SDSD. Circled region illustrates alleles found in dogs. Bootstrap values less than 70% are not shown.

Appendix 11: Interview Questions

1. What sorts of things do you think could be done with the dogs in Yarrabah?

2. What are some ways that Animal Control (AMW) can do this stuff good ways?

Appendix 12. Yarrabah Dog Care Day Posters. **These posters are property of the Yarrabah Aboriginal Shire Council and are not suitable (or approved) for use in other Aboriginal communities.**