Potential for transmission of zoonotic helminth infections among dingoes and dogs in the Wet Tropics of North Queensland, Australia.

Thesis submitted by

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BVSc (Hons) Uni Qld, BSc JCU

July 2019

for the degree of

Doctor of Philosophy

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ACKNOWLEDGMENTS

There are many people who I would like to thank for their contributions towards my PhD research and the compilation of this thesis. First and foremost I extend my sincere thanks to my supervisors: Dr Lee Skerratt, Dr Brad Congdon, Dr James Butler and Professor Chris Johnson. I am extremely fortunate to have had access to their wealth of experience during the course of my studies. All have been very supportive and have given me the freedom to pursue various projects without objection while providing insightful discussions about the research. Most importantly, I thank my supervisors for their much needed encouragement and motivation during the write-up of this thesis.

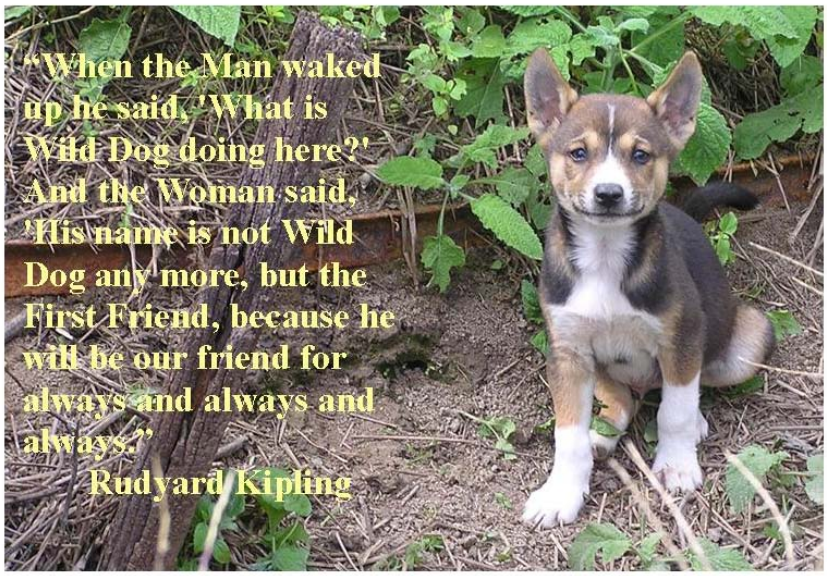
I am extremely grateful to Professor Andy Thompson from Murdoch University in Western Australia for taking me under his wing and introducing me to his amazing team and the wonderful world of molecular biology. Huge thanks to Aileen Elliot for allowing me to impose upon her lab space for an extended period, she endured my singing for months and was invaluable in assisting me in parasite identification. I would also like to thank Louise Pallant and Angela Reeves for their instruction on molecular techniques and wonderful friendship.

Funding for my PhD research and living expenses came from a number of sources. I am very grateful for a stipend from an Australian Postgraduate Award and a top up scholarship from the College of Public health, medical and veterinary sciences. The Australian Society of Parasitology (ASP) also provided an exchange program allowing me to attend Murdoch University to study molecular biology techniques. In addition, the ASP provided student scholarships allowing me to attend conferences in Tasmania and Western Australia. I would also like to thank the generosity of Assoc. Prof. Wayne Melrose and the late Prof. Rick Speare for sponsoring me to my first conference in Vlieland, Netherlands and further travel to Switzerland and Italy to meet and work with experts in the fields of Public Health and Parasitology. A thank you also to Prof. Giuseppe Cringoli, Assoc. Prof. Laura Rinaldi and staff from the University of Naples for their instruction on 'FLOTAC' copromicroscopic technique and wonderful coffee.

Field work for this project would not have been possible without the assistance of Dr Damian Morrant, his good humour and determination to track a dingo wherever it roamed, including up the back face of Walsh's pyramid, were truly admirable. Thank you also to Alfred Gray (Touché), Stephen Canendo (Uncle Bully), Aunty Shanna Mossman and Herman Sexton from Yarrabah Aboriginal Council for help with trapping and tracking dingos and community dogs. Special thanks also to Valerina and Andrew for kindly volunteering their dogs for tracking studies. Also, to everyone in the Yarrabah and Mossman Gorge Aboriginal communities for their ongoing support and assistance. I am also especially grateful to the many farmers and land owners who allowed me to set up remote cameras, trap dingoes and track these animals on their properties. Thank you for all the coffee and cake and wonderful stories.

Thank you also to Dan Metcalf and Adam McKeown from CSIRO Atherton, along with Matt Birch and Peter Box from Cairns Regional Council for providing dingo carcasses. I would also like to extend my gratitude to Assoc. Prof. Will Edwards for providing office and laboratory space with a fume hood for my sometimes stinky dingo samples. Thank you to Leanne Shillitoe, Kylie Brown, Jenni Paul, and Jonathan Tindall for administrative, technical and laboratory support.

Finally, I would like to say a special thank you to my family; my Mum, for all of her love and her support during my doctoral studies at JCU. Mum, you passed away before I got to finish my thesis but the lessons in life you and dad taught me were enough for me to know that I could continue and, through dedication and hard work, could achieve anything I put my mind to. Also, a very special thanks to my sisters Penny and Jacquie and my nephew Jackson for their love and support.



"You never fail, until you stop trying."

Albert Einstein.

DECLARATION ON ETHICS

Human ethics

The research presented and reported in this thesis was conducted in accordance with the National Health and Medical Research Council (NHMRC) *National Statement on Ethical Conduct in Human Research, 2007*. The proposed research study received human research ethics approval from the James Cook University (JCU) Human Research Ethics Committee (approval number H4264).

Signature: Date: 1st July, 2019.

Felicity Angela Smout

Animal ethics

The research presented and reported in this thesis was conducted in accordance with the National Health and Medical Research Council (NHMRC) *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 7th Edition, 2004* and the *Animal Care and Protection Act, 2001* (Qld). The proposed research study received animal ethics approval from the JCU Animal Ethics Committee (approval numbers A1495, A1546, A1821).

Signature: Date: 1st July, 2019.

Felicity Angela Smout

STATEMENT ON THE CONTRIBUTION OF OTHERS

My research was funded by the Australian Research Council (Linkage grant), Skyrail Rainforest Foundation, Terrain Natural Resource Management, Australian Rainforest Foundation and James Cook University (JCU). Travel and conference attendance were funded in part through the Australian Society for Parasitology, Assoc. Prof. Wayne Melrose and the late Prof. Rick Speare.

Some of my research required equipment and facilities external to my organisational unit at James Cook University, as well as collaboration and consultation with colleagues at James Cook University and other Australian institutions. The molecular biology laboratory at Murdoch University, Western Australia, provided facilities for my molecular work. CSIRO Atherton, Cassowary Coast Regional Council, Yarrabah Aboriginal Shire Council, Jumbun Aboriginal Community and Cairns Regional Council animal control officers provided specimens for my research.

My supervisors, Drs. Lee Skerratt, Brad Congdon, James Butler and Professor Chris Johnson gave advice throughout the project on research methodology, interpretation of results and manuscript and thesis preparation. I have summarised below the specific scientific and intellectual contributions of other individuals to each chapter of the thesis.

CHAPTER 2

Literature review

Published - paper 1: Smout, F., Schrieber L, Speare R, Skerratt LF. (2017). More bark than bite: Comparative studies are needed to determine the importance of canine zoonoses in Aboriginal communities. A critical review of published research. Zoonoses Public Health 64:495-504.

This published, peer-reviewed paper represents a review led by myself. I performed the majority of the literature review and paper write-up with substantial assistance from Layla Schrieber with critical appraisal and data organisation. All co-authors provided editorial input.

CHAPTER 3

Survey of helminth diseases in dingoes in the Wet Tropics of north-east Queensland.

Published - paper 2: Smout, F. A., Thompson, R. & Skerratt, L. F. (2013). First report of *Ancylostoma ceylanicum* in wild canids. International Journal for Parasitology: Parasites and Wildlife 2: 173-177.

This published, peer-reviewed paper represents my original study design, conduct, sample collection and processing, data organisation, data analysis, results interpretation and manuscript write-up. Lee Skerratt and Andrew Thompson provided assistance and guidance with data organisation and analysis along with editorial input.

CHAPTER 4

Survey of heartworm disease in dingoes in the Wet Tropics of north-east Queensland.

Published - paper 3: Smout, Felicity A., Skerratt, L.F., Butler, J., Johnson, C. & Congdon, B. (2016). Dingoes (*Canis dingo* Meyer, 1793) continue to be an important reservoir host of *Dirofilaria immitis* in low density housing areas in Australia. Veterinary Parasitology 215: 6-10.

This published, peer-reviewed paper represents my original study design, conduct, sample collection and processing, data organisation, data analysis, results interpretation and manuscript write-up. Lee Skerratt and Brad Congdon provided assistance and guidance with data organisation and analysis and James Butler provided access to CSIRO dingo carcasses. Chris Johnson assisted in acquiring funding and all co-authors provided editorial input.

CHAPTER 5

Survey of helminth diseases in dogs and soil in Wet tropics Indigenous communities.

Published - paper 4: Smout, Felicity A., Skerratt, L.F., Butler, J., Johnson, C., Congdon, B.. & Thompson, R.C. (2017). The hookworm *Ancylostoma ceylanicum*: An emerging public health risk in Australian tropical rainforests and Indigenous communities. One Health 3: 66-69.

This published, peer-reviewed paper represents my original study design, conduct, sample collection and processing, data organisation, data analysis, results interpretation and manuscript write-up. Lee Skerratt and Brad Congdon provided assistance and guidance with data organisation and analysis. Chris Johnson assisted in acquiring funding and all co-authors provided editorial input.

CHAPTER 6

Zoonotic helminth diseases in dogs and dingoes utilising shared resources in an Australian Aboriginal community.

Published- Paper 5: Smout, F.A., Skerratt, L.F., Johnson, C., Butler, J., Congdon, B. (2018). Zoonotic helminth diseases in dogs and dingoes utilising shared resources in an Australian Aboriginal community. Tropical Medicine and Infectious Diseases 3, 110.

This published, peer-reviewed paper represents my original study design, conduct, sample collection and processing, data organisation, data analysis, results interpretation and manuscript write-up. Lee Skerratt and Brad Congdon provided assistance and guidance with data organisation and analysis and James Butler provided access to CSIRO dingo carcasses. Chris Johnson assisted in acquiring funding and all co-authors provided editorial input.

THESIS ABSTRACT

Wild dogs (dingoes, free-ranging domestic dogs and hybrids) have the potential to pose a threat to biodiversity conservation and the health of domestic animals, livestock and humans in the Wet Tropics bioregion of north Queensland. The increasing human population in the Wet Tropics will inevitably result in more frequent interactions between people and wild dogs. One potential interaction is the transmission or 'spill-over' of diseases, including zoonotic parasites, from dingoes to the area's native fauna, livestock, domestic animals, human residents and visitors. Investigating all potential hosts and their interactions is hence necessary to understand and mitigate the possibility of 'spill-over' or 'spill-back' of zoonotic infection. Indigenous communities are at particular risk due to limited management of domestic dog health and the ability of community dogs to roam free, possibly contacting dingoes and their habitat and resources. Dogs remain an integral part of Indigenous community culture and the health and treatment of dogs is intrinsically linked to community health and well-being.

Research was undertaken in and around rural and remote Indigenous communities to understand the disease status of dingoes and sympatric community dogs. Using various parasitological techniques, combined with radio telemetry to track the movements of dingoes and free-roaming domestic dogs, risks of transmission of infection from dingoes to dogs and then people in Indigenous communities, or vice versa, were examined.

Faecal samples collected from tracked dingoes revealed 100% infection with the zoonotic hookworm *Ancylostoma caninum*, and one animal was infected with *Ancylostoma ceylanicum*; this is the first report of this parasite in dingoes. A similar result was found for necropsied dingoes; however, a much more elevated infection rate was seen in dingo scats. Those scats positively sequenced for hookworm, contained *A. ceylanicum, A. caninum* and *A. braziliense*, with *A. ceylanicum* the dominant species in Mount Windsor National Park, with a prevalence of 100%, but decreasing to 68% and 30.8% in scats collected from northern and southern rural suburbs of Cairns, respectively. I also observed, for the first time, the presence of *A. ceylanicum* infection in domestic dogs (21.7%) and soil (55.6%) in an Indigenous community and found it was present in soil samples from two out of the three popular tourist locations sampled.

Due to the ability of *A. ceylanicum* to cause a patent infection in humans, the zoonotic risk arising from this wild dog reservoir to communities in the Wet Tropics is of concern.

Domestic dogs also had a high prevalence of *A. caninum* with 100%, 96.4%, and 88.0% infection of tracked dogs, necropsied dogs and dog scats, respectively, but *A. ceylanicum* was not found. Similar levels of infection of the zoonotic roundworm *Toxocara canis* were found in dingoes and domestic dogs. However, whipworm *Trichuris vulpis* infection was far more prevalent in domestic dog necropsies (78.6%) than in dingo necropsies (3.7%). *Dirofilaria immitis* infection was found in high prevalence with 71% infection seen in dingoes in low density housing areas. This result highlights the importance of dingoes as reservoir hosts of heartworm disease and that the subsequent risk of infection to companion animals and humans depends on local factors such as housing density, possibly linked to chemotherapeutic heartworm control in domestic dogs and climate.

Eleven dingoes and seven free-roaming domestic dogs were fitted with GPS collars and tracked over an extended period. Dingo home ranges almost completely overlapped those of the domestic dogs and dingoes spent a substantial amount of time in areas used by dogs. I found that dingoes and dogs appeared to avoid direct contact however this spatial overlap in resource use presents an opportunity for the indirect spill-over and spill-back of zoonotic parasites, facilitated by the parasite's ability to survive for longer periods in the Wet Tropic's warm and humid conditions. Tracking and camera trap deployment in the Yarrabah community showed that the community rubbish tip and animal carcasses provided concentrated anthropogenic food sources for dogs and dingoes, and transmission risk is elevated in these locations.

Two dog health days were conducted in the Yarrabah Aboriginal community to provide free veterinary consultation for pets, provide community members with information about new dog laws and registration and to provide information about parasites infecting dogs and the possible public health risks associated with them. This resulted in the provision of treatment and veterinary consultation to 134 dogs and one cat along with the development of guidelines for domestic animal management which I prepared and presented to Yarrabah Council to assist in the introduction of registration of pets.

By using a “One Health” approach that integrated the disciplines of veterinary parasitology, epidemiology and ecological analysis of canids’ home range and resource use, I was able to establish the prevalence of parasitic pathogens and the current status of infection in dingoes and determine the pathways and mechanisms which lead to the potential risk of transmission of infections among dingoes, wildlife, domestic animals and humans. I determined that hot spots of infection transmission are likely to be sources of anthropogenic-derived food such as the rubbish tip, animal carcasses and public congregational areas such as school sporting grounds. Similar risks are likely to occur in other Indigenous communities in the Wet Tropics and warrant investigation and intervention.

Through collaboration with local, experienced environmental health workers I was able to achieve the overall aim of this project which was to determine, and provide workable and sustainable animal and health management practices to reduce the risk of spill-over of parasitic infection from dingoes to domestic dogs (or vice-versa). Mitigation measures should include exclusion fencing of the rubbish tip, effective disposal of animal carcasses, public education to increase community awareness about local zoonotic diseases and their prevention, along with regular chemoprophylactic therapy of community dogs and improved management of dogs and their diseases in Indigenous communities.

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CONFERENCES AND PRESENTATIONS

Smout, F. A. 2010 – Apunapima Environmental health worker workshop, Cairns, QLD – Oral presentation.

Smout, F. A. 2010 – Do populations of wild dogs threaten human health in Indigenous communities of the Wet Tropics? European Wildlife Disease Association. Healthy wildlife, healthy people, Vlieland, Netherlands – Oral and poster presentation. Appendix 1.

Smout, F. A., Constable, S.E. and Pye, R.J. 2011 – Dog management in Australia. One Health Congress, Melbourne, Victoria – Joint oral presentation with Animal Management in Rural and Remote Indigenous Communities and Vets Beyond Borders.

Smout, F. A. 2012 – Do populations of wild dogs threaten human health in Indigenous communities of the Wet Tropics? Australian Society of Parasitology conference, Launceston, Tasmania. – Oral and poster presentation. Appendix 2.

Smout, F. A., Skerratt, L.F. and Thompson, R.C.A. 2013 – First report of *Ancylostoma ceylanicum* in wild canids. Joint World Association for the Advancement of Veterinary Parasitology/Australian Society of Parasitology conference, Perth, WA – Oral and poster presentation, Poster Prize Winner. Appendix 3.

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Chapter 1

General Introduction

GENERAL INTRODUCTION

1.1 BACKGROUND

Zoonoses constitute a major public health problem worldwide and wildlife are now recognised as an important source of emerging human pathogens (Polley, 2005). Zoonoses can be defined as the diseases and infections that are naturally transmitted between vertebrate animals and humans. Wild animals can act as reservoirs of zoonotic agents and enable 'spill-over' of zoonotic pathogens (Kruse et al., 2004). More recently, it has become clear that free-ranging animals are responsible for most emerging infectious diseases in humans (Daszak et al., 2000). This is particularly the case in developing countries where disease transmission is enhanced by vectorial abundance, poor hygiene and close proximity to wildlife reservoirs and their habitats (Thompson and Conlan, 2011). Similar circumstances to those in developing countries are seen in many Indigenous communities in Australia today and free-roaming canids (domestic and wild), and the potential public health issues associated with them, have long been a concern. People who live in rural and remote communities are often surrounded by a diversity of wildlife and possibly, pathogen reservoirs. In addition, they can face considerable health disadvantages with reduced access to primary health care providers and health services. The Australian Government's ongoing commitment to "closing the gap" in chronic disease in Indigenous communities (COA, 2016) requires that dog health, a potentially hazardous environmental issue, needs to be addressed. Adequate and ongoing research and surveillance within communities is necessary in order to establish accurate estimates of disease risk and to define priorities at a local level (Traub et al., 2005). Investigations into the types of infections present, how these infections are transmitted between hosts and the risk factors that need to be mitigated to avoid such transmission are vital.

1.1.1 DINGOES AND DOGS AND THEIR RELATIONSHIP WITH INDIGENOUS AUSTRALIANS

Distributed widely throughout mainland Australia, the dingo (*Canis dingo* Meyer, 1793*)* is the country's largest terrestrial predator (mean weight 15 Kg) (Corbett, 1985; Johnson

et al., 2007) following its ability to out compete and possibly kill the generally larger and now extinct Tasmanian thylacine (*Thylacinus cynocephalus*) which previously held this title (Letnic et al., 2012). There is much ongoing debate about the origin of the dingo and the time frame for its arrival to the continent. It is believed to have been brought to Australia by Asian seafarers around 5000 years ago and remained effectively isolated from other dog populations since then (Savolainen et al., 2004). While fossil evidence indicates that the dingo has been in the country for at least 3,500 years (Corbett, 2001; Milham and Thompson, 1976), research into genetic diversity suggests a much older introduction at anywhere between 18,300 and 4,600 years ago (Oskarsson et al., 2011).

Aboriginal people adopted the dingo into their lives; however domestication of the dingo was only partial, with dingoes often foraging for their own food (Meggitt, 1965). Dingoes were used by Aborigines for companions, hunting aides, protectors and bed warmers (Smith and Litchfield, 2009). Interest may also have been generated by the fact that dingoes are placental mammals and effectively gave birth to young in a manner similar to humans; other than native rats and bats, all other Australian land mammals at that time were marsupials or monotremes (Wilks and Williamson, 1998). Dingoes were incorporated into the 'Dreamtime' creation stories that describe the origin of Aboriginal people, the land and the animals (Rose, 2000). 'Dog dreaming people' are elders responsible for dog-related traditional laws and customs including ceremonial rituals. In some communities, dogs are regarded as reincarnations of ancestors and connections to the spirit world (Donelan, 2005).

Domestic dogs (*Canis lupus familiaris*) have made a far more recent appearance in Australia. Arriving with European colonists in the 18th century (Savolainen et al., 2004), dogs are now widespread across the Australian continent both as owned and free-ranging (feral) animals. Domesticated dogs were easier to train than dingoes and assimilated better into people's homes (Kolig, 1978). Aboriginal people adopted the European dog to replace the dingo and today they remain an integral part of Indigenous community culture including origin myths (Parker, 2006). The concept of time proceeding as a straight line does not apply to all histories (Muecke, 1992). To keep pace with intense social change, and using incorporated method of time rather than straight line, many Aboriginal myths have replaced "dingo" with the now more familiar "dog" much as "dingo" may have replaced "thylacine" in the past (Parker, 2006). The

close association between Aboriginal people and dogs has resulted in numerous problems that did not occur previously with dingoes. Social constraints and lack of food often prevented young dingoes from breeding in the wild and when they did breed it was only once per year compared with domestic dogs which will breed twice per year along with having a greater number of pups per litter (Catling et al., 1992). These factors, combined with minimal husbandry, have led to dogs breeding excessively in communities and a resultant overpopulation of unwanted puppies and older dogs (Raw, 2001).

Whilst wild dogs can be a serious threat to livestock and native species there is also some sympathy in the community of the Wet Tropics for black and tan 'rainforest dingoes', which are generally regarded as a native species (Newsome and Corbett, 1985). This perspective is generating a growing resistance to broad-spectrum indiscriminate control methods such as poisoning and trapping, which may kill unique 'rainforest dingoes' along with dog/dingo hybrids and feral domestic dogs (Morrant, 2015). It has also been shown that top predators, such as dingoes, play a crucial role in maintaining prey biodiversity by controlling populations of introduced mid-sized predators such as cats, *Felis catus* and foxes, *Vulpes vulpes* (Johnson and VanDerWal, 2009). Many remaining Australian mammals would most likely benefit from the presence and positive management of dingoes (Johnson et al., 2007). Particularly, since recent research indicates that foxes are moving into the Wet Tropics region (Butler et al., 2008) and could provide a further threat to the biodiversity of the area.

1.2 STUDY AREA

The Wet Tropics of north-east Queensland extends from Townsville in the south to Cooktown in the north and encompasses an area of 1,998,790 ha (Fig. 1.1). This region contains remnant rainforest which holds globally-significant biodiversity and cultural values, recognised through its designation as the *Wet Tropics World Heritage Area* (WTWHA) (WTMA, 2004, 2013). Several Aboriginal communities occur within the Wet Tropics bioregion including Yarrabah Aboriginal community with a population of ~2494 people (Australian Bureau of Statistics, 2016.). Major towns such as Cairns also

occur within the region, with recreation and tourism bringing over 200 million visitors to the region each year (WTMA, 2013).

Figure 1.1 Map of Wet Tropics World Heritage Area of North Queensland

1.3 THE RESEARCH PROBLEM

Populations of pure dingoes are under considerable threat from hybridisation with domestic dogs. The degree of hybridisation appears to increase with proximity to human habitation (Stephens, 2011). The relatively higher proportions of hybrids found near populated areas are likely the result of increased contact between dingoes and domestic dogs in these areas (Elledge et al., 2006; Newsome and Corbett, 1985; Woodall et al., 1996).

Human activities such as urban expansion into previously underdeveloped areas of the Wet Tropics has compounded the wild dog problem, leading to subsequent increases in human-wild dog interaction and possibly the rate at which domestic dogs enter the feral population (Butler et al., 2014). In addition, lethal wild dog control programs in populated areas can have deleterious effects on dingo pack structure leading to an increased rate at which feral dogs are able to join packs (Fleming et al., 2001; Wallach et al., 2009).

Activities such as feeding of wild animals and improper disposal of rubbish could lead to an increase of dingoes around recreational and populated areas and the possibility of both dog attacks and the spread of disease to humans, their domestic dogs and livestock. The other major factor in the risk of disease spill-over from dingoes is human attitudes, knowledge and ultimately the management of the disease risk by people.

1.3.1 INVESTIGATING PARASITES AND ZOONOSES

Dingoes carry diseases of zoonotic importance, along with many that are transmissible to domestic and wild animals (Brown and Copeman, 2003). Determining the characteristics of this disease burden enables insight into the risk of disease transmission between these wild animals and among other animals and people.

In Australia, free-roaming canids and the potential public health issues associated with them have long been a concern in Australian Indigenous communities. These animals can include unrestrained domestic dogs from within the community, along with wild dogs and dingoes from surrounding areas. Unfortunately, some Aboriginal communities

often have a large population of malnourished and diseased dogs. Many of these dogs may have been dumped out in the bush and originate from other towns in the region. Those animals that manage to survive and make it into the community are often half starved and already have an elevated disease status (F. Smout, pers. obs.). Due to a lack of animal control procedures these stray dogs often survive by scavenging from the local rubbish tip or community rubbish bins and they utilise areas such as local parks and beaches. Many infectious agents are passed via the faecal-oral route and therefore may be indirectly transmitted in places used by dogs and the general public such as the local beach, park or swimming holes.

Previous researchers have documented various zoonotic organisms being carried by community dogs resulting in the suggestion that dogs may play a role in the human disease burden (Currie, 1995; Shield, 1992; Wilks, 2000). The effect of resultant dog health programs on Aboriginal health has been the focus of considerable debate. The main concern is that Aboriginal health funding is redirected to dog health under the assumption that improving dog health will improve community health (Currie, 1995). Several reviews have been published trying to assess this risk but none have explained their methods, nor critiqued the research using a specified system (Currie, 1995; Gaskin et al., 2007; Raw, 2001). Zoonotic and public health literature reviews have been scrutinised for their lack of methodological soundness in review techniques, because they are more likely to contain bias or errors (Waddell et al., 2009).

1.3.2 AIMS

This project aims to identify canine zoonotic helminth infections and potential risk practices that are currently most hazardous to communities in the Lowland Wet Tropics bioregion of northern Australia. In order to fulfil these aims, it is first necessary to pose many questions to establish the need for research into this area. An extensive and well critiqued literature review will determine if canine zoonotic diseases are a threat to humans in Indigenous communities and establish those diseases that have already been identified, where they have come from, what are the risks associated with these diseases and what measures have been instigated to mitigate this risk? Further studies can then be performed locally to establish if there is evidence of canine zoonotic helminth

infection in the area and if any novel parasitic diseases exist along with determining the possible reservoir host ie; dingoes in National Parks and State Forests and domestic dogs in Aboriginal communities. Do we have the right environmental conditions for dingoes to be reservoirs of these diseases in the Wet Tropics and what are the health security risks to local Indigenous communities and to Australian biosecurity? Given the tropical environmental conditions, could these parasites also be abundant in soil and spread infection through non-direct means such as per-cutaneous penetration and are there any activities which may increase the risk of disease transmission? Can these diseases be easily transmitted by 'spill-over' from dingoes to dogs and 'spill-back' from dogs to dingoes? And finally, what mitigation measures are necessary in order to reduce the potential risk of transmission of zoonotic infections to people in Indigenous communities?

The specific aims for this PhD are to determine:

(1) Current status of research on canine zoonotic diseases in Aboriginal communities;

(2) Parasitic helminth infection status of dingoes in the Wet Tropics using conventional and molecular diagnostic tools;

(3) Parasitic helminth infection status of domestic dogs in Aboriginal communities of the Wet Tropics using conventional and molecular diagnostic tools;

(4) Pathways, mechanisms and potential risk of transmission of zoonotic helminth infection among dingoes, domestic animals and humans, particularly in Indigenous communities, in the Wet Tropics;

(5) Methods to mitigate the potential risk of spill-over of zoonotic helminth infection among dingoes and domestic dogs in Indigenous communities in the Wet Tropics;

1.3.3 THESIS OUTLINE

This thesis is structured as a series of connected papers that have been published or are in the process of publishing at the time of thesis submission. Each paper is designed as an individual, stand-alone paper and for this reason there are some unavoidable repetitions, particularly within the methods sections, along with some minor cross-over

of specific aims above with regard to risk of transmission of infection and mitigation suggestions. The selection of pathogens to concentrate on was decided from initial necropsy findings in both dingoes and dogs along with relevance and zoonotic potential to Aboriginal communities in the Wet Tropics. Figure 1.2 illustrates the overall structure of the thesis.

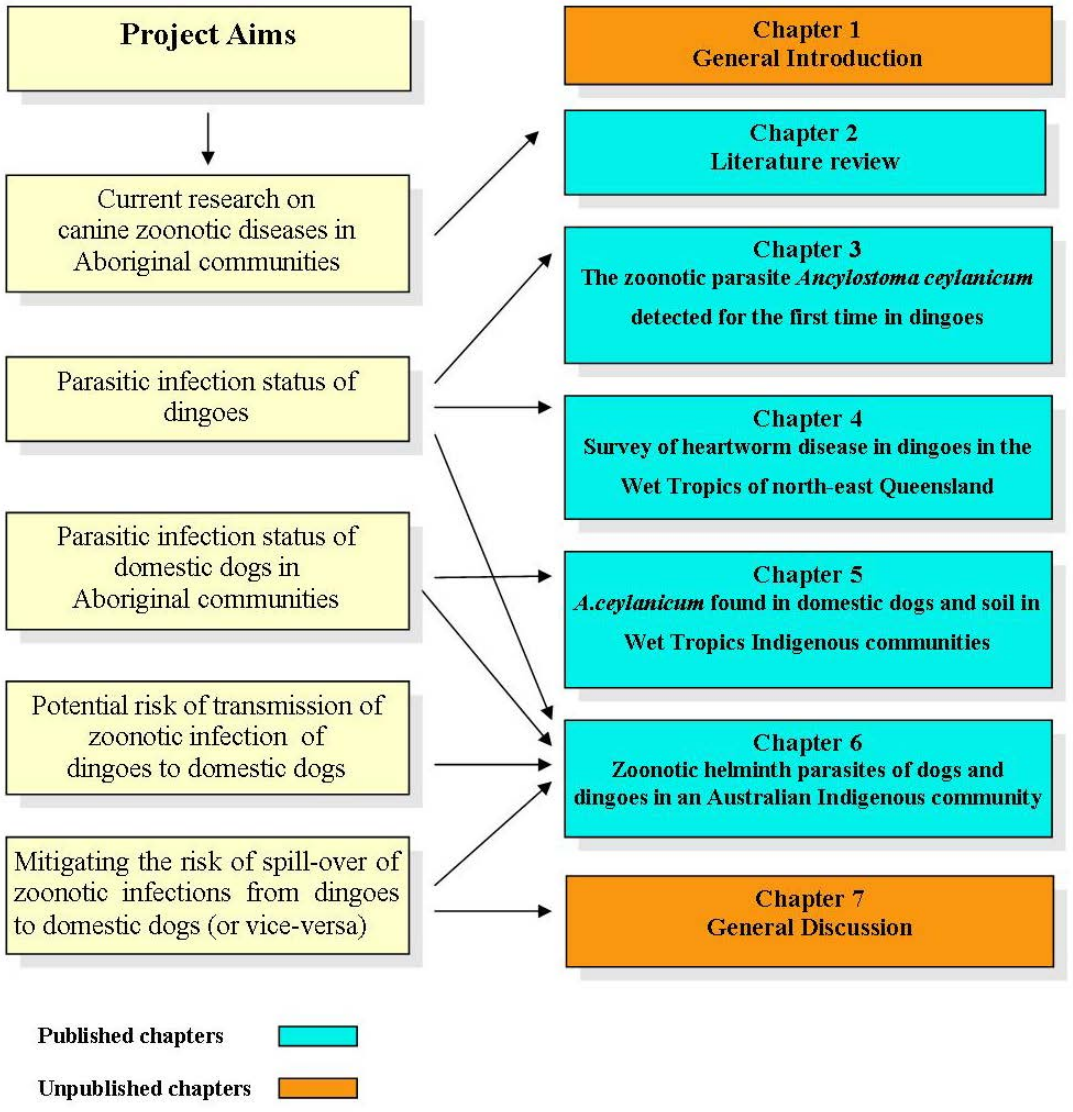

Figure 1.2 Thesis outline showing published and unpublished chapters.

Each chapter of this thesis is a manuscript as listed below. If published, the whole citation is included preceding the manuscript.

CHAPTER 1

General Introduction – Potential for transmission of zoonotic helminth infections among dingoes and dogs in Indigenous communities in the Wet Tropics of North Queensland, Australia.

In this chapter I discuss the background, aims, thesis outline and significance of the project.

CHAPTER 2

Literature review

Published - paper 1: Smout, F., Schrieber L, Speare R, and Skerratt LF. (2017). More bark than bite: Comparative studies are needed to determine the importance of canine zoonoses in Aboriginal communities. A critical review of published research. Zoonoses Public Health 00:1–10. https://doi.org/10.1111/zph.12354.

In this chapter I address thesis aims point (1), determining the current status of research on canine zoonotic diseases in Aboriginal communities. This literature review critiques over forty years of peer-reviewed literature concerned with the transmission of canine zoonotic diseases in Australian Aboriginal communities. It is the fundamental starting point to determine what canine zoonoses are present in Aboriginal communities, the implications of these diseases and what research is further necessary.

CHAPTER 3

Survey of gastro-intestinal helminth diseases in dingoes in the Wet Tropics of north-east Queensland.

Published - paper 2: Smout, F. A., Thompson, R. & Skerratt, L. F. (2013). First report of *Ancylostoma ceylanicum* in wild canids. International Journal for Parasitology: Parasites and Wildlife 2: 173-177.

This chapter investigates canine zoonotic parasites in dingoes at a local level to determine base line data of the infections present in the wild canine population around Indigenous communities and if any novel infections are present. I address thesis aims point (2) here. Through the utilisation of molecular and traditional diagnostic tools I was able to detect, for the first time, the zoonotic parasite *Ancylostoma ceylanicum* in wild dogs/dingoes. Due to the ability of *A. ceylanicum* to cause a patent infection in humans, the zoonotic risk arising from this wild dog reservoir to communities in the Wet Tropics is of concern. I also discuss how infections such as *A. ceylanicum* could be transmitted among dingoes, free-roaming domestic dogs and humans.

CHAPTER 4

Survey of heartworm disease in dingoes in the Wet Tropics of north-east Queensland.
Published - paper 3: Smout, Felicity A., Skerratt, L.F., Butler, J., Johnson, C. & Congdon, B. (2016). Dingoes (*Canis dingo* Meyer, 1793) continue to be an important reservoir host of *Dirofilaria immitis* in low density housing areas in Australia. Veterinary Parasitology 215: 6-10.

This chapter follows on from chapter 3 and again addresses thesis aims point (2). I specifically investigated *Dirofilaria immitis* in dingoes in the region as I suspected an elevated prevalence of infection from previous studies and from the many positive heartworm infections found in dingoes I had necropsied. Through personal communications with several local veterinarians I was told of a distinct lack of infection in domestic dogs around Cairns. Thus, I found that dingoes serve as a reservoir host for this parasite in areas of low density housing.

Dirofilaria immitis is responsible for heartworm disease in dogs and cats and human zoonotic filariosis in tropical and temperate regions throughout the world. I discuss the use of chemotherapeutic heartworm prevention in domestic dogs as a way of controlling the infection in the resident dingo population.

CHAPTER 5

Survey of helminth diseases in dogs and soil in Wet tropics Indigenous communities.

Published - paper 4: Smout, Felicity A., Skerratt, L.F., Butler, J., Johnson, C., Congdon, B. & Thompson, R.C. (2017). The hookworm *Ancylostoma ceylanicum*: An emerging public health risk in Australian tropical rainforests and Indigenous communities. One Health 3: 66-69.

In this chapter I address thesis aims point (3). By investigating the status of zoonotic infections in dogs and soil samples from several Indigenous communities and popular tourism locations in the Wet Tropics I was able to ascertain, for the first time, that the parasite *A. ceylanicum* was present in domestic dogs in an Indigenous community and in soil at tourism sites and was a substantial public health risk in the region. I suggest that mitigation measures such as ongoing dog health and control programs employing local Aboriginal residents should be established in communities. Collaboration with Aboriginal health workers is recommended to enable capacity building at the community level and to increase responsibility and ownership of a dog management solution.

CHAPTER 6

Zoonotic helminth diseases in dogs and dingoes utilising shared resources in an Australian Aboriginal community.

Published - Paper 5: Smout, F.A., Skerratt, L.F., Johnson, C., Butler, J., Congdon, B. (2018). Zoonotic helminth diseases in dogs and dingoes utilising shared resources in an Australian Aboriginal community. Tropical Medicine and Infectious Diseases 3, 110.

In this chapter I again further address thesis aims (3), (4) and (5) by surveying the parasitic infections of free-roaming dogs in an Indigenous community and the dingoes in the surrounding region. This was important to establish the types of parasitic infections present in the community and determine the risk of transmission of infection between dogs and dingoes and how this may happen. I placed GPS tracking collars on the dogs and dingoes to establish their home ranges and potential overlap of habitat. I found that dingo home ranges almost completely overlapped that of the domestic dogs and dingoes spent a substantial amount of their time in areas used by dogs. However, at no time did dingoes and dogs utilise the same area at the same time. I also suggest mitigation measures to reduce risk of infection transmission within the community.

CHAPTER 7

General discussion.

This final chapter summarises findings of the previous chapters and again addresses thesis aim point (5) where I suggest concluding mitigation methods to reduce the public health risk of canine zoonotic diseases and recommend management strategies for dogs and their diseases in Indigenous communities in the Wet Tropics.

Tables and figures are illustrated throughout this thesis and additional information has been added to appendices. I have created all tables and figures present in this thesis, unless stated otherwise.

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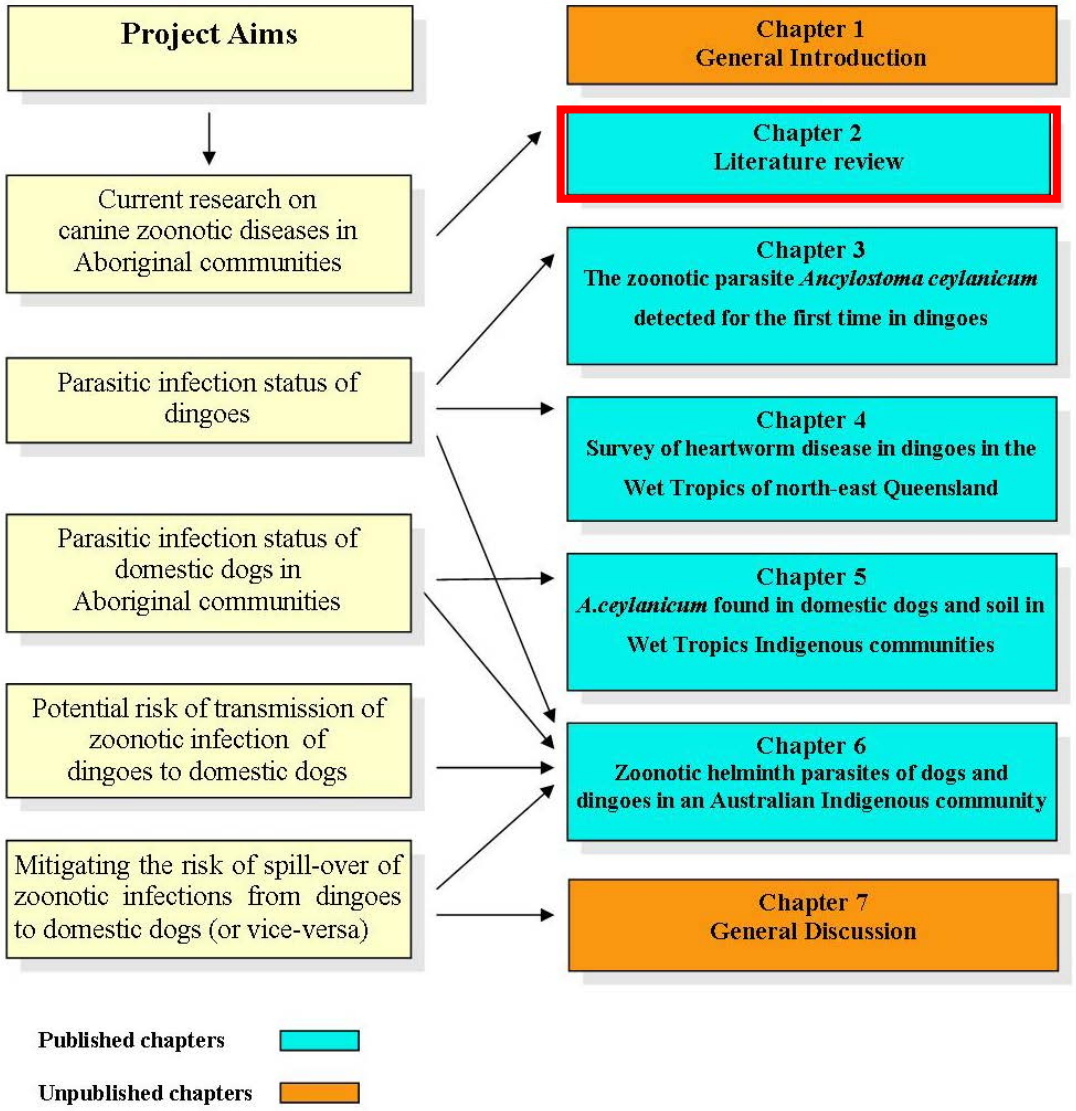
CHAPTER 2.

In this chapter I perform a critical review of the published research on canine zoonotic diseases in Aboriginal communities over the past forty years**.**



Published paper 1

Citation: Smout, F., Schrieber L, Speare R, Skerratt LF. (2017). More bark than bite: Comparative studies are needed to determine the importance of canine zoonoses in Aboriginal communities. A critical review of published research. Zoonoses Public Health 00:1–10. https://doi.org/10.1111/zph.12354

TITLE: MORE BARK THAN BITE: COMPARATIVE STUDIES ARE NEEDED TO DETERMINE THE IMPORTANCE OF CANINE ZOONOSES IN ABORIGINAL COMMUNITIES. A CRITICAL REVIEW OF PUBLISHED RESEARCH.

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Supplementary material: Appendices 4 and 5.

2.1 SUMMARY:

The objective of this review is to identify and critique over forty years of peer-reviewed literature concerned with the transmission of canine zoonoses to Aboriginal people and determine the zoonotic organisms documented in dogs in Australian Aboriginal communities. A systematic literature search of public health, medical and veterinary databases identified 19 articles suitable for critical appraisal. Thirteen articles documented the occurrence of recognised zoonotic organisms in dogs in Aboriginal communities including, *Toxocara canis*, *Dirofilaria immitis*, *Streptococcus dysgalactiae*, *Rickettsia felis*, *Sarcoptes scabiei* and *Giardia*. Currently, there is definitive evidence indicating that dogs act as a reservoir for human scabies in Aboriginal communities. However, there is a need for large scale, high quality, comparative studies of dogs and humans from the same household to assess the occurrence and importance of transmission of *S. scabiei* and other diseases between dogs and humans. These studies should use current genetic and molecular techniques along with traditional techniques to identify and type organisms in order to better understand their epidemiology. This review has revealed that there is a lack of high quality, comparative studies to determine whether dogs are contributing to human disease by transmitting zoonoses. My recommendations differ significantly from current public health policy and may have substantial implications for human and dog health.

2.2 KEY WORDS: Aboriginal, zoonoses, dogs, parasites, scabies.

2.3 IMPACTS:

- This critical review of over 40 years of published research reveals a lack of high quality, comparative studies to determine whether dogs are contributing to human disease in Aboriginal communities.
- The aim of this paper is to provide the public health audience with a summary of zoonotic organisms that have been found in dogs and humans within Aboriginal Australian communities.
- A better understanding of the epidemiology of zoonotic diseases is essential in order to direct health care funding where it is most needed.

2.4 INTRODUCTION

For decades, the shared environment of Australian Aboriginal people and unhealthy dogs has raised public health concerns because of the assumed risk of zoonoses. In many Aboriginal communities dogs are often diseased and malnourished, reflecting the condition of stray or unwanted dogs from mainstream communities (Raw, 2001).

Historically, Aboriginal Australians adopted the dingo (*Canis lupus dingo*) into their community (Meggitt, 1965). Following the colonisation of Australia, Aboriginal people embraced the European dog (*Canis lupus familiaris*) which has resulted in numerous problems that were not previously seen. Dingoes breed once per year (Catling et al., 1992; Corbett, 1995) compared with domestic dogs which will breed twice per year and have a greater number of pups in their litters (Catling et al., 1992). This has resulted in many communities being burdened with canine overpopulation and a poor state of dog health which not only affects animal welfare but human social welfare (Constable, 2008). This is a cause for concern and has resulted in the implementation of numerous dog health programs since the mid 1980s (English, 2000).

Previous researchers have documented various zoonotic organisms being carried by community dogs resulting in the suggestion that dogs may play a role in the human

disease burden (Currie, 1995; Shield, 1992; Wilks, 2000). The effect of resultant dog health programs on Aboriginal health has been the focus of considerable debate. The main concern is that Aboriginal health funding is redirected to dog health under the assumption that improving dog health will improve community health (Currie, 1995). The debate was largely extinguished in the Northern Territory following the research conducted by Walton et al (2004; 1999), that used micro-satellite typing to show that scabies mites from dogs and humans group separately in a phylogenetic dichotomous tree which they suggested demonstrated separate transmission cycles. The ramifications of this have been that many communities are now under the impression that dogs pose no significant public health risks. Several reviews have been published trying to assess this risk but none have explained their methods, nor critiqued the research using a specified system (Currie, 1995; Gaskin et al., 2007; Raw, 2001). Zoonotic and public health literature reviews have been scrutinised for their lack of methodological soundness in review techniques, because they are more likely to contain bias or errors (Waddell et al., 2009).

Therefore, despite over 40 years of research, I present here the first critical review of canine zoonoses in Australian Aboriginal communities, using a systematic methodology.

The aim of this paper is to provide the public health audience with a summary of zoonotic organisms that have been found in dogs and humans within Aboriginal Australian communities, their zoonotic potential and their importance to the human disease burden based on evidence and methodological soundness. Through this review I can better assess the public health risks that dogs pose in Aboriginal Australian communities.

I identify directions for future high quality, evidence based research to address current gaps in knowledge. My recommendations differ significantly from current public health policy and have substantial implications for human and dog health.

2.5 METHODS

2.5.1 LITERATURE SEARCH STRATEGY

A database search of several public health, medical and veterinary databases including Medline, Web of Science, Embase, Scopus, Biosis reviews, APAIS Health, Cinahl, Zoological record, CABI Abstracts, EBM Reviews was undertaken using combinations of the words, 'zoonoses' OR 'zoonotic' OR 'disease' OR 'parasites' AND 'dogs' AND 'Australia' AND 'Aboriginal'. Frequent authors were also searched and searches through the reference lists of eligible papers were also studied and included if eligible.

2.5.2 SELECTION OF ARTICLES FOR REVIEW

Database searches returned 43 articles, a further nine articles were retrieved by studying the reference lists of all papers and searching for common authors. Of these, 19 articles were eligible for inclusion. These articles have been summarised in Appendix 4. Only articles that were consistent with the aim and were published in peer reviewed journals were eligible for inclusion (Figure 2.1).

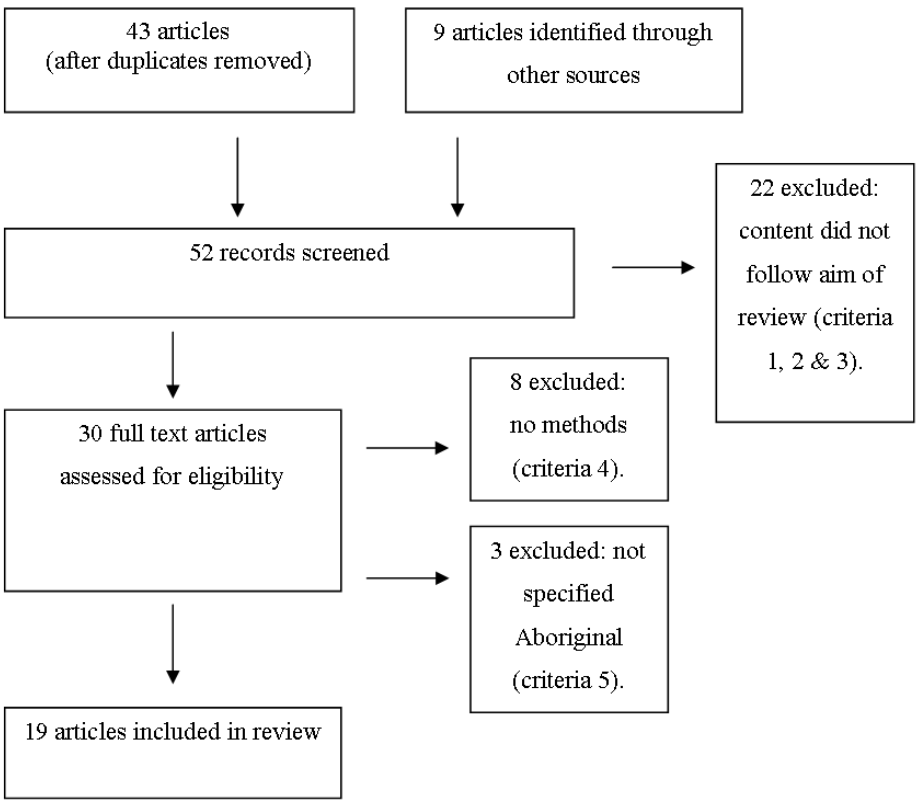

Figure 2.1 Flow diagram of papers through the exclusion and critiquing process

2.5.3 EXCLUSION CRITERIA

A checklist was developed and papers excluded if:

1. They did not contain any zoonotic information pertaining to dogs (or did not distinguish between companion animals).
2. They did not contain any information about an organism recognised as a zoonosis. (However, research was included if the organism's ability to cause disease in humans was still unknown).
3. The main aim was to test the efficiency of a microbiological technique.
4. They did not describe their methods of research.
5. They did not specify whether the research pertained to Aboriginal people or dogs from their communities (or a location commonly known as an Aboriginal community).

2.5.4 CRITIQUING TOOLS

Appendix 5 illustrates how included articles were critiqued using the Crowe Critical Appraisal Tool (CCAT).(Crowe and Sheppard, 2011) Briefly, the CCAT is an appraisal tool that allows reviewers to evaluate a paper by dividing it into categories and scoring each category out of five based on descriptors. There are a total of eight categories;

1. Preamble (Title, Abstract and text overall).

2. Introduction (Background and Objective).

3. Design (Research design, measure, bias).

4. Sampling (method, size, protocol).

5. Data collection (method, protocol).

6. Ethical matters (participant ethics and researcher ethics).

7. Results (analysis, integration, interpretation, outcome).

8. Discussion (interpretation, generalisation, conclusion).

Two reviewers used the Crowe critical appraisal tool to systematically summarise the strengths and weaknesses of each study by following the above criteria. Reference was made to Dohoo et al. (2003) and Lewis-Beck et al. (2004) for information regarding sampling and research design.

2.5.5 ETHICAL MATTERS

Ethics approval was not required for this review as there was no human or animal intervention. The authors state there are no conflicts of interest or funding sources to be declared.

2.6 RESULTS

The majority of research in this area is not recent (i.e. many papers with large sample sizes were published before 1994). Most studies were opportunistic, had small sample sizes and did not compare pathogens in humans with those in dogs. The few comparative studies almost never compared dogs and people from the same household. Furthermore, the pathogenicity of many of the organisms found has not been determined.

Of the 19 papers included in the review, six were short contributions (Hii et al., 2011; Jenkins and Andrew, 1993; Lee and Hampson, 1996; Meloni et al., 1988; Schnagl and Holmes, 1978; Thompson et al., 1993a). I did not find any eligible papers prior to 1974 with the bulk of studies undertaken between 1990 and 2000. Papers were published in medical, veterinary, public health and parasitology journals. The majority were in medical journals however, after 2000, this shifted to parasitology. Four papers determined the prevalence of a wide range of parasites (Jenkins and Andrew, 1993; Meloni et al., 1993; Shield et al., 2015; Thompson et al., 1993a). The remaining 15 papers targeted 12 zoonotic organisms (Table 2.1).

Table 2.1 Number of published papers according to zoonotic organism and symptoms recorded in people.

Organism	Number of papers/Reference	Symptoms in people
Dirofilaria immitis	1/Welch & Dobson (1974a)	Pulmonary lesions, retinal granuloma.
D. immitis and *Toxocara canis*	1/Welch et al. (1979)	Visceral larval migrans, Ocular larval migrans
Microsporum canis	1/Kaminski & Green (1977)	Rare cause of Tinea capitis
Coronavirus-like particles	2/Schnagl et al. (1978) Schnagl & Holmes (1978)	Unknown, possible gastroenteritis
Giardia spp.	2/Meloni et al. (1988) Hopkins et al. (1997)	Acute diarrhoea, weight loss and abdominal pain.
Spirochaetes	2/Lee & Hampson (1994) Lee & Hampson (1996)	Unknown, possible cause of diarrhoea and dehydration
Parasites (Wide range)	4/Meloni et al. (1993) Jenkins & Andrew (1993) Thompson et al. (1993) Shield et al. (2015)	Range of acute gastroenteritis
Sarcoptes scabiei	2/Walton et al. (1999) Walton et al (2004)	Skin lesions, secondary bacterial infection
Ancylostoma spp.	1/Palmer et al. (2007)	Eosinophilic enteritis, cutaneous larval migrans
Blastocystis	1/Parkar et al. (2007)	Unknown
Rickettsia felis	1/Hii et al (2011)	Fever, rash, headache, abdominal pain, nausea, vomiting, and diarrhoea, as well as central nervous system involvement (photo-phobia, hearing loss, and/or meningism)
Streptococcus dysgalactiae (SDSE)	1/Schrieber et al (2014)	Unknown

Two reviewers using CCAT appraisal methods were often in agreement on scores and differed by only 1 or 2 points occasionally (See Appendix 4 for averaged results). Most papers received a low score for design, sampling, data collection and ethical matters.

2.6.1 DESIGN AND SAMPLING

Only five of 19 articles described the research design of the study albeit briefly (Hopkins et al., 1997; Meloni et al., 1993; Schrieber et al., 2014; Walton et al., 2004; Walton et al., 1999). Five were purely observational and documented the presence of zoonotic organisms in dogs only (Hii et al., 2011; Jenkins and Andrew, 1993; Lee and Hampson, 1996; Palmer et al., 2007; Thompson et al., 1993a). Thirteen also examined samples from humans to see whether disease transmission had occurred.

The most renowned comparative studies conducted on possible transfer of zoonotic organisms from dogs to people in Aboriginal communities are those of Walton et al. (2004; 1999). The studies included a large number of scabies mites but a limited number of hosts, 16 people and 17 dogs. Within household comparisons were only applicable to mites from a four-week-old baby and three puppies. The results of these studies are discussed further on in this paper.

Welch & Dobson (1974a) conducted research to assess the prevalence of antibodies to the dog heartworm, *Dirofilaria immitis* in Caucasian and Aboriginal Australians. This was achieved by screening blood samples for anti *D. immitis* antibodies in both Aboriginal and Caucasian sera and compared with a miniature mass radiography for lesions that appeared to coincide with *D. immitis*. The Aboriginal sample was quite large (n = 323), however the random Caucasian sample consisted of only 38 individuals. The miniature mass radiography of Aboriginal participants, completed by the Queensland Department of Health, found no lesions. In comparison, five Caucasians from Queensland were found to have lesions in the same year, but it is unknown whether they were the same individuals who participated in the study. Regardless, the

prevalence of canine dirofilariasis significantly correlated with the mean titre of individuals testing positive to anti- *D. immitis* antibodies, indicating that *D. immitis* prevalence in dogs will increase a person's exposure.

Lee & Hampson (1992) found that Aboriginal people from various communities in the Kimberley area had intestinal spirochaetal bacteria in their faeces. In addition, another study of dogs from Fitzroy Crossing and Jarvis Bay were also found to have morphologically similar spirochaetes in their faeces to humans (Lee and Hampson, 1996). Multilocus enzyme electrophoresis of spirochaetes isolated from a dog with diarrhoea were closely related genetically to spirochaetes recovered from Aboriginal children with whom the dog lived (Lee and Hampson, 1994).

Kaminski & Green (1977) conducted a large scale study on the prevalence of Tinea capitis in Aboriginal communities. In the community of Maningrida they found that 25.3% of children with tinea capitis were due to the "Maningrida" type variant of *Microsporum canis*. This variant was also found in four cats and two dogs in the community.

Schrieber et al. (2014) reported the finding of an identical strain of *Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE), also known as group G and C streptococci, in the throat of a child and their dog. This study specifically used samples from a human and a dog from within the same household.

The only other study I could find that conducted comparative studies on potentially zoonotic organisms isolated from dogs and people in Aboriginal communities was that of Schnagl and Holmes (1978; Schnagl et al., 1978) who found coronavirus-like particles in faeces from both dogs and humans in Aboriginal communities. However, it is largely unknown what these particles are, what they do and whether their presence in both dogs and people indicate disease.

The sample sizes for five papers (Meloni et al., 1993; Shield et al., 2015; Thompson et al., 1993a; Welch et al., 1979; Welch and Dobson, 1974b) were considerable, providing precise estimates of prevalence in dogs in the Kimberley region (Meloni et al., 1993;

Thompson et al., 1993a), various locations around Queensland (Welch and Dobson, 1974b), Central Australia (Welch et al., 1979) and Arnhem Land (Shield et al., 2015). Two papers were from the same study, although considered different organisms (Meloni et al., 1993; Thompson et al., 1993a). The prevalence of *Toxocara canis* and *Dirofilaria immitis* differed markedly among locations, highlighting the need for dog control and education programs to be targeted towards the risks faced by each community.

The study by Jenkins & Andrew(1993) had a much smaller sample size of 15, presumably because it was an opportunistic study made possible by a culling program by the local council. Therefore, the results indicate 'presence of an organism' rather than prevalence or disease freedom.

Parkar et al (2007) compared PCR detection directly from faeces to *in vitro* propagation for the detection of *Blastocystis.* The study included dogs sourced from Aboriginal communities but did not state the communities from which the dogs were sourced nor include samples from people in the communities for comparison.

Hii et al (2011) also used PCR assays to assess the prevalence of *Rickettsia felis* in dogs at Maningrida Aboriginal community in the Northern Territory. This was an opportunistic study of 130 dogs included in a desexing program operated by the Animal Management in Rural and Remote Indigenous Communities (AMRRIC) organisation.

2.6.2 ETHICAL MATTERS

Fourteen papers published from 1974-2007 did not discuss ethical approval of their study; nine of these included the use of human data. Only seven out of 19 papers scored any points in the ethical matters category. Only two papers discussed the use of informed consent when taking samples from Aboriginal people and their dogs (Schrieber et al., 2014; Walton et al., 1999). Many papers thanked nurses and participants and two papers recognized the support of the community council (Jenkins and Andrew, 1993; Welch et al., 1979).

While informed consent is not mentioned in many of the papers, it may still have been received. Regardless papers should clearly state the ethical procedures undertaken within Aboriginal communities whether samples are coming from humans or their dogs.

2.6.3 Sarcoptes results

Walton et al. (1999) stated that the human derived scabies mites and the dog derived scabies mites in the same Aboriginal community had different transmission cycles based on them grouping in separate clusters within a phylogenetic dichotomous tree. However, a reanalysis of the data using reticulated networks rather than dichotomous trees to represent the evolutionary history of *S. scabiei* in the Aboriginal community showed that both human to human and dog to human transmission cycles occur and that both are important for control programs (Morrison, 2005). Therefore, the method of analysis can significantly affect interpretation of results. In this case, failure to consider reticulate evolution led to an important zoonotic transmission pathway being overlooked.

2.7 DISCUSSION

The continuing health disparities seen between Indigenous and non-Indigenous Australians are often related to socioeconomic factors and the harsh living conditions experienced within rural and remote Indigenous communities. Some of these health issues could be attributed to canine zoonotic diseases due to the presence of large free-ranging dog populations and the general lack of veterinary services in these areas. Often zoonotic diseases are not reported and instead labelled under symptom based headings such as gastroenteric disease, which not only down plays their importance but reduces the likelihood of identifying zoonotic organisms and risk practices associated with their transmission.

2.7.1 SKIN INFECTIONS:

2.7.1.1 *Microsporum canis*

Fungal skin infections are common amongst Aboriginal people living in the humid regions of northern Australia. A granular variant of *Trichophyton rubrum* was reported to be responsible for the most common endemic ringworm cases (Green and Kaminski, 1973; Koh et al., 2003). Kaminski and Green (1977) isolated a variant of *Microsporum canis* from 21 Aboriginal children suffering from tinea capitis in the Maningrida community and found two dogs to be possible reservoirs of this variant.

2.7.1.2 Scabies

Scabies is a debilitating skin condition in Aboriginal communities caused by the mite *Sarcoptes scabiei*. It is important because the resultant trauma to the skin can lead to subsequent bacterial infection. In some Aboriginal communities scabies has been shown to underlie up to 70% of streptococcal pyoderma (Currie and Carapetis, 2000). Dog-derived scabies mites have been experimentally shown to burrow, lay eggs and defecate in human skin initiating papular lesions (Estes et al., 1983).

Smith and Claypoole (1967) documented 22 cases of human infestation with *Sarcoptes scabiei* var *canis* in which members of a household were living with a dog diagnosed with sarcoptic mange. The characteristic features included the sudden onset of intensely pruritic papules and vesicles in areas of contact with pets, extreme difficulty in demonstrating mites in humans and excellent responses to treatment with scabicides.

The treatment of household dogs with sarcoptic mange is important in preventing and treating human cases of scabies. The initial reaction is sufficient for secondary bacterial infection (Smith and Claypoole, 1967) with bacteria such as *Streptococcus pyogenes* or *Staphylococcus aureus*, which can lead to post infective complications including acute post streptococcal glomerulonephritis (Bandi and SaiKuMar, 2013; Hoy et al., 2012).

The studies conducted by Walton et al.(2004; 1999) have been used as evidence of a lack of zoonotic transmission of the scabies mite. However, reanalysis of the data using more appropriate methods showed that dog to human transmission occurred multiple times and was an important component of the epidemiology of human scabies

(Morrison, 2005). Given this and the above evidence from other studies the conclusions of Walton et al (2004; 1999) that "control programs for human scabies in endemic areas do not require resources directed against zoonotic infection from dogs," are incorrect. Successful mitigation of the effects of scabies in Aboriginal communities must include the control of sarcoptic mange in dogs.

2.7.2 RESPIRATORY INFECTIONS:

2.7.2.1 *Dirofilaria immitis* is a filarial nematode responsible for heartworm in dogs and can be transmitted to humans by mosquitoes. Because mosquitoes are the vector, *D. immitis* transmission is more common in areas where mosquitoes are endemic. This may explain the high frequency of *D. immitis* in blood mounts of dogs sourced from Queensland communities compared with those from Central Australia (Welch et al., 1979). A recent study in the Wet Tropics of Far North Queensland found *D. immitis* at high prevalence (72.7%) in wild dingoes in low density housing areas (chapter 4) (Smout et al., 2016). The heartworm life cycle has five larval stages (L1 - L5). Adult heartworms are generally present in the pulmonary arteries but may be found in the right ventricle, right atrium and caudal vena cava in heavy infections. Mature females release microfilariae (L1) into the host's bloodstream. The vector mosquito that feeds on an infected dog, the primary host, picks up the microfilariae where they undergo two moults to L3 stage. The mosquito transmits infective larvae (L3) when it subsequently feeds on a human, the secondary host. In the dog, the larvae mature in the muscle sheath, subcutaneous and adipose tissue to L5 stage and the immature adults migrate to the heart and pulmonary artery where they mature to adults. A similar transmission pattern has been proposed for humans where adult nematodes are eventually washed into the pulmonary artery and become lodged in the lungs causing pulmonary nodules (Narine et al., 1999). These pulmonary nodules have been mistaken for tuberculosis and metastatic tumours (Narine et al., 1999; Ro et al., 1989). Aboriginal people have extremely high rates of tuberculosis with 6.2 cases per 100,000 population in 2008 versus 0.9 cases per 100,000 population of non-Indigenous people born in Australia (Barry et al., 2012). In 1974, Welch & Dobson (1974b) reported that fluorescent antibody tests (FAT) found 65 (20.1%) of 323 sera collected from Aboriginal participants had positive titres of human anti-*D. immitis* antibodies. This correlated significantly with the prevalence of canine dirofilariasis. All of the Aboriginal

participants underwent miniature mass radiography for tuberculosis by the Queensland Department of Health, however, no *D. immitis* like lesions were found in the lungs. These results could indicate that a high exposure to *D. immitis* may provide protective immunity against infection in people in Aboriginal communities (Welch and Dobson, 1974b).

2.7.2.2 *Toxocara canis*

Although classically associated with ocular and visceral larva migrans, *Toxocara* infection is now known to manifest more commonly as non-classic or covert toxocariasis where clinical signs include wheezing and asthma, pulmonary infiltrates, and eosinophilia (Feldman and Parker, 1992; Sharghi et al., 2000). Welch et al. (1979) reported *T. canis* in about 75% of dogs from most areas in Queensland. Although a recent national study found a low prevalence of *T. canis* in domestic dogs in veterinary clinics and refuges (1.2%) (Palmer et al., 2008), a recent wild dog survey, which may better reflect the zoonotic risk from free ranging community dogs, reported prevalences of 46% (chapter 3) (Smout et al., 2013). Mizgajska (2001) concluded that the prevalence of human toxocariasis is proportional to soil contamination with infective eggs of *Toxocara* spp. Shield et al. (2015) found 21% of people seropositive for T. canis in their studies in Arnhem Land in the mid 1990's. Toxocariasis is now being heralded as the most common human parasitic worm infection in the United States and with its high prevalence in developing countries, it is considered that its global importance may be greatly underestimated (Hotez and Wilkins, 2009).

2.7.3 GASTROENTERIC INFECTIONS:

2.7.3.1 *Giardia*

Giardia duodenalis (syn *Giardia intestinalis*; *G. lamblia*) is the most common intestinal parasite of humans in developed countries (Thompson, 2000). The highly variable symptoms of giardiasis include persistent diarrhoea, abdominal pain and rapid weight loss (Thompson et al., 1993b). It is now commonly thought that although dogs can carry strains of *Giardia* which are potentially infective to humans, transmission mainly occurs

among humans (Hopkins et al., 1997; Robertson and Thompson, 2002). In support of this Hopkins et al. (1997) found differences in genotypes of *Giardia* isolated from 13 Aboriginal people from Fitzroy Crossing and nine dogs that had been culled from the same area. They found that the samples separated into four different genetic groups. All of the human and three dog isolates were contained in groups 1 and 2, whilst groups 3 and 4 consisted entirely of *Giardia* samples from dogs. In contrast, Traub et al. (2004) studied zoonotic *Giardia* transmission in a remote community in India and found that *Giardia* isolates derived from dogs were placed within the human genetic groupings. Furthermore, humans residing in a house that owned dogs and where one dog was infected with *Giardia* were significantly more likely to be infected, than humans that did not have a dog or a dog infected with *Giardia*. In addition, genetically identical isolates were found in dogs and humans from the same household in two cases. Together these are taken as strong evidence in support of the potential for zoonotic transmission.

The contrasting results seen between the Aboriginal and the Indian communities may be because dogs from Aboriginal communities experience a higher level of interaction with other dogs and have less opportunity to eat human faeces (Traub et al., 2004). *Giardia* isolates are prone to competitive exclusion, enabling selection of host specific *Giardia* assemblages (Thompson, 2000). It is also possible that the source of dog samples within the Aboriginal study is biased and may therefore be masking zoonotic transmission. According to Hopkins et al. (1997) the dog isolates used in the study were retrieved from culled dogs from the same area as the human participants (n=9). However, there is nothing to suggest that the dogs used in the study were from the same household as human participants or that the dogs were owned by anyone. Generally, dogs that are euthanased by councils are unhealthy, stray dogs. Sampling from the stray/wild dog population might bias the result because the major form of *Giardia* transmission, dog to dog, would predominately select for dog specific *Giardia* assemblages. Further research is required to ascertain the potential and frequency of zoonotic transmission of *Giardia* from dogs to their owners in Aboriginal communities.

2.7.3.2 *Ancylostoma* spp.

Palmer et al. (2007) investigated the public health significance of hookworms in Australia and found that *Ancylostoma caninum*, the dog hookworm, had the highest prevalence (14%) in Aboriginal communities. It is interesting to note that with such a high incidence of *A. caninum* in communities, I didn't recover any reports of eosinophilic enteritis. An Australian study implicated *A. caninum* as the leading cause of human eosinophilic enteritis (Prociv and Croese, 1990). It is possible that this disease is masked by other health issues in Aboriginal communities.

The detection of *Ancylostoma ceylanicum* for the first time in Australia in 10.9% of domestic dogs found positive for hookworm (Palmer et al., 2007) and in Australian wild dogs (chapter 3) (Smout et al., 2013) warrants further investigation given this parasite's potential to infect dogs and cats and cause a patent infection in humans (Inpankaew et al., 2014; Ngui et al., 2012). Koehler et al.(2013) recorded the first two cases of *A. ceylanicum* in humans in Western Australia and suggest, as there was no record of travel outside of Australia, the infection was autochthonous and derived from dogs or cats.

2.7.3.3 *Rickettsia felis*

R. felis, the agent of flea-borne spotted fever in humans, is typically transmitted through the bite of an infected cat flea (*Ctenocephalides felis*).Clinical infection ranges from fever, headaches, chills, muscle aches, joint pain and possible eschar at the bite site to a more severe, multi-systemic disease as a result of a widespread vasculitis (Maina et al., 2012; Teoh et al., 2016). Molecular techniques have been used to identify *R. felis* infection in cat fleas from multiple sites in Western Australia (Schloderer et al., 2006). Williams et al. (2011) reported *R. felis* infection in two adults and three children from Victoria. Previously, many infections in Australia may have been misdiagnosed as murine typhus as serological diagnosis was not specific and was typically confounded by cross-reactivity with typhus group rickettsiae (Teoh et al., 2016). Dogs are often infested with cat fleas and infected dogs can appear physically healthy, which may be characteristic of reservoir hosts of *R. felis* (Hii et al., 2011). Further study is needed to determine the pathogenicity of this infection in dogs.

2.7.4 ZOONOSES THAT ARE EVIDENT IN ABORIGINAL COMMUNITY DOGS BUT HAVE NOT YET BEEN RESEARCHED.

2.7.4.1 Coronavirus-like particles

Schnagl et al. (1978) reported that coronavirus-like particles were equally prevalent in humans with or without symptoms of diarrhoea. The proportion of children who excreted the viral particles increased with age. Viruses found in humans and dogs were indistinguishable morphologically (Schnagl et al., 1978). The authors concluded that given there are few reports of canine enteric coronaviruses it is difficult to gauge how widespread and important they are, not only as a health risk to dogs, but also to humans (Schnagl and Holmes, 1978).

2.7.4.2 Intestinal spirochaetes

Lee & Hampson (1992, 1994, 1996) investigated intestinal spirochaetes in faecal samples of humans, pigs and dogs. Although they are found more commonly when diarrhoea is present, their pathogenicity is still largely unknown. Spirochaetes are possibly commensal organisms of the intestine that are flushed out during bouts of diarrhoea (Leach et al., 1973). Therefore, it is unknown what impact spirochaetes may have on Aboriginal health.

2.7.4.3 *Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE)

Schrieber et al. (2014) identified an identical strain of SDSE from pharyngeal swabs of a child and dog from the same household. Once considered a commensal organism recent studies have shown that through horizontal gene transfer SDSE may be gaining virulence genes from *Streptococcus pyogenes,* thus elevating its potential importance as a human pathogen (Brandt and Spellerberg, 2009).

2.8 RECOMMENDATIONS

Identifying all factors including those of the shared environment that may or may not contribute to disease is extremely important to the improvement of health outcomes for Aboriginal Australians. This review has revealed that there have not been enough high quality comparative studies to determine whether dogs are contributing significantly to the disease burden of Aboriginal communities by transmitting zoonoses.

Aboriginal health researchers have expressed concern that further research into canine zoonoses may result in government funding being shifted from child health to fund dog health programs, irrespective of the evidence of whether it will substantially benefit the health of Aboriginal children (Currie, 1995). It is outside the scope of this paper to predict future funding decisions and distribution of resources. However, I believe that I have shown that there is insufficient scientific evidence to prove that zoonotic transmission is not important. There is evidence that zoonotic transmission is occurring with *S. scabiei* being transmitted from dogs to people. A coordinated approach is necessary and dog health should be added to the list of potentially hazardous environmental issues to be addressed within Aboriginal communities. Dog health programs are still extremely important for animal welfare and providing relevant information to pet owners in Aboriginal Australian communities regardless of zoonotic potential. Furthermore, as there can be no disputing the significant problem of dog bites as a serious canine zoonotic issue; (Abreu and D'adonna, 2009) the safety of community members from pack dogs should be motivation enough for the employment of animal control officers in Aboriginal communities.

Despite 30 years of dog health programs, and over 40 years of research, I could only find 19 studies that had published their findings in peer reviewed journals. I am aware of many other research projects which have been conducted in Aboriginal communities, such as that of Wilks and Williamson (1998), but the results of these studies could not be found in peer reviewed journals and may result in research being repeated in an already over-researched Aboriginal population.

In addition, the small amount of comparative studies conducted almost never included people and dogs from the same household. Studies which only used culled dogs for

isolates are not always reliable as most are stray dogs with minimal human contact. While it can be assumed that funding constraints make it difficult to invest time in building relationships and employing local Aboriginal research assistants and research candidates, the benefits of local knowledge would far outweigh these issues and ensure an accurate sample of the community as a whole, improving the quality, extent and impact of research results.

Canine zoonotic organisms that still need to be studied in Aboriginal Australian communities include: *Ancylostoma ceylanicum, Strongyloides stercoralis, Campylobacter,* Zoonotic *Salmonella, Streptococcus* spp., *Staphlococcus* spp., *Leptospira interrogans, Echinococcus granulosus, Dipylidium caninum, Spirometra erinacei, Cryptosporidium* and *Cystoisospora canis*.

Current public health approaches to helminth infections are directed at investigating anthroponotic routes of infection (Crompton et al., 2003). Whereas addressing zoonotic origins may be more appropriate. Identification of human parasitic infections such as *Trichuris trichiura* using egg morphology alone may be inadequate and may have previously led to cases of misidentification due to morphological similarity to *T. vulpis* (Dunn et al., 2002). The recent findings of *A. ceylanicum* in dogs (Palmer et al., 2007), dingoes (chapter 3) (Smout et al., 2013) and humans in Australia should ensure caution is used when diagnosing infections such as *A. duodenale* in humans(Koehler et al., 2013). The development of advanced, PCR-based techniques allows for differentiation between hookworm species using DNA isolated from eggs in faeces and soil (Traub et al., 2008) and enables a better understanding of the epidemiology of *A. ceylanicum* infection (chapter 3) (Smout et al., 2013).

To achieve the most from further research it would be wise to invest time in the Aboriginal community, communicate with relevant community groups and workers and employ Aboriginal locals (such as the animal management worker) on the project team. The establishment of early contact with community based health services can result in collaboration with Aboriginal health workers and other research projects that may already be underway in the community. It is extremely important to conduct comparative studies using samples from dogs and humans from the same household, along with samples from the stray/wild dog population. Samples from both necropsied

and live animals would be ideal. And finally, it is imperative that results are published in peer reviewed journals for the benefit of everyone involved, present and future.

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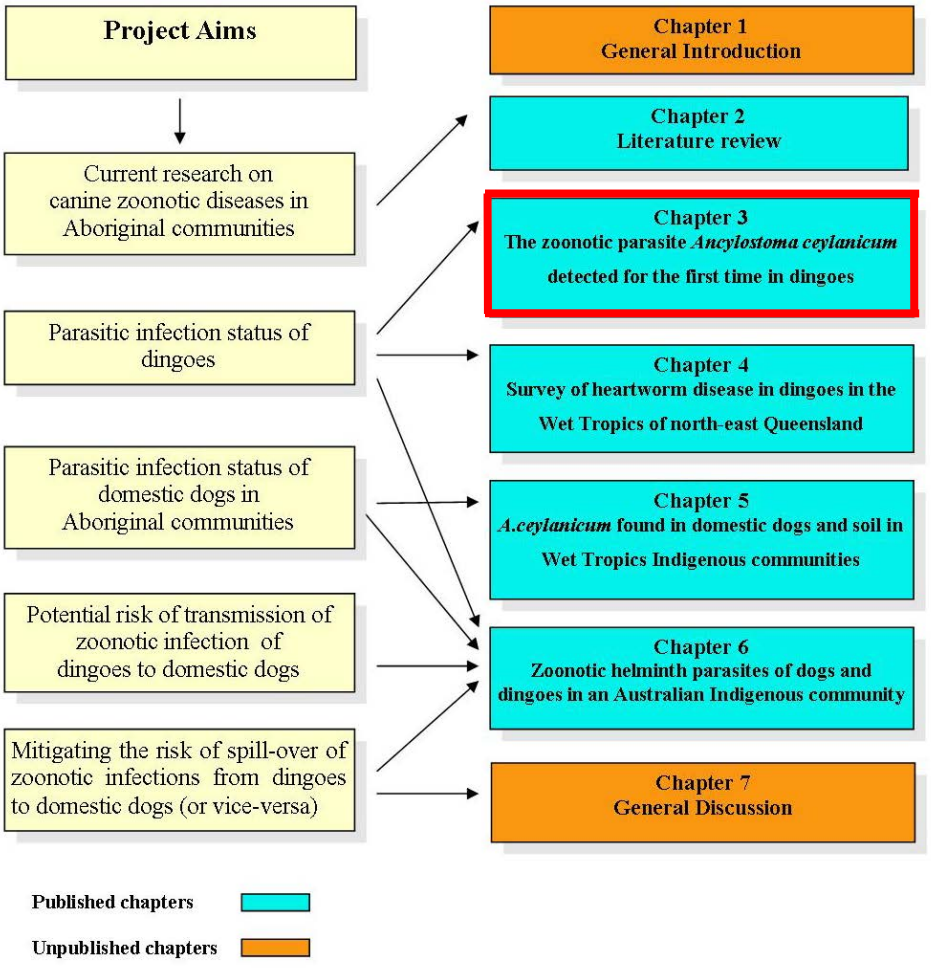
Supporting information:

Appendix 4 - Summary of articles included in review.

Appendix 5 - CCAT analysis

CHAPTER 3.

Survey of gastro-intestinal helminth diseases in dingoes in the Wet Tropics of north-east Queensland. After establishing that canine zoonotic infections are a threat in Indigenous communities, I investigate zoonotic infections in dingoes at a local level to determine base line data of the infections present in the wild canine population around the Wet Tropics and if any novel infections are present. Through the utilisation of molecular and traditional diagnostic tools I was able to detect, for the first time, the zoonotic parasite *Ancylostoma ceylanicum* in dingoes. Due to the ability of *A. ceylanicum* to cause a patent infection in humans, the potential zoonotic risk arising from this wild dog reservoir to communities in the Wet Tropics is of concern. I also discuss how infections such as *A. ceylanicum* could be transmitted among dingoes, free-roaming domestic dogs and humans.



Published - paper 2

Citation: Smout, F. A., Thompson, R. & Skerratt, L. F. (2013). First report of *Ancylostoma ceylanicum* in wild canids. International Journal for Parasitology: Parasites and Wildlife 2: 173-177.

Graphical abstract

TITLE: FIRST REPORT OF *ANCYLOSTOMA CEYLANICUM* IN WILD CANIDS.

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Supplementary material: Appendix 6.

3.1 ABSTRACT:

The parasitic nematode *Ancylostoma ceylanicum* is common in dogs, cats and humans throughout Asia, inhabiting the small intestine and possibly leading to iron-deficient anaemia in those infected. It has previously been discovered in domestic dogs in Australia and this is the first report of *A. ceylanicum* in wild canids. Wild dogs (dingoes and dingo hybrids) killed in council control operations (n=26) and wild dog scats (n= 89) were collected from the Wet Tropics region around Cairns, Far North Queensland. All of the carcasses (100%) were infected with *A. caninum* and three (11.5%) had dual infections with *A. ceylanicum*. Scats, positively sequenced for hookworm, contained *A. ceylanicum, A. caninum* and *A. braziliense*, with *A. ceylanicum* the dominant species in Mount Windsor National Park, with a prevalence of 100%, but decreasing to 68% and 30.8% in scats collected from northern and southern rural suburbs of Cairns, respectively. Due to the ability of *A. ceylanicum* to cause a patent infection in humans, the zoonotic risk arising from this wild dog reservoir to communities in the Wet Tropics should be determined.

3.2 KEY WORDS: *Ancylostoma ceylanicum*, Dingo, Canine, Hookworm, Zoonosis, Australia

3.3 INTRODUCTION:

Ancylostoma ceylanicum is a common hookworm of domestic dogs and cats in countries throughout Asia (Conlan et al., 2012; Traub et al., 2007; Yoshida et al., 1968). This parasite has also been reported in South America (Rep and Heinemann, 1976), Africa (Baker et al., 1989; Schuster et al., 2009), New Guinea (Anten and Zuidema, 1964), and more recently, Australia (Palmer et al., 2007).

Heavy infection can result in bloody diarrhoea and iron-deficient anaemia (Carroll and Grove, 1984). It was long thought that *A. ceylanicum* was a synonym of *A. braziliense*, which has led to some confusion. Biocca (1951) demonstrated that the two are distinct species. Human infection with *A. ceylanicum* was previously considered to be abnormal and unimportant (Hotez et al., 2004; Lane, 1913; Yoshida et al., 1968). Subsequent studies, however, have revealed that this parasite can cause severe abdominal

discomfort and diarrhoea (Carroll and Grove, 1986; Hsu and Lin, 2012; Tu et al., 2008) as well as cognitive impairment (Wijers and Smit, 1966) and should be considered to be of significant zoonotic importance (Conlan et al., 2012; Mahdy et al., 2012; Ngui et al., 2012; Thompson and Conlan, 2011; Traub et al., 2008).

In wild animals, *A. ceylanicum* has been identified in wild felids including the Asian golden cat (*Viverricula malaccensis),* the leopard cat (*Felis bengalensis)* and the civet (*Felis temminchii)* (Biocca, 1951; Chowdhury and Schad, 1972). Here, I look at the geographical distribution of hookworm species in the Cairns region of northern Australia and report for the first time *A. ceylanicum* in wild canids, specifically in the dingo (*Canis lupus dingo*).

3.4 MATERIALS AND METHODS

The wild dog of Australia, otherwise commonly known as the dingo (*Canis lupus dingo*) is distributed widely in all states with the exception of Tasmania. European settlement has led to hybridisation of the wild dog with the domestic dog and this has resulted in fewer pure dingoes. The degree of hybridisation was not investigated in the animals used in this study and so I have chosen to use the term dingoes, as all of the animals examined resembled dingoes morphologically, including features such as a larger palatal width, longer rostrum, shorter skull height and wider top ridge of skull when compared with domestic dogs (Crowther et al., 2014; Newsome et al., 1980)

3.4.1 STUDY AREA AND COLLECTION OF SPECIMENS

The study area was restricted to localities within the Wet Tropics World Heritage Area in north-east Queensland, Australia. This region was further sub-divided into four localities, Mount Windsor National Park (NP), northern Cairns (rural areas around Cairns outer northern suburbs eg. Barron), southern Cairns (rural areas around Cairns outer southern suburbs eg. Walsh's Pyramid) and Atherton. Eighty-nine wild dog scats were collected over a 12 month period during 2010/11 from Mt. Windsor NP, northern and southern Cairns locations. Twenty-six wild dog carcasses were supplied by Cairns Council animal control officers and local landholders following routine control measures from 2007 onwards around farmland and outer suburbs of northern and

southern Cairns and Atherton. No dingoes were killed specifically for this study. All of the specimens were bagged and frozen as soon as possible following collection and information regarding collection date and location were noted. All protocols were reviewed and approved by James Cook University Animal Ethics Committee (Approval no. A1546).

3.4.2 NECROPSY TECHNIQUE AND PARASITE PRESERVATION

Twenty-six dingoes (15 males and 11 females) ranging in age from four weeks to over five years of age (mean age approximately 22 months), were thoroughly examined for ectoparasites by combing the entire body surfaces within a white necropsy tub. External observations covered body condition, skin and hair coat, eyes, ears and mucous membranes.

Internal observations covered the heart and lungs which removed en bloc and examined along with thoracic viscera and larynx. Examination of the abdominal viscerafollowed with removal of the spleen and gall bladder. The stomach and intestines were excised and the stomach, small intestine, and large intestine were each ligated at the junctions and examined separately. The intestinal lumen was exposed via an incision along its length and the contents washed into a 250-µm aperture sieve. Stomach washings were also examined for the presence of helminths. A representative sample of stomach contents was preserved in 10% formalin for use in a separate ecological study to determine the species of prey consumed. Intestines were then passed between the examiner's thumb and forefinger several times to scrape off any attached worms whilst a visual inspection was made of the mucosa. All contents were washed thoroughly and preserved in 70% ethanol for later microscopical examination. Faecal samples were also collected directly from the large bowel and preserved in 5% Sodium acetate-acetic acid-formalin (SAF) fixative for microscopy and 80% ethanol for molecular procedures.

Gross examination of lymph nodes, liver, adrenal glands, kidneys, urinary bladder, uterus, ovaries, testicles and prostate gland was undertaken with several cuts made through organs to investigate for internal masses. Unfortunately facilities for freezing intestines at -80C were not available in the field however personal protection required for risk group two pathogens was adhered to including gloves, lab coat and eye

protection. A biological safety cabinet was also used when handling faecal matter and soil samples.

3.4.3 MICROSCOPIC EXAMINATION

All specimens were transported to the School of Veterinary and Biomedical Sciences, Murdoch university, Western Australia. Intestinal contents were examined under dissecting and compound microscopes. Positive identification of *A. ceylanicum* was established using those criteria documented in Biocca's (1951) paper on the morphological differentiation of *A. braziliense* and *A. ceylanicum*. Where present, at least fifty individual hookworms were identified before deciding on the species present. In samples where *A. ceylanicum* was detected, all hookworms were identified.

Faecal scats and necropsy-collected faeces were examined by simple smear technique, where faeces were mixed on a slide with a small volume of water, and those samples positive for strongyle eggs noted. The smear technique was chosen due to the high number of samples to be examined and after several floatation and sedimentation techniques failed to reveal any further parasites. Given the high number of positive samples detected it was decided to include all samples for molecular analysis.

3.4.4 GENOMIC DNA EXTRACTION

DNA was extracted directly from faeces using a Promega Maxwell® 16 research instrument system and tissue kit. The final DNA elution was prepared in 300µl of elution solution and stored at -20˚C until required.

In order to confirm morphological identification, male *A. ceylanicum* specimens from two separate animals, and male *A. caninum* specimens also underwent molecular identification. Worms were washed and DNA was extracted using an Epicentre MasterPure™ Complete DNA and RNA Purification Kit according to the manufacturer's instructions.

3.4.5 MOLECULAR METHODS – PCR

A direct PCR assay modified from Traub et al. (2008) was used for the DNA amplification of hookworm species. A forward primer RTHWIF (5'-GATGAGCATTGCWTGAATGCCG-3') and reverse primer RTHWIR (5'-GCAAGTRCCGTTCGACAAACAG-3') were used to amplify an approximately 485 bp and 380 bp section of the internal transcribed spacer-1 (ITS-1), 5.8S and internal transcribed spacer-2 (ITS-2) regions of *Ancylostoma* spp. The PCR assay was prepared in a volume of 25µl consisting of 1 X PCR buffer, 2 mM $MgCl_2$, 0.4 mM of each dNTP, 10 pmol of each primer, 1.0 U *Taq* DNA polymerase (Biotech International, Perth, Australia) and 1 µL of template genomic DNA. Due to the presence of inhibitors, DNA template often needed to be diluted to 1:2 or 1:4 concentration. PCR cycling conditions consisted of a pre-heating step at 95˚C for 5 min. This was followed by 40 cycles of 95˚C for 30 s (denaturing), 60˚C for 30 s (annealing), 72˚C for 30 s (extension), a final extension of 72˚C for 7 min and a holding temperature of 14˚C. Cycling was performed on an Applied Biosystems 2720 Thermal Cycler. The verification of the PCR product was established on a 1.5% agarose gel dyed with SYBR®Safe DNA gel stain (Appendix 6).

3.4.6 DNA SEQUENCING OF CANINE HOOKWORM

DNA sequencing was conducted on all positive samples. PCR products were purified using an Agencourt® AMPure® XP PCR purification kit. DNA was quantified using a spectrophotometer and sequenced using an ABI 3730XL 96 capillary DNA sequencer (Applied Biosystems using Big Dye version 3.1 dye terminators). All chromatograms were viewed using Finch TV Version 1.4.0 (Geospiza Inc.). Dual infections were characterized by the presence of overlapping nuclotide peaks at specific positons in the chromatograms which corresponded to the specific hookworm species. Sequences were compared to a variety of GenBank *Ancylostoma* spp. submissions for similarity.

3.4.7 DATA ANALYSIS

Prevalence was calculated by dividing the number of samples positive for each hookworm species by the total number of samples positive for hookworm in each location (Table 3.1). The significance ($p < 0.05$) of the difference between hookworm species and location was determined using the two-sided Fisher's Exact test (Kirkman, 1996 (accessed 10.05.16).).

3.5 RESULTS AND DISCUSSION

Sixty-three of the 89 scats (70.7%) were PCR positive for hookworm. Of these, 49 samples returned clear and readable sequences. Chromatograms showed low signal strength on repeatedly unreadable samples, this often corresponded with low spectrophotometer DNA readings.

BLAST results showed a 99% or greater homology to previously published sequences with GenBank accession nos. DQ78009 for *A. ceylanicum* and JQ812694 for *A. caninum.* One sample, from northern Cairns, was 100% homologous with Genbank accession no. DQ438069.1 for *A. braziliense.*

From the 26 wild dog intestines examined, only the hookworms *A. ceylanicum* and *A. caninum* were found. BLAST results from sequences of postively identified *A. ceylanicum* samples NSD25 (Fig. 4.1) and NSD26 (both from northern Cairns) were 100% homologous with GenBank accession no. DQ780009 for *A. ceylanicum.* Positively identified *A. caninum* from sample NSD20 (from southern Cairns) was 100% homologous with DQ438073 for *A. caninum* confirming that morphological identification of samples was correct. Other gastrointestinal helminths detected during necropsies were *Toxocara canis* (46%), *Trichuris vulpis* (0.04%) and the tapeworms, *Spirometra erinacei* (46%) and *Dipylidium caninum* (0.04%).

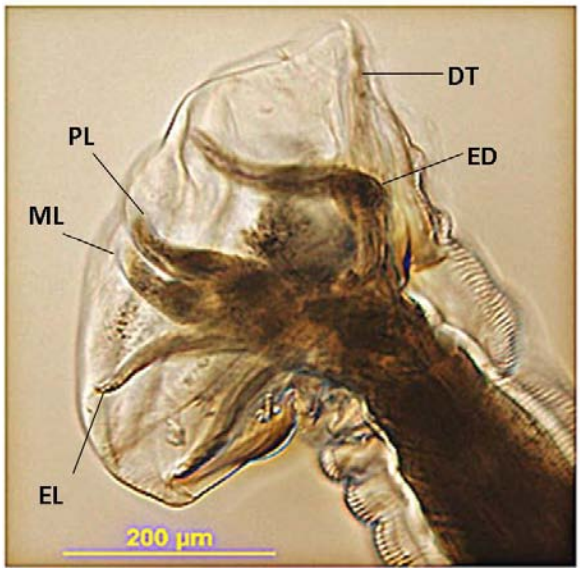

Figure 3.1 Lateral view of male bursa of *A. ceylanicum* clearly showing divergent externolateral ray (EL) and closely associated mediolateral (ML) and posteriolateral (PL) rays. The externodorsal (ED) ray is indicated at the attachment point to the dorsal trunk (DT).

Prevalence results for both scats and necropsies are illustrated in Table 3.1 and Figure 3.2. From the 26 wild dog carcasses examined, all were infected with *A. caninum* (100%) and three (11.5%) contained *A. ceylanicum* and *A. caninum* together as dual infections from northern and southern regions of Cairns whilst Atherton dingoes were solely infected with *A. caninum* (100%). The prevalence of *A. ceylanicum* in wild dog scats was highest at Mt. Windsor NP (100%) followed by northern Cairns (68%) and finally scats from southern Cairns (30.8%). A single infection of *A. braziliense* (4%) was identified in a scat collected from northern Cairns. Fisher's Exact test on frequency analysis showed that prevalence of hookworm species in wild dog scats varied significantly with location ($p = 0.0004$).

Table 3.1 Location and prevalence of hookworm species from wild dog scats and wild dog necropsies.

Parasite	Location	Number of positive samples (% prevalence)			
		Scats*/n		Necropsy†/n	
Ancylostoma ceylanicum	MtWindsor NP	11/11	(100)	-	
	Northern Cairns	17/25	(68)	2/2	(100)
	Southern Cairns	4/13	(30.8)	1/17	(5.9)
	Atherton	-		0/7	(0)
Ancylostoma caninum	MtWindsor NP	5/11	(45.5)	-	
	Northern Cairns	19/25	(76)	2/2	(100)
	Southern Cairns	11/13	(84.6)	17/17	(100)
	Atherton	-		7/7	(100)
Ancylostoma braziliense	MtWindsor NP	0/11	(0)	-	
	Northern Cairns	1/25	(4)	0/2	(0)
	Southern Cairns	0/13	(0)	0/17	(0)
	Atherton	-		0/7	(0)
Dual infections *(A. ceylanicum* and *A. caninum)*	MtWindsor NP	5/11	(45.5)	-	
	Northern Cairns	12/25	(48)	2/2	(100)
	Southern Cairns	2/13	(15.4)	1/17	(5.9)
	Atherton	-		0/7	(0)

*PCR, † Morphological identification.

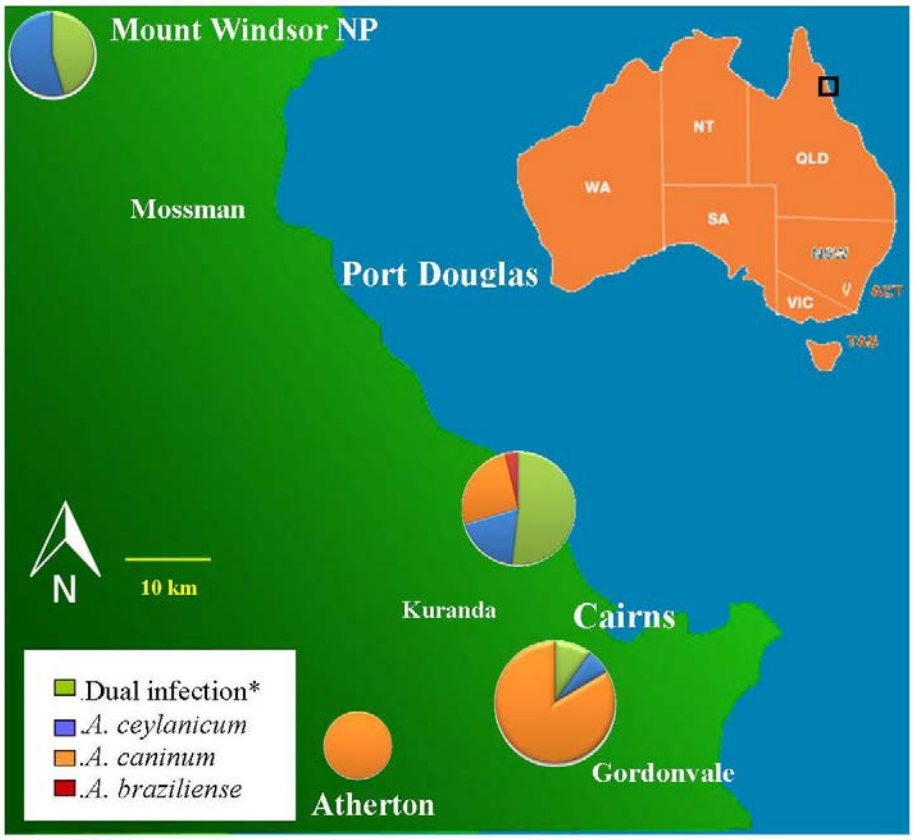

Figure 3.2 Hookworm populations in dingoes and scats collected in north-east Queensland, Australia. (Circle size reflects the number of positive samples collected in the surrounding area; the segments indicate the composition of the population according to species, as represented in Table 3.1). *Dual infection indicates infection with both *A. ceylanicum* and *A. caninum*.

This study reports, for the first time, the presence of the hookworm *A. ceylanicum* in wild canids and the first occurrence of this parasite in Far North Queensland, Australia. It also demonstrates that *A. ceylanicum* is the dominant hookworm species of wild canids in Mt Windsor National Park. This area has a history of logging (Crome et al., 1992), and over the past several decades there is evidence of rainforest recovery and expansion (Tng et al., 2012). A study by Palmer et al. (2007) found *A. ceylanicum* in other regions of Australia and although the survey did include Cairns, Far North Queensland, it looked only at domestic dogs. Previous reports of *A. ceylanicum* in Australia (Adams, 2003; Gasser et al., 1996; Stewart, 1994) were a result of misidentification of *A. braziliense* as *A. ceylanicum* (e Silva et al., 2006; Traub et al., 2007).

Previous studies on pound dogs in Cairns (Setasuban and Waddell, 1973) and wild dogs near Townsville (Brown and Copeman, 2003), approximately 350km south of the current study area, reported the prevalence of *A. caninum* at 100% and 90% respectively. These prevalences are similar to the findings in this study for the dingoes necropsied. (Table 3.1).

Further gastrointestinal helminths, found during necropsies, were *Spirometra erinacei* at a prevalence of 46%. This is consistent with previous findings in North Queensland (Brown and Copeman, 2003). Only one animal (0.04%) was found to be infected with *Dipylidium caninum;* this figure is substantially less than the previous report of 59% by Brown and Copeman (2003). A small number of fleas, *Ctenocephalides felis* and *C. canis*, the intermediate hosts of *D. caninum*, were seen on some of the dingoes. As many of these animals were shot by farmers during pest control operations, they may have been left exposed for a period of time after death, whereupon fleas would leave the carcass as it cooled.

Interestingly, the zoonotic tapeworm, *Echinococcus granulosus* was not found in animals in this study. Banks et al. (2006), previously identified a pocket of high cystic echinococcosis infection in cattle on the Atherton Tableland, therefore, the parasite is present in the region. As the main definitive host of *E. granulosus,* dingoes have often been described with high worm burdens (Baldock et al., 1985). Wallabies, abundant in the region, have previously proven to be the most susceptible macropod to hydatid cyst infection (Durie and Riek, 1952), and can form a major dietary constituent of dingoes. As the majority of my study animals were under two years of age, it is possible that they generally targeted smaller prey such as rats (*Rattus rattus*), bandicoots (*Perameles nasuta*) and in one pup's unfortunate case, an echidna (*Tachyglossus aculeatus*), all of which are not thought to carry hydatid infection.

The detection of *Toxocara canis* at a prevalence of 46% is of some concern due to the zoonotic potential of this parasite. *T. canis* is predominantly a parasite of young dogs (Kelly, 1977), therefore, an increased prevalence in my study animals may be expected. The single finding of the canine whipworm, *Trichuris vulpis,* is believed to be the first report of this parasite in a wild dog in Far North Queensland.

A. braziliense is known to occur in North Queensland (Beveridge, 2002; Stevenson and Hughes, 1980) and the finding of a single infection from a scat is consistent with previous reports of low prevalences in the region (Heydon, 1929; Setasuban and Waddell, 1973).

A recent paper by Conlan et al. (2011) described *A. ceylanicum* as the most neglected of all human hookworm species. Some studies have reported insignificant findings in several clinical studies involving *A. ceylanicum* infection in healthy well-fed adults (Carroll and Grove, 1986; Wijers and Smit, 1966). However, these findings should be balanced by reports where significant clinical disease has been seen such as that reported by Anten and Zuidema (1964) suggesting that severity is dependent on host and environmental factors as occurs with most diseases.

Dingoes used in this study were generally 'problem' animals killed in council control operations. They were frequently found to be utilising areas close to people and farms and therefore may have been in close contact with domestic dogs in which *A. caninum* is the dominant hookworm infection. In contrast, the majority of wild dog scats collected were found further afield in National Parks and rural areas, away from human habitats and in areas dominated by rainforest or green corridors (areas of habitat designated as wildlife corridors between regions). This sampling bias may be one of the reasons for the higher prevalence of *A. ceylanicum* found in scats. Also, although some care was taken to collect scat samples following rainy periods to ensure they were recently deposited, the collection of multiple scats from the same individuals cannot be ruled out. Therefore, some differences may be due to sampling, and infection incidences in various locations, may not be as high as those stated.

The results showed a significant difference between species of hookworm present in scats and location. These differences cannot be attributed to reasons such as season or altitude as *A. ceylanicum* was found in scats collected year round and at elevations ranging from seven meters above sea level (Barron) up to approximately 1000 meters (Mt. Windsor NP). Again, higher frequencies of *A. ceylanicum* were present in scats collected from more remote locations (100% from Mt. Windsor NP). It is possible, therefore, that *A. ceylanicum* is the more abundant hookworm in areas of rainforest vegetation and that in locations where domestic dogs, the reservoir host of *A. caninum*,

are present there may be a spill-over of infection from domestic dogs that influences the hookworm species present in the wild dog population.

Given that *A. ceylanicum* has previously been found in cats and wild felids, further investigation is necessary to evaluate the hookworm population of domestic and feral cats in the region. Future studies should also concentrate on animals in potentially high risk Indigenous communities to determine the extent of *A. ceylanicum* infection in Far North Queensland and to assess the risk of zoonotic transmission and infection.

The zoonotic potential of this parasite should not be underestimated. Indigenous communities are at particular risk because of the limited management of domestic dog health and the presence of free-roaming community dogs that can be exposed to parasite eggs and larvae in soil contaminated by wild dogs. Together with the warm, moist conditions of the tropics this provides an ideal scenario for the success of soil-transmitted helminth infections.

3.7 ACKNOWLEDGMENTS

I would like to thank CSIRO Atherton, Cairns Council and Damian Morrant for assistance with sample collection. I received funding assistance from the Australian Society of Parasitology researcher exchange training and travel award. I would also like to thank Aileen Eliott, Louise Pallant and Angela Reeves for their advice and expertise with microscopic and PCR analysis.

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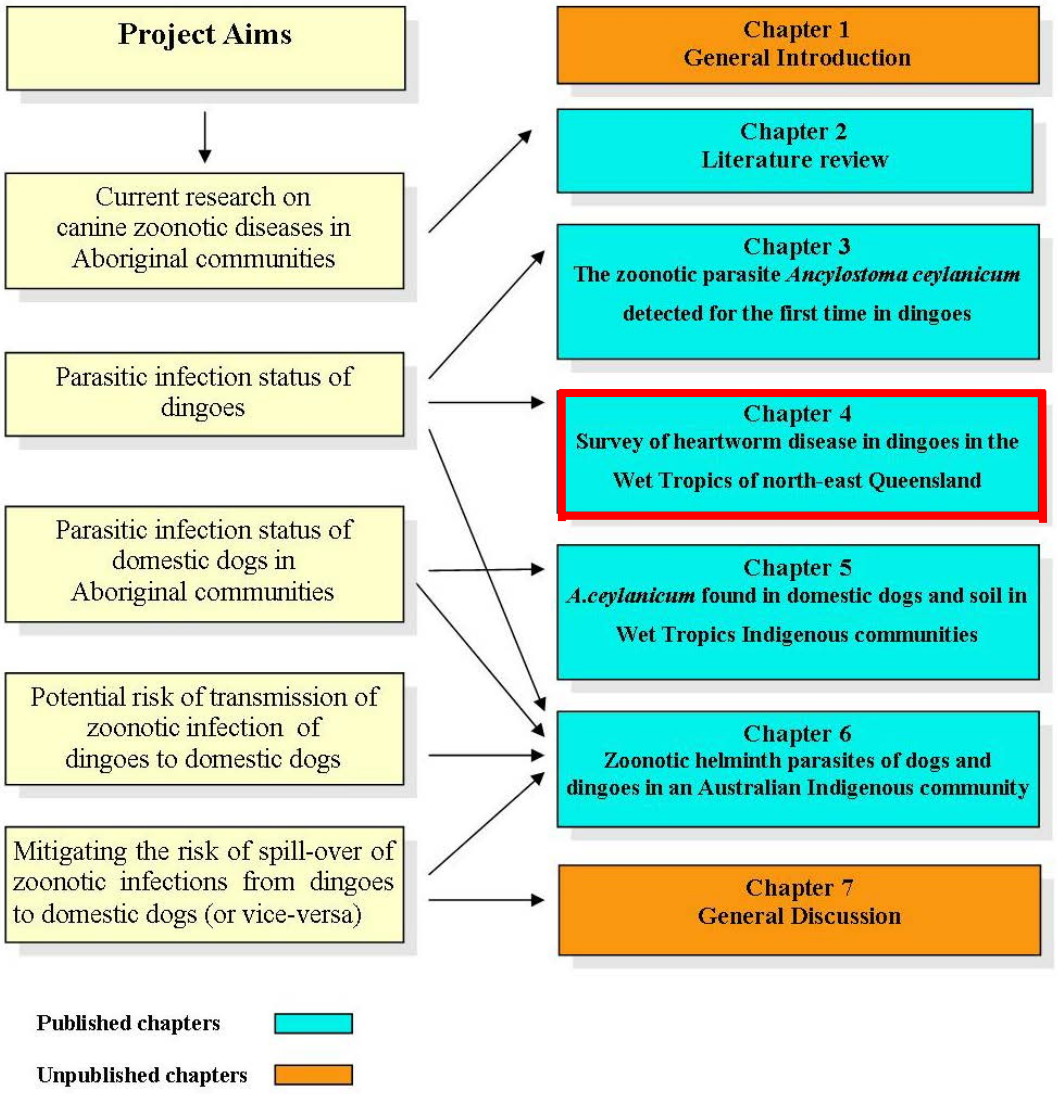
Supporting information:

Appendix 6: Photos of hookworm agarose gel electrophoreses with dilutions from lab book.

CHAPTER 4.

Survey of heartworm disease in dingoes in the Wet Tropics of north-east Queensland. This chapter follows on from chapter 3 and again addresses thesis aim (2). I specifically investigated *Dirofilaria immitis* in dingoes in the region as I suspected an elevated prevalence of disease from previous studies and from the many positive heartworm infections found in dingoes I had necropsied. Through personal communications with several local veterinarians I was told of a distinct lack of infection in domestic dogs around Cairns. Thus, I found that dingoes serve as a reservoir host for this parasite in areas of low density housing.


Project Aims
Current research on canine zoonotic diseases in Aboriginal communities
Parasitic infection status of dingoes
Parasitic infection status of domestic dogs in Aboriginal communities
Potential risk of transmission of zoonotic infection of dingoes to domestic dogs
Mitigating the risk of spill-over of zoonotic infections from dingoes to domestic dogs (or vice-versa)
Chapter 1 General Introduction
Chapter 2 Literature review
Chapter 3 The zoonotic parasite Ancylostoma ceylanicum detected for the first time in dingoes
Chapter 4 Survey of heartworm disease in dingoes in the Wet Tropics of north-east Queensland
Chapter 5 A.ceylanicum found in domestic dogs and soil in Wet Tropics Indigenous communities
Chapter 6 Zoonotic helminth parasites of dogs and dingoes in an Australian Indigenous community
Chapter 7 General Discussion
Published chapters
Unpublished chapters

Published - paper 3

Citation: Smout, Felicity A., Skerratt, L.F., Butler, J., Johnson, C. & Congdon, B. (2016). Dingoes (*Canis dingo* Meyer, 1793) continue to be an important reservoir host of *Dirofilaria immitis* in low density housing areas in Australia. Veterinary Parasitology 215: 6-10.

TITLE: DINGOES (*CANIS DINGO* MEYER, 1793) CONTINUE TO BE AN IMPORTANT RESERVOIR HOST OF *DIROFILARIA IMMITIS* IN LOW DENSITY HOUSING AREAS IN AUSTRALIA.

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4.1 ABSTRACT:

Heartworm (*Dirofilaria immitis*) is a parasitic nematode responsible for canine and feline cardiopulmonary dirofilariasis and human zoonotic filariosis in both tropical and temperate regions throughout the world. Importantly, this study in the Wet Tropics of Far North Queensland found *D. immitis* remains at high prevalence (72.7%) in wild dingoes in low density housing areas in Australia. This prevalence is equivalent to the highest levels seen in wild dogs in Australia and represents an ongoing risk to domestic dogs, cats and humans. In contrast, in higher density residential areas prevalence was significantly lower (16.7%, p=0.001). It is possible that chemotherapeutic heartworm (HW) prevention in domestic dogs in these higher density housing areas is helping to control infection in the resident dingo population. Five dingoes killed in council control operations around Atherton, a non-endemic HW region in the Wet Tropics, were all negative for HW likely due to the colder climate of the region restricting transmission of the disease. This survey highlights the importance of dingoes as reservoir hosts of HW disease and that the subsequent risk of infection to companion animals and humans depends on local factors such as housing density, possibly linked to chemotherapeutic HW control in domestic dogs and climate. My findings show that veterinary clinicians need to ensure that pet owners are aware of HW disease and do not become complacent about HW chemoprophylaxis in areas which support dingo populations.

4.2 KEY WORDS: *Dirofilaria immitis*, Dingo, Canine, Heartworm, Zoonosis.

4.3 INTRODUCTION:

Very little is known about the epidemiology of heartworm (HW) disease caused by the filarial nematode, *Dirofilaria immitis*, within wild and rural domestic dog (or human) populations. Concerns have been raised that wild canids constitute an important on-going reservoir for HW and other parasitic diseases that may be transmissible to domestic dogs and humans (Polley, 2005). Reports of canids that act as reservoirs for HW infection include jackals, foxes and wolves in Serbia (Penezić et al., 2014) coyotes in California (Sacks, 1998), the red fox in Australia (Marks and Bloomfield, 1998;

Mulley and Starr, 1984) and the dingo in the dry tropics of northern Australia (Brown and Copeman, 2003; Starr and Mulley, 1988).

Dirofilaria immitis is a serious and potentially life-threatening parasite of canines and felines. It is responsible for canine cardiopulmonary dirofilariasis, otherwise known as HW disease, in both tropical and temperate regions throughout the world. *D. immitis* infections are widespread in those regions of Australia where the climate is suitable for mosquito vectors of the genera *Aedes, Culex* and *Anopheles* (Welch et al., 1979).

Human infections are also possible. Many reported cases are asymptomatic but pulmonary infection may cause radiological coin lesions of the lung (Rena et al., 2002; Ro et al., 1989; Theis, 2005). This can result in radiological misdiagnosis of a primary or metastatic lung tumour, leading to invasive procedures to achieve a definitive diagnosis (Lee et al., 2010; Theis, 2005). Ocular dirofilariasis caused by *D. immitis* has also been reported in Australia (Moorhouse, 1978).

Historically, the presence of this nematode in domestic dogs and dingoes (or wild dogs) is well documented in Australia (Brown and Copeman, 2003; Carlisle and Atwell, 1984; Coman, 1972; Dunsmore and Shaw, 1990; Kelly, 1977; Starr and Mulley, 1988). However, there is a marked lack of recent literature on current distribution and prevalence, with most surveys dating back to the 1970s and 1980s. Previously, the prevalence of HW was reported to have been as high as 77% in domestic dogs from Townsville, a growing city of several hundred thousand people located in the dry tropics region of north Queensland (Aubrey and Copeman, 1972), but this decreased to about 15% in adult pound dogs in 2001 (Brown and Copeman, 2003). This decline is thought to have been due to the widespread use of effective prophylaxis in domestic dogs with macrocyclic lactones first used in Australia in 1994 (Brown and Copeman, 2003; Holm-Martin and Atwell, 2004). However, a subsequent high prevalence of 75% in Townsville in 2003 contradicts this explanation (Brown and Copeman, 2003) and highlights the need for a greater understanding of the epidemiology of HW.

Very high prevalences of *D. immitis* infections have been reported in domestic dogs from far north Queensland Aboriginal communities on the western side of Cape York Peninsula, with 88% and 90% in Kowanyama and Aurukun, respectively (Welch et al., 1979). A correspondingly high prevalence of anti-*D. immitis* antibodies and relatively elevated antibody titres were seen in the human population (Welch and Dobson, 1974). These communities have limited or no access to veterinary care and minimal management of domestic dog health.

Little is known about the transmission of HW from dingoes to domestic dogs and potentially humans. The availability of standing water used by mosquito vectors is thought to be the most important risk factor and is influenced by temperature and rainfall (Carlisle, 1969). Previous studies have found that heartworm infection is most common in domestic dogs living in areas close to permanent bodies of water, where mosquito populations are high (Welch et al., 1979). However, the abundance of a pathogen in a reservoir host is known to vary with population-specific factors such as demography and behaviour (Carlisle, 1969).

Canine HW infection can be effectively prevented by chemoprophylactic treatment of animals (Boreham and Atwell, 1983). Over the past two decades successful programs have been available to domestic dogs resulting in reduced reports of infection. A recent study on HW infection in dog shelters across South Australia, New South Wales and Queensland reported prevalences ranging from 0 to 2.2% (Mitchell, 2012).

Therefore, in order to better predict the current and potential on-going threat of HW infection it is important to understand its epidemiology in wild canids. The first step in assessing the potential HW threat posed by dingoes to both domestic dogs and humans is to establish prevalence in wild populations under a range of ecological conditions. This study presents the results of the first survey for *D. immitis* in dingoes in the Wet Tropics of Far North Queensland, Australia; a potential high-risk region where the combination of high rainfall, humidity and temperature are favourable to increased mosquito populations. These results are compared with those found for other dingo populations previously surveyed in locations in northern Queensland.

4.4 MATERIALS AND METHODS

Australia's largest land predator, the dingo (*Canis dingo* Meyer, 1793) is distributed widely in all states of Australia with the exception of Tasmania. European settlement has led to hybridisation of dingoes and domestic dogs and this has resulted in fewer genetically pure bred wild dingoes in many areas (Ritchie et al., 2012; Woodall et al., 1996). I have chosen to use the term dingo for all animals referred to in this study as all resembled dingoes morphologically, including features such as a larger palatal width, longer rostrum, shorter skull height and wider top ridge of skull when compared with domestic dogs (Crowther et al., 2014; Newsome et al., 1980).

4.4.1 STUDY AREA AND COLLECTION OF SPECIMENS

The study was conducted in two sections of the Wet Tropics World Heritage Area in north-eastern Queensland, Australia: the Cairns coastal region and Atherton Tablelands, a mid-elevation plateau (600-900 m). Cairns has a tropical climate with strongly seasonal rainfall of approximately 2,000 mm annually. Mean monthly temperatures range from 20̊ to 29˚ C. Eleven dingoes were trapped in the Cairns coastal region between October 2010 and May 2013. These animals were trapped in natural forested areas, often bordering cane farms, away from houses and will be referred to as 'wild' dingoes for the purposes of this study. Five mls of whole blood were collected from the jugular vein of each animal and stored in tubes containing EDTA anti-coagulant under refrigeration. As these 11 dingoes were then fitted with collars and enlisted in a GPS tracking study, necropsies were not performed.

Seventeen dingo carcasses ranging in age from seven months to greater than five years were supplied for necropsy by Cairns Regional Council animal control officers and local landholders. These dogs were killed during control programs, in or adjacent to, agricultural farming land and/or the outer suburbs of northern and southern Cairns and Atherton from 2007 onwards and will be referred to as 'urban fringe' dingoes for this study. No dingoes were specifically killed for this study. All protocols were reviewed and approved by James Cook University Animal Ethics Committee (Approval no. A1546).

4.4.2 NECROPSY FOR ADULT D. IMMITIS

At necropsy, the heart and lungs were excised and right ventricle and pulmonary arteries were examined without magnification for pathology and the presence of adult *D. immitis.* Other organs were also examined for evidence of ectopic migration of adult HW. Further details such as age, sex and body condition score of the animals were recorded.

4.4.3 DETECTION OF ADULT D. IMMITIS ANTIGEN

Blood samples from trapped animals were tested for circulating antigen, as per manufacturer's instructions, using a commercial ELISA SNAP test kit for heartworm (IDEXX Laboratories Inc., Rydalmere, NSW.): Antigen tests are now the test of choice for heartworm detection because of their high specificity (97%) and sensitivity (84%) (Atkins, 2003).

4.4.4 BLOOD SMEARS

Thin blood smears from the 11 wild dingoes were also stained with Diff Quik and examined under a light microscope to further test for the presence of microfilariae. *D. immitus* microfilariae were identified morphologically and distinguished from *Acanthocheilonema* (syn. *Dipetalonema*) *reconditum*, a filarial parasite of the subcutaneous tissues and fascia of canids, according to existing descriptions (Kelly, 1973; Sawyer et al., 1963). *Dirofilaria repens* is not known to occur in Australia (Stringfellow et al., 2002).

4.4.5 DATA ANALYSIS

Prevalence was calculated by dividing the number of samples positive for heartworm by the total number of samples tested for heartworm in each age group and sex (Table 4.1). The significance ($p < 0.05$) of the difference in probability of infection between wild dingoes and urban fringe dingoes, sex and age were determined using the two-sided Fisher's exact test (Kirkman, 1996 (accessed 10.05.16).).

A fifty kilometre square area surrounding the site where the animal was trapped or killed was evaluated for level of human 'footprint' or development-density of housing and farm buildings present. Development-density was compared at locations where dingoes were sampled according to the HW infection status of dingoes using an independent samples t-test. These comparisons were carried out using IBM SPSS Statistics for Windows, Version 19.0. (Armonk, NY: IBM Corp).

4.5 RESULTS

Twenty-eight dingoes were sampled from three areas within the Wet Tropics, Cairns urban fringe (n=12), adjacent natural forest areas surrounding Cairns (n=11) and Atherton Tablelands, approximately 100 km distant to Cairns (n=5). Overall, 10 of 28 dingoes were infected with HW (36%, 21%-54% Wilson C.I.) from the Cairns and Atherton regions. All 5 dingoes killed in Atherton were negative for adult HW.They consisted of two males and one female under two years of age and one male and one female over two years old.

From around Cairns, eight of 11 live wild dingoes (72.7%) were positive for *D. immitis* as indicated by an antigen ELISA test, four females under two years old and four males over two years old, (Table 4.1, Figure 4.1). Seven of the eight test positive dingoes (87.5%) demonstrated low circulating antigen levels and one, a three year old male, had a high antigen titre. Positive ELISA test results were confirmed via visual identification of microfilaria in thin blood smears. All animals were trapped from October 2010 to May 2013 and appeared to be well muscled and in a healthy condition, although three lactating females were noted to be slightly underweight, likely due to the energy expenditure involved in milk production and reduced time for feeding while caring for pups.

Table 4.1 Prevalence of heartworm infection in wild and urban fringe dingoes by age and sex from the Cairns region. Five negative dingoes from Atherton were not included in the table.

		Number of positive samples (% prevalence)					
Age	**Sex**	**Wild*/n**		**Urban fringe†/n**		**Total/n**	
< 2 years old	Male	0/1	-	0/1	-	0/2	-
	Female	4/5	(80)	0/2	-	4/7	(57.1)
Sub-total		**4/6**	**(66.7)**	**0/3**	**-**	**4/9**	**(44.4)**
> 2 years old	Male	4/4	(100)	1/6	(16.7)	5/10	(50)
	Female	0/1	-	1/3	(33.3)	1/4	(25)
Sub-total		**4/5**	**(80)**	**2/9**	**(22.2)**	**6/14**	**(42.9)**
Total		**8/11**	**(72.7)**	**2/12**	**(16.7)**	**10/23**	**(43.5)**

*Snap test and microfilaria morphology, † Adult nematode morphological identification.

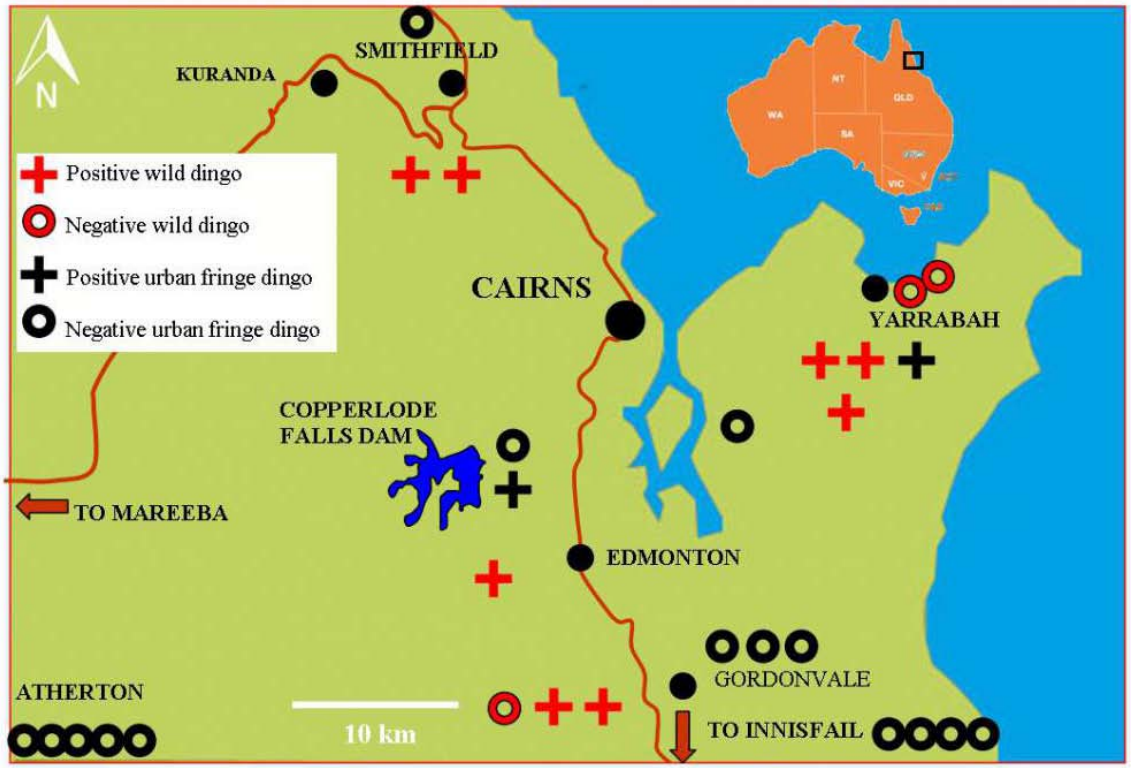

Figure 4.1 Heartworm in dingoes in north-east Queensland, Australia.

Two (16.7%) of 12 urban fringe dingoes killed in the Cairns region, a male and a female both over two years old, were infected with adult HW as determined by necropsy (Table 4.1). No evidence of pathology was seen in the right ventricle or pulmonary artery where the worms were located. This may be due to the relatively light infection with fewer than ten adult worms seen in each animal. Both male and female nematodes were collected from the two animals.

HW infection was significantly more likely in wild compared with urban fringe dingoes in the Cairns region, OR = 13.3 (1.3 – 172.6, 95% C.L.) ($p = 0.01$, Fisher's Exact Test). No significant difference was seen for sex ($p > 0.99$) or age ($p = 0.67$) in relation to infection status with a two-tailed Fisher's exact test.

A comparison using all Cairns region sample sites showed no significant difference in the mean urban housing development-density at which infected or non-infected animals occurred (Independent t test: $t_{21} = 1.89$, $p = 0.17$, $n=23$). I then repeated this analysis removing the Mt Sheridan sampling location (n=2, one infected and one non-infected

dingo). This site was anomolous in that pockets of recent high-density urban development abutted large expanses of national park or state forest. For this reason Mt Sheridan was not able to be catagorized as either a low- or high-density site. My second analysis found that throughout the remaining sampling locations infection occurred at a significantly lower mean urban housing development-density (Independent t test: t_{19}= 2.53, p = 0.023, n=21) and that variation in the density of development was greater at uninfected sites (Levene's test: $F_{1, 19}$ = 14.10, p = 0.001, n=21). Combining these results suggests that it is possible for an animal to be free from infection at any given human housing density, but that infections occur significantly more often when the density of human development is below an area of ~4 km^2/50 km^2 total area.

4.6 DISCUSSION.

Overall, the prevalence of HW disease in dingoes of the Far North Queensland Wet Tropics coastal region is moderate. In dingoes caught outside of urban areas it is equivalent to the highest levels seen in wild dogs in Australia. My result of 72.7% HW prevalence in wild trapped dingoes from Cairns region is similar to that reported in a previous study on wild dogs undertaken near Townsville, ~350km south of the current study area (prevalence of 75%) (Brown and Copeman, 2003). Townsville is located in the dry tropics and has an average annual rainfall of 1143 mm compared with 2000 mm in Cairns.

If average rainfall and its influence on the availability of standing water are important determinants of disease prevelance then these two results seem somewhat paradoxical. However, above average rainfall was recorded for the several years leading up to the Brown and Copeman (2003) study including the wettest year on record with 2400 mm of rain in 2000 (Australian Bureau of Meteorology (BoM)). This increased rainfall may have contributed to the elevated HW prevalence seen in the Townsville region through providing a better breeding climate for vector mosquitoes. A study of animals trapped in the same area of Townsville two years later, following several years of closer to average annual rainfall, recorded a HW prevelence of only 12.5% in adult wild dogs (Mochankana, 2005). A similar effect of climate on HW prevalence was shown in

Brisbane in 1968 where the prevalence of infection was approximately double that reported in previous studies following a particularly wet summer when mosquito abundance was high and prolonged (Carlisle, 1969).

I observed a lower HW prevalence of 16.7% in urban fringe dingoes that were considered to be 'problem' animals and were killed by landholders and council in dog control operations. They were frequently found to be utilising areas close to people and farms where domestic pets may have had chemoprophylactic HW prevention and therefore may have spent less time where infected mosquito vectors are present. In contrast, the majority of wild trapped dingoes were found further afield, in National Parks and rural areas, away from human habitats, and in areas dominated by rainforest or green corridors (areas of habitat designated as wildlife corridors between regions). This may be one of the reasons for the higher prevalence of *D. immitis* in wild trapped animals. An evaluation of the area of human "footprint" or housing development-density surrounding the site where the animal was trapped or killed supports this finding; there being a general trend towards a higher probability of infection where density of human development was below an area of ~4 km^2/50 km^2.

Studies have suggested that infection is usually greater in older male dogs (Carlisle, 1969; Welch et al., 1979). Consistent with these findings, my results showed a greater degree of infection in males aged over two years compared with younger males. I also saw an increased rate of infection in younger females however none of these findings were significant. Of these four females, three were lactating and subsequent tracking and surveillance located their dens. These were in areas of dense vegetation and close to a permanent water source. Previous reports have found that heartworm infection is most common in dogs living in such areas (Welch et al., 1979).

I did not find any HW infection in the five urban fringe dingoes from the Atherton Tablelands, where HW disease is not considered endemic (Carlisle and Atwell, 1984). Atherton has an average daily minimum below 14° C for six months of the year. Thus, the lack of disease in the region is consistent with the colder climate at this higher

elevation. Fortin and Slocombe (1981) found that *D. immitis* larvae do not develop in mosquitoes at temperatures below 14˚ C.

However, reports of canine HW in traditionally non-endemic areas (Bowman et al., 2009; Otranto et al., 2009; Simón et al., 2005) are becoming increasingly common. Environmental and ecological changes as a result of human and natural disturbances can be expected to continue to influence the spread of zoonotic parasitic diseases (Patz et al., 2000). Genchi et al.(2005) have suggested that if the climatic trend of increasing temperatures, humidity and rainfall continues, filarial infection, including HW, will continue to spread into previously infection free areas of Europe as has already occurred in northern regions of the United States of America and Canada (Zimmerman et al., 1992).

A recent study of mosquitoes in north Queensland rainforests found their abundance and diversity was highest in lowland areas, where mean annual temperatures were high. This suggests that global warming will increase the diversity and abundance of disease vectors at all elevations (Hilbert, 2010). Urban expansion and the replacement of rainforests with human modified lands dramatically increases the abundance of sun-loving mosquitoes such as *Anopheles*, vector mosquitoes for *D. immitis* (Yasuoka and Levins, 2007). Creating grasslands adjacent to rainforests may increase the susceptibility of species in both habitats to novel disease transmission by increasing the vector community on rainforest edges (Steiger et al., 2012).

The results from the wild trapped dingoes presented here are expected for animals that are not receiving any form of preventative HW treatment and are residing in habitats suitable for large numbers of mosquito vectors. These animals may constitute a significant source of infection for susceptible dogs and humans. Anthropogenic factors such as urban expansion into previously undeveloped areas continue to bring domestic animals and humans into increasing contact with wildlife reservoirs of zoonotic pathogens (Daszak et al., 2007).

Currently the risk from HW appears to be mitigated by regular chemoprophylactic therapy of domestic animals which reduces the number of infected mosquito vectors in

the area and lowers the risk of infection to domestic pets and people. This prevention therapy may also have a spill-over effect and consequently reduce the risk of HW infection in dingoes living in the same region. However, a note of caution is warranted, a lack of recent *D. immitis* prevalence studies in far north Queensland together with two decades of successful chemoprophylaxis programs resulting in reduced reports of infection by Veterinary clinicians in Cairns (F. Smout, pers. comm.) may lead to complacence and reduced compliance of pet owners to seek preventative treatment.

Veterinarians understandably play a vital role in the 'One Health" approach to disease prevention (Baneth, 2012). My findings show that veterinary clinicians need to continue to educate pet owners about HW disease risk and advise owners on HW chemoprohylaxis in areas which support dingo populations. An annual HW chemoprophylaxis program for pets, with a yearly injection, rather than a daily or monthly program may serve to improve owner compliance and ensure HW protection for both pets and their owners (Rohrbach et al., 2011).

4.7 CONFLICT OF INTEREST STATEMENT

The authors have no financial or personal relationship with other people or organisations that could have inappropriately influenced their work.

4.8 ACKNOWLEDGMENTS

The authors would like to thank CSIRO Atherton, Cassowary Coast Regional Council, Yarrabah Aboriginal Shire Council, Cairns Regional Council animal control officers, Matt Birch and Peter Box, Damian Morrant, Dr. Sarah Gill, Alfred Gray Jnr, Stephen Canendo, Shanna Mossman and Herman Sexton for assistance with sample collection and dingo trapping. Partial financial support was also provided by the Australian Research Council, Skyrail Rainforest Foundation, Terrain NRM and Australian Rainforest Foundation.

4.9 ADDENDUM

In light of ever increasing anthelmintic resistance in dogs, untreated dingoes may serve as an important refugium of unexposed parasites which may help to dilute out anthelmintic resistant strains (Pers. comms. E. Jenkins). Also, the practice of mosquito larviciding in and around settlements was not investigated .This possibility could also account for the finding of lower prevalence in urban fringe dingoes.

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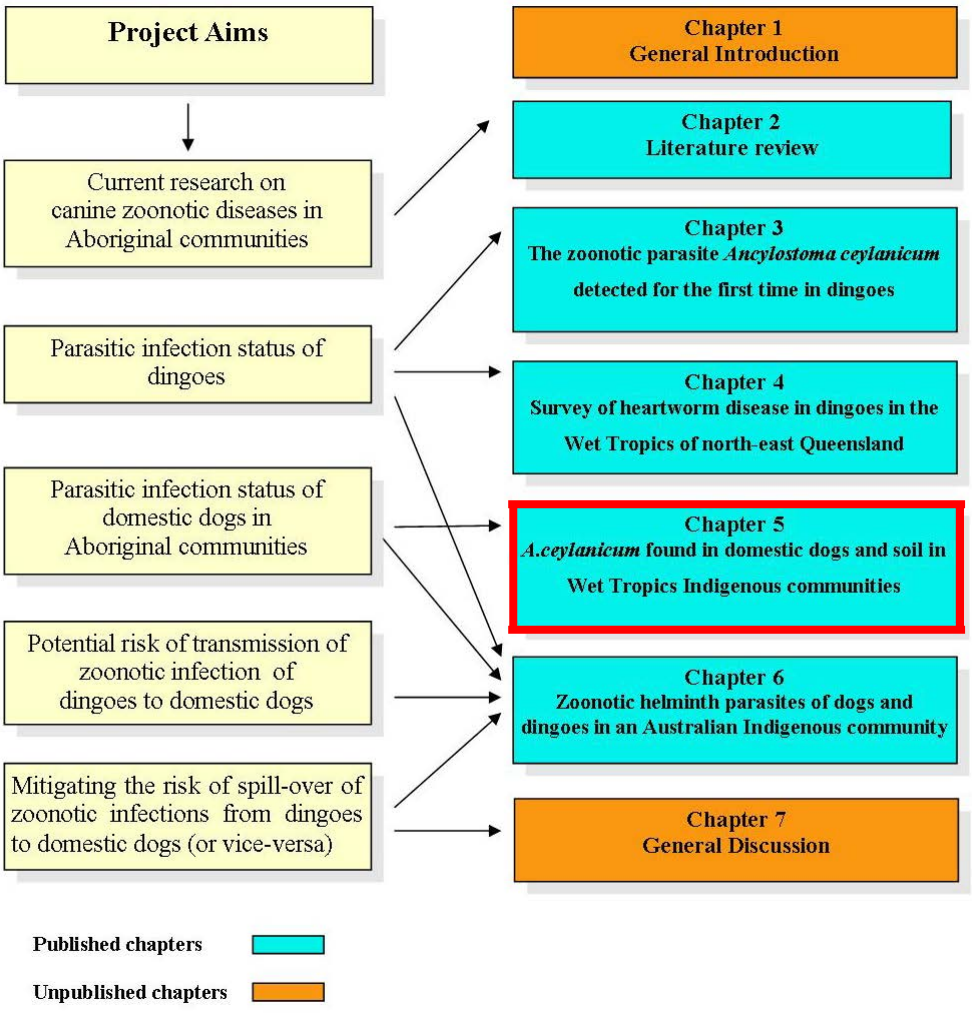
CHAPTER 5.

Survey of helminth infections in dogs and soil in Wet tropics Indigenous communities. After establishing that zoonotic infections were present in the dingoes locally I needed to investigate domestic dogs. In this chapter I address one aim (3). By investigating the status of zoonotic infections in dogs and soil samples from several Indigenous communities and popular tourism locations in the Wet Tropics I was able to ascertain, for the first time, that the parasite *A. ceylanicum* was present in domestic dogs in an Indigenous community and in soil at tourism sites and was a substantial public health risk in the region



Published - paper 4

Citation: Smout, Felicity A., Skerratt, L.F., Butler, J., Johnson, C., Congdon, B.. & Thompson, R.C. (2017). The hookworm *Ancylostoma ceylanicum*: An emerging public health risk in Australian tropical rainforests and Indigenous communities. One Health 3: 66-69.

Graphical abstract

TITLE: THE HOOKWORM *ANCYLOSTOMA CEYLANICUM*: AN EMERGING PUBLIC HEALTH RISK IN AUSTRALIAN TROPICAL RAINFORESTS AND INDIGENOUS COMMUNITIES.

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5.1 ABSTRACT:

Ancylostoma ceylanicum is the common hookworm of domestic dogs and cats throughout Asia, and is an emerging but little understood public health risk in tropical northern Australia. I investigated the prevalence of *A. ceylanicum* in soil and free-ranging domestic dogs at six rainforest locations in Far North Queensland that are Indigenous Australian communities and popular tourist attractions within the Wet Tropics World Heritage Area. By combining PCR-based techniques with traditional methods of hookworm species identification, I found the prevalence of hookworm in Indigenous community dogs was high (96.3% and 91.9% from necropsy and faecal samples, respectively). The majority of these infections were *A. caninum.* I also observed, for the first time, the presence of *A. ceylanicum* infection in domestic dogs (21.7%) and soil (55.6%) in an Indigenous community. *A. ceylanicum* was present in soil samples from two out of the three popular tourist locations sampled. My results contribute to the understanding of dogs as a public health risk to Indigenous communities and tourists in the Wet Tropics. Dog health needs to be more fully addressed as part of the Australian Government's commitments to "closing the gap" in chronic disease between Indigenous and other Australians, and encouraging tourism in similar locations.

5.2 KEYWORDS: *Ancylostoma ceylanicum*, dogs, canine, Aboriginal, hookworm, zoonosis

5.3 INTRODUCTION:

Human hookworm infections have been attributed mainly to *Necator americanus* and *Ancylostoma duodenale* (Bethony et al., 2006; De Silva et al., 2003) while *Ancylostoma ceylanicum*, a common hookworm of domestic dogs and cats throughout Asia (Conlan et al., 2012; Traub et al., 2007; Yoshida et al., 1968), has been largely ignored. This is despite knowledge that *A. ceylanicum* can cause patent enteric infections in humans (Anten and Zuidema, 1964; Bonne, 1937; Carroll and Grove, 1986). Concern about this

parasite in tropical Australia has been growing following its recent discovery in humans in Western Australia (Koehler et al., 2013), domestic dogs in Western Australia, Victoria, Queensland and the Northern Territory (Palmer et al., 2007) and dingoes in Far North Queensland (chapter 3) (Smout et al., 2013).

The Wet Tropics bioregion of Far North Queensland contains remnant rainforest which holds globally-significant biodiversity and cultural values, and these are recognised by its designation as the Wet Tropics World Heritage Area (WTWHA) (WTMA, 2004, 2013). The WTWHA is a major tourist attraction and many locations within or on its periphery are both culturally important for Indigenous communities and also visited by tourists (Gratani et al., 2014; Gratani et al., 2016; Sangha et al., 2011). Free-ranging domestic dogs and dingoes (or 'wild dogs') are widespread, and interact in close proximity to people in the region (chapter 4) (Smout et al., 2016). Indigenous Australians in tropical communities are at particular risk from *A. ceylanicum* and *A. caninum* infection due to the limited health management of domestic dogs and the presence of free-roaming community dogs that may have been exposed to parasite eggs and larvae in soil contaminated by dingoes (chapter 3) (Smout et al., 2013). Along with the faecal oral route for infection, the larvae can also penetrate the skin of humans, as well as their canine or feline host. Therefore people coming into contact with contaminated soil or sand also risk infection (Robertson and Thompson, 2002). Consequently, when developing public health protocols in Indigenous communities the role of the dog in the transmission of hookworm infection to humans should also be considered, since successful control of infection may require better management and treatment of dogs. Chemotherapy focusing on the human population alone is unlikely to be successful (Thompson and Conlan, 2011).

The recent development of advanced, PCR-based techniques capable of differentiating between hookworm species using DNA isolated from eggs in faeces and soil (Traub et al., 2008) enables a better understanding of the epidemiology of *A. ceylanicum* infection. Here, I investigate the geographical distribution of *A. caninum* and *A. ceylanicum* in tropical Far North Queensland using this latest technology. I report for the first time the presence of *A. ceylanicum* in domestic dogs and soil in Indigenous communities, and locations also frequented by tourists.

5.4 MATERIALS AND METHODS:

5.4.1 STUDY AREA AND COLLECTION OF SPECIMENS

The study area was conducted at six localities within the WTWHA of north-east Queensland, Australia. The region was further sub-divided into six localities, three Indigenous communities (Mossman, Yarrabah and Jumbun) and three tourist locations (Mossman Gorge, Lake Placid and Murray Upper) (Figure 5.1).

Figure 5.1 The Wet Tropics World Heritage Area in Far North Queensland, and the location of study sites.

In total, 130 soil samples were collected from locations within the study sites with high concentrations of human activity such as around picnic tables, water holes and school yards and playgrounds. Soil samples collected from Jumbun community were omitted from this study due to inconclusive results. Samples were collected with disposable wooden paddles and placed in individual zip locked plastic bags to avoid cross contamination. Eighty-six faecal samples were collected from free-ranging domestic dogs in these sites in November and December 2011 (Table 5.1). In addition, 27

domestic dog carcasses were supplied by Yarrabah Aboriginal Council Animal Control Officers between December 2010 and December 2011. No domestic dogs were killed specifically for this study. All of the specimens were necropsied immediately and faecal samples collected. All protocols were reviewed and approved by James Cook University Animal Ethics Committee (Approval no. A1546).

5.4.2 NECROPSY TECHNIQUE AND PARASITE PRESERVATION

The stomach and intestines of the 27 domestic dog carcasses (9 males and 18 females) ranging in age from 10 weeks to over eight years of age (mean age approximately 2.5 years), were excised. The stomach, small intestine, and large intestine were each ligated at the junctions and examined separately. The intestinal lumen was exposed via an incision along its length and the contents washed into a 250-µm aperture sieve. Stomach washings were also examined for the presence of helminths. Intestines were then passed between the examiner's thumb and forefinger several times to scrape off any attached worms whilst a visual inspection was made of the mucosa. All contents were washed thoroughly and preserved in 70% ethanol for later microscopical examination. Faecal samples were also collected directly from the large bowel and preserved in 5% SAF for microscopy and 80% ethanol for molecular procedures (chapter 3) (Smout et al., 2013).

5.4.3 MICROSCOPIC EXAMINATION

All specimens were transported to the School of Veterinary and Life Sciences, Murdoch University, Western Australia. Intestinal contents were examined under dissecting and compound microscopes. Positive identification of *Ancylostoma* species was established using criteria documented in Biocca's (1951) paper. Where present, at least fifty individual hookworms were identified before deciding on the species present. Faecal scats, necropsy-collected faeces and soil were examined by simple smear technique, where faeces or soil were mixed on a slide with a small volume of water, and those samples positive for strongyle eggs noted. Given the high number of positive samples detected it was decided to include all samples for molecular analysis (chapter 3) (Smout et al., 2013).

5.4.4 GENOMIC DNA EXTRACTION

DNA was extracted directly from faeces using a Promega Maxwell® 16 research instrument system and tissue kit. The final DNA elution was prepared in 300µl of elution solution and stored at -20˚C until required. In order to confirm morphological identification, male *A. caninum* specimens also underwent molecular identification. Worms were washed and DNA was extracted using an Epicentre MasterPure™ Complete DNA and RNA Purification Kit according to the manufacturer's instructions (chapter 3) (Smout et al., 2013). To save on analysis time and costs batches of five closely collected, individual soil samples were pooled resulting in 26 pooled samples. DNA was extracted using a PowerMax® Soil DNA isolation kit and stored at -20˚C until required.

5.4.5 MOLECULAR METHODS – PCR

A direct PCR assay modified from Traub et al. (2008) was used for the DNA amplification of hookworm species. A forward primer RTHWIF (5'-GATGAGCATTGCWTGAATGCCG-3') and reverse primer RTHWIR (5'-GCAAGTRCCGTTCGACAAACAG-3') were used to amplify an approximately 485 bp and 380 bp section of the internal transcribed spacer-1 (ITS-1), 5.8S and internal transcribed spacer-2 (ITS-2) regions of *Ancylostoma* spp. The PCR assay was prepared in a volume of 25µl consisting of 1 X PCR buffer, 2 mM $MgCl_2$, 0.4 mM of each dNTP, 10 pmol of each primer, 1.0 U *Taq* DNA polymerase (Biotech International, Perth, Australia) and 1 µL of template genomic DNA. Due to the presence of inhibitors, DNA template often needed to be diluted to 1:2 or 1:4 concentration. PCR cycling conditions consisted of a pre-heating step at 95˚C for 5 min. This was followed by 40 cycles of 95˚C for 30 s (denaturing), 60˚C for 30 s (annealing), 72˚C for 30 s (extension), a final extension of 72˚C for 7 min and a holding temperature of 14˚C. Cycling was performed on an Applied Biosystems 2720 Thermal Cycler. The verification of the PCR product was established on a 1.5% agarose gel dyed with SYBR®Safe DNA gel stain (chapter 3) (Smout et al., 2013).

5.4.6 DNA SEQUENCING OF CANINE HOOKWORM

DNA sequencing was conducted on all positive samples. PCR products were purified using an Agencout® AMPure® XP PCR purification kit. DNA was quantified using a spectrophotometer and sequenced using an ABI 3730XL 96 capillary DNA sequencer (Applied Biosystems using Big Dye version 3.1 dye terminators). All chromatograms were viewed using Finch TV Version 1.4.0 (Geospiza Inc.). Dual infections were characterized by the presence of overlapping nuclotide peaks at specific positons in the chromatograms which corresponded to the specific hookworm species. Sequences were compared to a variety of GenBank *Ancylostoma* spp. submissions for similarity.

5.4.7 DATA ANALYSIS

Prevalence of a species of hookworm within positive samples was calculated by dividing the number of samples positive for each hookworm species by the total number of samples positive for hookworm in each location (Table 5.1). The significance ($p < 0.05$) of the difference between hookworm species and location was determined using the two-sided Fisher's Exact test (Kirkman, 1996 (accessed 10.05.16).).

5.5 RESULTS:

Of the twenty-seven domestic dog intestines examined from Yarrabah, 26 were infected with *A. caninum* (96.3%). Twenty-four of the 26 pooled soil samples (80.8%), and 79 out of 86 dog faecal samples (91.9%) were PCR positive for hookworm. Of these, 21 soil samples and 63 faecal samples returned clear and readable sequences. For repeatedly unreadable samples, chromatograms showed low signal strength, which often corresponded with low spectrophotometer DNA readings.

BLAST results showed a 99% or greater homology to previously published sequences with GenBank accession nos. DQ78009 for *A. ceylanicum* and JQ812694 for *A. caninum.* Positively identified *A. caninum* from sample NYB14 (from Yarrabah) was

100% homologous with DQ438073 for *A. caninum* confirming that morphological identification of samples was correct.

From the 63 positive canine faecal samples examined, 62 were infected with *A. caninum* (98.4%), one had a sole infection of *A. ceylanicum* (1.6%) and four (6.3%) contained a mixed infection of *A. ceylanicum* and *A. caninum* together. These dual infections occurred only in dogs from the Mossman community while Yarrabah dogs were solely infected with *A. caninum* (100%) (Table 5.1). The prevalence of *A. ceylanicum* in soil was highest at Mossman Gorge and Lake Placid (100%) followed by Mossman (55.6%) and Yarrabah communities (25%). Fisher's exact test on frequency analysis showed that prevalence of hookworm species in dog faeces varied significantly with location (p = 0.018). No significant difference was seen for soil in relation to location (p = 0.171).

Table 5.1 Study sites and prevalence of hookworm species from positive samples of soil and dog faeces

Location	***A. caninum*/n**		***A. ceylanicum*/n**		**Dual infection/n**	
Communities						
Mossman (f)	22/23	95.7%	5/23	21.7%	4/23	17.4%
Mossman (s)	8/9	88.9%	5/9	55.6%	4/9	44.4%
Yarrabah (f)	34/34	100%	0/34	0%	0/34	0%
Yarrabah (s)	8/8	100%	2/8	25%	2/8	25%
Jumbun (f)	6/6	100%	0/6	0%	0/6	0%
Tourist locations						
Mossman Gorge (s)	1/1	100%	1/1	100%	1/1	100%
Lake Placid (s)	0/1	0%	1/1	100%	0/1	0%
Murray Upper (s)	2/2	100%	0/2	0%	0/2	0%

(f) faeces, (s) soil

5.6 DISCUSSION:

Prevalence of hookworm found in domestic dogs in the study sites was high. The most predominant species, *A. caninum* is not currently perceived as a severe zoonotic threat as the resultant cutaneous larval migrans infection is self-limiting in humans. Although, *A. caninum* infection can also result in eosinophilic enteritis and associated intestinal hypersensitivity (Prociv and Croese, 1996). This study reports, for the first time, *A. ceylanicum* infection in domestic dogs and soil in a Queensland Indigenous community and in soil in protected areas frequented by tourists. Due to this parasite's potential for patent enteric infection in humans, this is a public health concern, and consideration should be given to addressing and mitigating risks for infection.

The local Mossman Indigenous community, Kuku Yalanji, is situated 75 km north of Cairns (Figure 5.1) and four km from Mossman (the nearest town). It has an estimated resident population of 100 people (Australian Bureau of Statistics, 2011 (accessed 29.04.16).-b). The community has built an eco-tourism centre which attracts 350,000 visitors annually who come to see the rainforest in this part of the Wet Tropics World Heritage Area. Visitors can swim in the local water holes and sunbake on the banks of the Mossman River. *A. ceylanicum* is able to infect a host through percutaneous penetration along with the faecal-oral route. Hence the warm, moist conditions at this site may provide ideal conditions for contracting soil-transmitted helminth infections.

Free-roaming domestic dogs are often present around the local medical centre in Mossman where several positive *A. ceylanicum* faecal and soil samples were collected. As is often the case in the tropical North during the warmer months, many of the residents, especially children, are often bare-foot thus coming into direct contact with potentially infected soil.

Located at the base of the Barron Gorge 15 km north-west of Cairns, Lake Placid is a large natural lake formed by the Barron River (Figure 5.1). It is popular amongst local and international tourists as well as kayaking enthusiasts. The pet-friendly, Lake Placid Tourist Park is located next to Lake Placid recreational area, where a positive sample for *A. ceylanicum* was collected. Although the entrance to the recreational area is clearly signposted that domestic dogs are prohibited, these rules are not always adhered to (F. Smout *pers. obs.*).

Jumbun is a small Indigenous community with a population of 104 people (Australian Bureau of Statistics, 2011 (accessed 29.04.16).-a), located at Murray Upper approximately 160 km south of Cairns (Figure 5.1). Due to a recent local council animal management program, only 10 dogs were present in the community at the time of the study, although the dog numbers can vary greatly (A. Miller pers. comms.). All faecal samples collected here were positive for *A. caninum* (100%).

Yarrabah Aboriginal community is located to the south-east of Cairns (Figure 5.1), with a population of 2494 (Australian Bureau of Statistics, 2016.). Free-ranging dogs are abundant in the community and are often seen wandering in the surrounding WTWHA rainforest, where dingoes are also present (F. Smout *pers. obs.*). Interestingly, none of the dogs necropsied, nor any of the canine faecal samples were positive for *A. ceylanicum.* However, two of the soil samples collected here did return positive results, and these came from the primary school and the high school playgrounds. Further investigation should be undertaken here of the *A. ceylanicum* status of cats in the community, since they may have been the source of infection and may have used the sandy playground of the schools for defaecation. A previous study in a Western Australia Aboriginal community found poor sanitation and promiscuous defaecation by children to also be factors contributing to hookworm infection (Reynoldson et al., 1997). Therefore, if *A. ceylanicum* is present in humans in the community, infection could be spread by similar behaviour.

While *A. ceylanicum* may not be ubiquitous throughout Far North Queensland, further research needs to be undertaken to determine the true extent of infection in this region. Previous research has shown *A. ceylanicum* to be the predominant hookworm infection in the dingo population approximately 40 km north-west of Mossman Gorge (chapter 3) (Smout et al., 2013). It is possible that *A. ceylanicum* is the more abundant hookworm in these rainforest areas, and that in locations where domestic dogs, the reservoir host of *A. caninum*, are present such as in the Indigenous communities here, there may be a spill-over of infection from domestic dogs that influences the hookworm species present in the dingo population (chapter 3) (Smout et al., 2013).

Our understanding of the epidemiology involved in the transmission of helminth infections in Indigenous Australians has been greatly enhanced through the use of molecular techniques. The Australian Government's ongoing commitment to

encouraging tourism in the WTWHA (WTMA, 2000) and to "closing the gap" in chronic disease in Indigenous communities (COA, 2016) requires that dog health, a potentially hazardous environmental issue, needs to be addressed. Current public health approaches to helminth infections are directed at investigating anthroponotic routes of infection (Crompton et al., 2003). My results suggest that addressing zoonotic origins may be more appropriate and dogs must be considered as a reservoir of human infections when using mass drug administration for controlling hookworm infection in the human population (Thompson, 2015).

Ongoing dog health and control programs employing local Aboriginal residents should be established in communities with the collaboration of Aboriginal health workers to enable capacity building at the community level in order to increase responsibility and ownership of a dog management solution. A 'One Health' approach through integration of ecological, veterinary and human health incorporating the diagnosis and treatment of diseases of both humans and dogs is necessary for the development of more targeted and cost effective community health programs.

5.7 CONFLICT OF INTEREST STATEMENT:

The authors have no financial or personal relationship with other people or organisations that could have inappropriately influenced their work.

5.8 ACKNOWLEDGMENTS:

The authors would like to thank staff from Bamanga Bubu Ngadimunku Inc. at Mossman Gorge for assistance with sample collection. Thank you also to Alfred Gray Jnr, Stephen Canendo, Shanna Mossman and Herman Sexton, Yarrabah Aboriginal Shire Council and Jumbun Aboriginal Community. I received partial financial support from the Australian Research Council, Skyrail Rainforest Foundation, Terrain NRM, Australian Rainforest Foundation and the Australian Society of Parasitology researcher exchange training and travel award. I would also like to thank Aileen Eliott, Louise Pallant and Angela Reeves for their advice and expertise with microscopic and PCR analysis.

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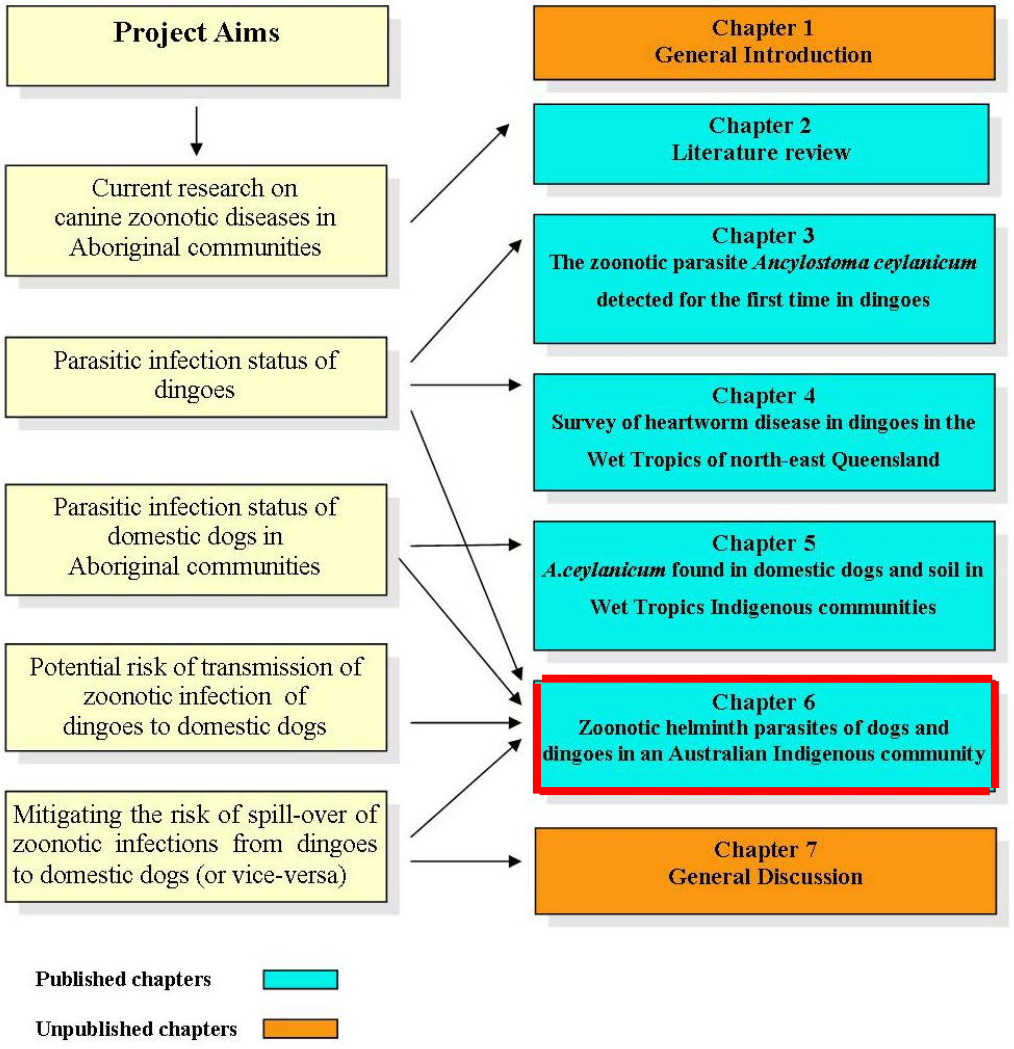
CHAPTER 6.

In this chapter I again further address thesis aims (3), (4) and (5) by surveying the parasitic infections of free-roaming dogs in an Indigenous community and the dingoes in the surrounding region. This was important to establish the types of parasitic infections present in the community and determine the risk of transmission of infection between dogs and dingoes and how this may happen. I placed GPS tracking collars on the dogs and dingoes to establish their home ranges and potential overlap of habitat. I found that dingo home ranges almost completely overlapped that of the domestic dogs and dingoes spent a substantial amount of their time in areas used by dogs.



Published- Paper 5

Citation: Smout, F.A., Skerratt, L.F., Johnson, C., Butler, J., Congdon, B. (2018). Zoonotic helminth diseases in dogs and dingoes utilising shared resources in an Australian Aboriginal community. Tropical Medicine and Infectious Diseases 3, 110.

Graphical abstract

TITLE: ZOONOTIC HELMINTH DISEASES IN DOGS AND DINGOES UTILISING SHARED RESOURCES IN AN AUSTRALIAN ABORIGINAL COMMUNITY

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6.1 ABSTRACT:

The impacts of free-roaming canids (domestic and wild) on public health have long been a concern in Australian Indigenous communities. I investigated the prevalence of zoonotic helminth diseases in dogs and sympatric dingoes, and used radio telemetry to measure their spatial overlap, in an Aboriginal community in the Wet Tropics of Australia. Samples collected from dingoes and dogs showed high levels of infection with the zoonotic hookworm *Ancylostoma caninum*. Dingoes were also positive for *A. ceylanicum* infection (11.4%) but dogs were infection free. Whipworm, *Trichuris vulpis,* infection was far more prevalent in necropsies of domestic dogs (78.6%) than dingoes (3.7%). Dogs were free from *Dirofilaria immitis* infection, while dingoes recorded 46.2% infection. Eleven dingoes and seven free-roaming domestic dogs were fitted with Global Positioning System collars and tracked over an extended period. Dingo home-ranges almost completely overlapped those of the domestic dogs. However, dingoes and dogs did not utilise the same area at the same time, and dogs may have avoided dingoes. This spatial overlap in resource use presents an opportunity for the indirect spill-over and spill-back of parasites between dogs and dingoes. Tracking and camera traps showed that the community rubbish tip and animal carcasses were areas of concentrated activity for dogs and dingoes.

6.2 KEY WORDS: Dingo, Dogs, Aboriginal, Diseases, Canine, Zoonoses.

6.3 INTRODUCTION:

Globally, the risk of disease transmission among free-roaming dogs (*Canis familiaris*), wildlife and humans is a growing concern, driven largely by the burgeoning population of domestic dogs (Gompper, 2014; Hughes and Macdonald, 2013; Knobel et al., 2014). In Australia, free-roaming canids and the potential public health issues associated with them have long been a concern in Indigenous communities. These animals can include unrestrained domestic dogs from within the community, along with wild dogs and dingoes (*Canis dingo* Meyer, 1793) from surrounding areas. Urban expansion into

previously undeveloped areas around communities has brought people and their pets into closer contact with dingoes, resulting in antagonistic interactions and stakeholder conflict (Butler et al., 2014). One potential interaction is the transmission or 'spill-over' of diseases, including zoonotic parasites, from dingoes to dogs and thus to humans. Investigating all potential hosts and their interactions is hence necessary to understand and mitigate 'spill-over' and 'spill-back' of zoonotic infection (chapter 2) (Smout et al., 2017a).

It is estimated that soil-transmitted helminths infect 2 billion people worldwide with many of these infections occurring in Australia's closest neighbours, South-East Asia (Gordon et al., 2017). Some of the parasites of zoonotic importance in the Wet Tropics bioregion of northern Queensland include the common roundworm, *Toxocara canis* and the dog hookworm, *Ancylostoma caninum*. These worms can cause 'visceral larval migrans' and 'cutaneous larval migrans' respectively in humans, along with a range of symptoms. Shield et al. (2015) found prevalences as high as 28% and 21% for *Strongyloides stercoralis* and *T. canis* infection respectively in children and adults in a remote Aboriginal community of the Northern Territory. The recent finding that *Ancylostoma ceylanicum* occurs in domestic dogs (chapter 5) (Palmer et al., 2007; Smout et al., 2017b), dingoes (chapter 3) (Smout et al., 2013) and humans (Koehler et al., 2013) in Australia is of further concern, due to the much higher prevalence of this parasite than previously thought (Gordon et al., 2017) and its potential to cause patent infections resulting in significant illness in humans (Carroll and Grove, 1986; Hsu and Lin, 2012).

Another recent study in Queensland has revealed that dingoes are a reservoir host for heartworm (*Dirofilaria immitis*) in low density housing areas (chapter 4) (Smout et al., 2016). Heartworm is spread by mosquito vectors and is a potentially life-threatening disease for canines. Although infection in humans is generally asymptomatic, the parasite can cause infarction and nodule formation in the lungs (Kochar, 1985; Malik et al., 2016).

Understanding the interactions between the multiple hosts and vectors of canid diseases in and around human settlements is critical to public health management (Butler et al., 2004; Kennedy et al., 2018). Given that many helminth diseases are often spread through the faecal-oral route, faecally-contaminated areas of mutual resource use may

be important locations for disease transmission via exposure to shedding pelage and faeces, even when no direct contact has taken place (Polley, 2005; Robertson et al., 2000).

Traditionally, dingoes accompanied Aboriginal people as hunting aides, companions and protectors. Dogs have replaced dingoes in most communities and, to some extent, hold the same sacred position (Wilks, 2000). Due to this complex relationship, dogs are often not restrained to house yards and are given the freedom to come and go as they please. Veterinary services for Indigenous communities are limited (Kennedy et al., 2018), have offered little chemoprophylactic therapy for community dogs, or education about dog population management or disease risks and have involved inappropriate mass culling of dogs without informed consent from animal owners (Hardaker, 2008). The result of this has often been a quick return to pre-cull numbers and a loss of trust in agencies responsible for management of dogs.

The aim of the present study was to determine the prevalence and intensity of helminth parasite infection of domestic dogs and dingoes in a Wet Tropics Indigenous community and identify 'hot-spots' for potential transfer of disease by evaluating the spatial and temporal use of habitat and resources by sympatric dogs and dingoes. I used my results to identify interventions that may reduce the risk of disease transmission between hosts, and thus to improve public health management. These results can then be extrapolated to other Indigenous communities in northern Australia in order to better manage free-roaming dog and dingo populations, and broader biosecurity risks and threats.

6.4 MATERIALS AND METHODS

6.4.1 STUDY AREA

The study was conducted in and around Yarrabah Aboriginal community, in the rural lowlands of the Wet Tropics bioregion in north-eastern Queensland, Australia (Figure 6.1). This bioregion contains remnant rainforest which holds globally-significant biodiversity and cultural values. These values are recognised by its designation as the

Wet Tropics World Heritage Area (WTWHA) (WTMA, 2004, 2013). Yarrabah is 53 km by road from the regional city of Cairns. It has a tropical climate with strongly seasonal rainfall of approximately 2,000 mm per annum. Mean monthly temperatures range from 20˚C to 29˚C. The community is bordered by ocean on two sides and Mount Yarrabah, with an elevation of 602 m to the north-west. Vegetation is varied, ranging from estuarine mangroves to coastal swamps of melaleuca, eucalyptus and palm species. The study area covered approximately 160 km^2 and is bordered by and includes State Forest and National Park.

Yarrabah has a population of 2494 people (Australian Bureau of Statistics, 2016.). This community was chosen due to its proximity to a known dingo population, as well as being representative of other Indigenous communities in the Wet Tropics and elsewhere in northern Australia.

6.4.2 DINGOES

6.4.2.1 Dingo necropsy samples

Twenty-seven dingo carcasses (15 males and 12 females) were supplied by Cairns Regional Council animal control officers and local landholders following routine control measures from 2007 to 2012. These dingoes originated from farmland adjacent to Yarrabah as well as from the outer suburbs of northern and southern Cairns and Atherton. No animals were killed specifically for this study. All of the specimens were bagged and frozen as soon as possible following collection and information regarding collection date and location were noted.

The stomach and intestines were excised and all contents were washed thoroughly and preserved in 70% ethanol for later microscopical examination. The heart and lungs were also excised and right ventricle and pulmonary arteries were examined for the presence of *D. immitis.* Further details such as age, sex and body condition score (Purina Body Condition System 1-9, Nestle Purina Pet Care Center, Rhodes, NSW) were recorded.

6.4.2.2 Dingo trapping

Twelve dingoes (seven females and five males) were trapped either in the outer suburbs of the Cairns/Gordonvale region adjacent to Yarrabah, or within the Yarrabah community and fitted with Global Positioning System (GPS) tracking collars.

Ten of the twelve dingoes were trapped using Oneida Victor® Soft Catch® traps (Oneida Victor Inc. Ltd., USA) during a concurrent study undertaken in the area (Morrant et al., 2017a). Traps were monitored using Trapsite VHF transmitters (Telonics, Mesa, Arizona) attached to the trap chain. Trap sites were also visually inspected each morning.

Two further dingoes, one female and one male, were trapped within the Yarrabah community on November 2012 and May 2013 respectively. Due to the presence of many wandering domestic dogs, large cage traps were used here. Traps were placed in shaded areas, baited with raw chicken carcasses and visually inspected at least twice daily.

6.4.2.3 Processing dingoes:

An intra-muscular injection of Domitor® (medetomidine hydrochloride, Pfizer Australia Pty. Ltd., West Ryde, NSW) was used to sedate the animals. To further reduce risk to field workers, dingoes were placed on a restraint board with straps over their neck and loins and fitted with a muzzle. Animals were examined for the presence of external parasites and faecal samples were collected. Age and body condition scores were estimated and animals were weighed (Shimano 45kg stainless weighing scale). Five mL of blood was collected from the jugular vein of each animal and stored in ethylenediaminetetraacetic acid (EDTA) tubes and refrigerated at 4 ˚C for antigen testing with 24 hours. Animals were implanted with a microchip (Trovan 956 ISO, Microchips Australia Pty. Ltd., Australia) on the dorsal midline between the shoulder blades using a 12-gauge implanter needle (Trovan Deluxe [IME] Implanter, Microchips Australia Pty. Ltd., Australia).

6.4.2.4 GPS tracking:

All dingoes were fitted with a Tellus™2A GPS tracking collar (Followit AB, Sweden). Following processing, the sedative was reversed with Antisedan® (atipamezole hydrochloride, Pfizer Australia Pty. Ltd., West Ryde, NSW) and the dingoes were monitored until they moved away from the trap site.

GPS collars were programmed to record a location (waypoint) every two hours for fourteen days (alternating between odd and even hours at one-week durations), and then every ten minutes for half a day (alternating between before noon and after noon each time) and every two hours for the rest of the day on the fifteenth day (Morrant et al., 2017a).

The collars also included a release mechanism that could be manually activated via a remote communication device (RCD-04, Followit AB, Sweden); this device was also used to remotely download data from collars. A timed-release mechanism was set to release ten months after deployment for collars not manually released. GPS data were screened to remove location errors by removing two-dimensional (2-D) locations with a positional dilution of precision (PDOP) < 5. (Lewis et al., 2007; Morrant et al., 2017a).

Maps were created by entering waypoints into Google Earth using Excel to Keyhole Markup Language (KML) file through Earth Point Tools for Google Earth 2017/2018 (http://www.earthpoint.us/ExcelToKml.aspx).

6.4.3 DOMESTIC DOGS

6.4.3.1 Faecal and tissue samples

Fifty faecal samples were collected from free-ranging domestic dogs within the Yarrabah urban area. Heart, stomach and intestinal samples were collected from 28 domestic dog carcasses (9 males and 19 females), supplied by Yarrabah Aboriginal Council Animal Control Officers between December 2010 and December 2011.. All of

the specimens were necropsied, as per section *2.2.1.* above, immediately following euthanasia.

6.4.3.2 Domestic dog tracking

Seven domestic dogs (two females and five males) were recruited from three households within the community and fitted with motion detector GPS-data loggers (i-gotU GT-600, Mobile Action Technology, Taiwan) attached to a 40mm wide synthetic collar. The dimensions of the loggers were 46 x 41.5 x 14mm with a weight of 37grams. Individual loggers were further protected and water proofed by being wrapped inside plastic film, placed inside a plastic zip lock bag and then covered with black Heatshrink tubing.

Data loggers were set to record a position every 41 seconds and were changed twice per week in order to retrieve maximum data per animal. Battery life on trackers was between two – five days with four days being the usual case. Community dogs were tracked from October 2012 to September 2013. Movements were observed and mapped on Google Earth. Trapping site for TD11 and the rubbish tip were monitored with camera traps over a six month period (PC900 HyperFire™, Reconyx, USA and DLC Covert II, Covert Scouting Cameras Inc., USA).

6.4.4 PARASITOLOGY TECHNIQUES

6.4.4.1 Detection of adult *D. immitis* antigen

Blood samples from trapped animals were tested for circulating antigen, as per manufacturer's instructions using a commercial ELISA SNAP test kit for heartworm (IDEXX Laboratories Inc., Rydalmere, NSW): sensitivity 84% and specificity 97% (Atkins, 2003). The IDEXX SNAP test detects the glycoprotein found in the reproductive tract of the mature female *Dirofilaria immitis* worm thus further tests, as described below, were undertaken to ensure those animals with low, or male only, worm burdens were not missed.

6.4.4.2 Blood smears

Thin blood smears from the 12 dingoes were also stained with Diff Quik and examined under a light microscope to further test for the presence or absence of microfilariae. Where samples returned an antigen negative result and no microfilaria were seen on a stained slide, a further test was performed by microscopically investigating for microfilariae in the region above the buffy coat of a spun blood sample in a microhematocrit tube (Rizzo and Ware, 1989). *Dirofilaria immitis* microfilariae were identified morphologically and distinguished from *Acanthocheilonema* (syn. *Dipetalonema*) *reconditum*, a filarial parasite of the subcutaneous tissues and fascia of canids, according to existing descriptions (Kelly, 1973; Sawyer et al., 1963). *Dirofilaria repens* is not known to occur in Australia (Stringfellow et al., 2002).

6.4.4.3 Faecal examination

Faecal samples (5 g if available) were examined macroscopically for the detection of proglottids and then screened by direct microscopy for the presence of worm eggs and larvae. Further molecular analysis in the form of direct PCR was undertaken on all fifty of the dog faecal samples and eight of the twelve dingo samples (66.7%) to determine *Ancylostoma* species present. DNA sequencing was conducted on all positive PCR samples (chapter 3) (Smout et al., 2013; Traub et al., 2008).

6.4.4.4 Microscopic examination

Intestinal contents were examined under dissecting and compound microscopes. Positive identification of *Ancylostoma* spp. was established using those criteria documented by Biocca (1951). Where heavy infections were present, at least fifty individual hookworms were identified before recording the *Ancylostoma* spp. present. As dogs were housed together prior to euthanasia, ectoparasite prevalence was not focussed on in this study; however microscopic examination was used to identify fleas, ticks and mites seen.

6.4.5 DATA ANALYSIS

Prevalence of infection was calculated on all dingo and dog samples by dividing the number of samples positive for parasite infection by the total number of samples collected. Home range was estimated using 100% minimum convex polygon (MCP100) (Mohr, 1947). The area of the polygon was measured using Google Earth co-ordinates and entering data into Earth Point Tools for Google Earth 2017 (http://www.earthpoint.us/Shapes.aspx).

6.4.6 ETHICS

All protocols were reviewed and approved by James Cook University Human and Animal Ethics Committees (Approval nos. H4264, A1495 and A1546).

6.5. RESULTS

6.5.1 PARASITE INFECTIONS

Twelve dingoes were trapped between October 2010 and May 2013. One dingo (TD05) was lost and not able to be tracked following release, but has been included in the parasite infection data (Table 6.1). All dingoes appeared to be well muscled and in a healthy condition although four lactating females were slightly underweight. Body condition scores ranged from three (underweight) to five (ideal). Faecal samples revealed 100% infection with *A. caninum* with one animal (12.5%) returning a positive PCR result for *A. ceylanicum*. A similar result was also obtained for necropsied dingoes of 100% and 11.1% infection for *A. caninum* and *A. ceylanicum*, respectively (Table 6.1). Further molecular identification to differentiate *A. caninum* from *A. ceylanicum* was not undertaken on the faecal samples of four of the dingoes, as they were trapped after the completion of laboratory work. No skin lesions were seen on any dingoes.

Table 6.1 Number of infected and prevalence of helminth infections in free-roaming domestic dogs and dingoes.

(% prevalence)	Necropsy dingo† (n = 27)	Collared dingo* (n = 12)	Total dingoes (n = 39)	Necropsy dog† (n = 28)	Collared dog* (n = 7)	Dog scats* (n = 50)	Total dogs (n = 85)
Nematoda							
Ancylostoma caninum	27 (100) (91-100)§	8/8 (100) 4NI (78-100)	35/35(100) (93-100)	27 (96) (86-100)	7 (100) (77-100)	44 (88) (78-98)	78 (92) (85-98)
Ancylostoma ceylanicum	3 (11) (0-24)	1/8 (13) 4NI (0-38)	4/35 (11) (0-23)	0	0	0	0
Toxocara canis	12 (44) (27-62)	4 (33) (9-58)	16 (41) (26-56)	13 (46) (29-64)	2 (28.6) (0-58)	10 (20) (9-31)	25 (29) (20-39)
Trichuris vulpis	1 (4) (7-14)	1 (8) (0-28)	2 (5) (0-14)	22 (79) (63-94)	2 (28.6) (0-58)	17 (34) (21-47)	41 (48) (38-59)
Dirofilaria immitis	10 (37) (20-55)	8 (67) (43-91)	18 (46) (31-61)	0	NI	NI	0
Cestoda							
Dipylidium caninum	1 (4) (7-14)	0	1 (3) (0-10)	20 (71) (55-88)	0	0	20 (24) (14-33)
Spirometra erinacei	12 (44) (27-62)	6 (50) (25-75)	18 (46) (31-61)	0	0	3 (6) (0-14)	3 (4) (0-8)

† Necropsy samples.

* Faecal samples.

§95% confidence intervals.

NI = Not investigated.

The physical condition of domestic dogs varied. All collared dogs (n = 7) were well muscled and healthy (BCS = 5, ideal); however the stray, necropsied animals (n = 28)

ranged in body condition score from the minimum of one (very poor) to seven (over weight) with the majority scoring two - three (79%). Domestic dogs had an overall high prevalence of *A. caninum* with 100%, 96.4%, and 88.0% infection in collared dogs, necropsied dogs and domestic dog scats (n = 50), respectively (Table 6.1). *Ancylostoma ceylanicum* was not identified in domestic dogs.

Similar levels of infection with *Toxocara canis* were seen in dingoes (41%) and domestic dogs (29.4%) overall; however *Trichuris vulpis* and *Dipylidium caninum* infections were far more prevalent in domestic dog necropsies (78.6% and 71.4%, respectively) than in dingo necropsies (3.7% for both). The reverse was seen for *Spirometra erinacei* infections with 46.1% and 3.5% overall prevalence in dingoes and dogs, respectively. It is also noted that dogs were free from *Dirofilaria immitis* infection, while dingoes had an overall infection rate of 46.2%. All trapped dingoes that returned positive antigen tests were also microfilariemic positive (66.7%). No dingoes were antigen negative but microfilariemic positive. Many of the necropsied dogs (71%) also showed signs of chronic skin diseases from which *Sarcoptes scabiei* and *Demodex canis* mites were identified. *Ctenocephalides felis* and *C. canis* along with the common dog tick, *Rhipicephalus sanguineus.*were also found on dogs.

6.5.2 HOME RANGES AND RESOURCE USE

A total of 1253 and 308 tracking days were analysed for dingoes and domestic dogs, respectively. Mean home range size for dingoes was 44.8 ±11.38 km^2, and 2.3 ±0.59 km^2 for dogs (Table 6.2). Female dogs ranged further than male dogs with a mean home range of 3.8 ±1.35 km^2 and 1.7 ±0.50 km^2 respectively. In contrast male dingoes had larger ranges than females, with home range averages of 56.2 ±22.37 km^2 and 38.3 ±13.30 km^2, respectively, but the difference was not significant (t-test: t = 0.7387, d.f = 9, $P > 0.05$).

Table 6.2 Home range areas (MCP100) of 11 tracked dingoes and seven free-roaming domestic dogs

Dingo/Dog	Sex	Mass(kg) [BCS]		Duration of tracking (days)	Home range (km^2)	Location
Dingo						
TD01	Male	27	[5]	79	107.3*	Walsh's Pyramid
TD02	Male	21	[5]	195	76.8*	Mount Peter
TD03	Male	21.5	[4]	120	34.5*	Glen Boughton
TD04	Female	17	[3]	150	57.1*	Walsh's Pyramid
TD06	Female	13	[3]	122	6.9*	Old Smithfield
TD07	Female	9	[3]	101	8.1*	Old Smithfield
TD08	Female	13	[3]	171	79.0*	Walsh's Pyramid
TD09	Female	15	[4]	202	85.9*	Glen Boughton
TD10	Female	14.5	[4]	96	26.1*	Walsh's Pyramid
TD11	Female	14	[3]	17	5.1	Yarrabah
TD12	Male	14	[4]	58	6.2	Yarrabah
Mean					44.8 (±11.38)	
Dog						
CD01	Female (desexed)	14	[5]	67	2.4	Yarrabah
CD02	Male	30	[5]	28	2.7	Yarrabah
CD03	Male	33	[5]	80	2.6	Yarrabah
CD04	Male	32	[5]	15	0.6	Yarrabah
CD05	Male	7	[5]	26	2.2	Yarrabah
CD06	Male	38	[5]	65	0.4	Yarrabah
CD07	Female	30	[5]	27	5.1	Yarrabah
Mean					2.3 (±0.59)	

*Data from concurrent study (Morrant et al., 2017a).

Of the 11 dingoes tracked, the two trapped within the Yarrabah community (TD11 and TD12) spent the majority of their time there. One other female (TD09) trapped at Glen Boughton, approximately six kms away, was recorded on the southern side of the community on one day only. TD11 and TD12 regularly entered the community and utilised areas around the police station, hospital and high school (Figure 6.1).

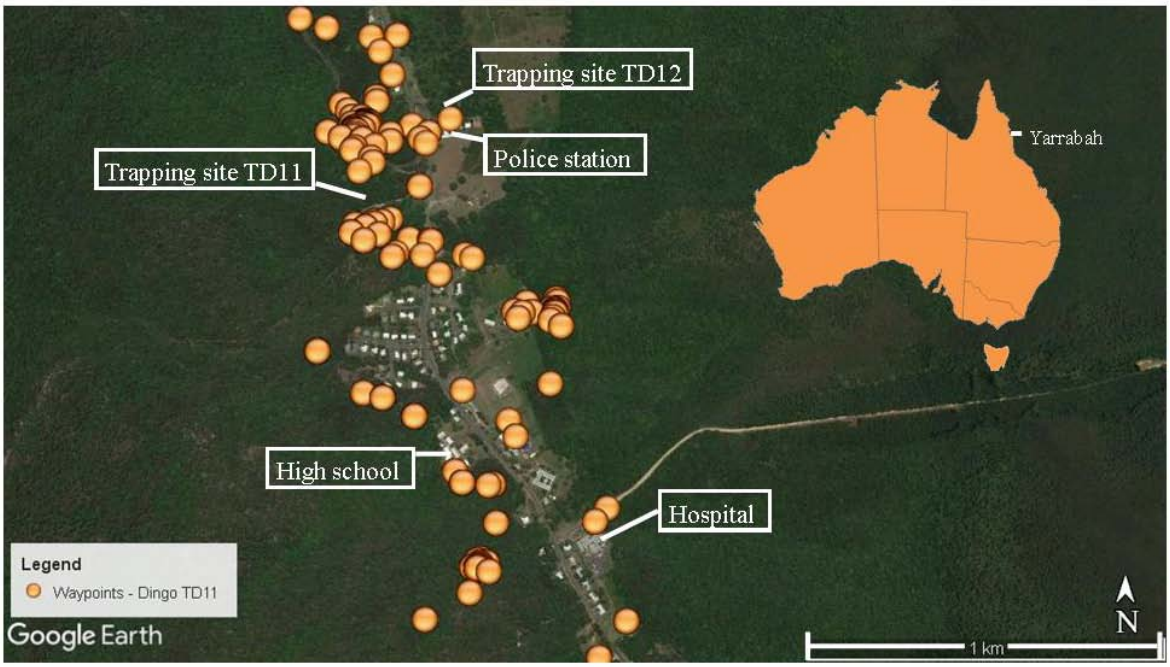

Figure 6.1 Waypoints collected in Yarrabah over a seven day period (7th November 2013 to 14th November 2013) for female dingo TD11.

Five collared dogs from one household ventured out on forays almost daily, with the majority of these trips to the rubbish tip for short periods. These five dogs spent a considerable amount of time, on average two to three hours per day, outside of urban boundaries. The i-gotU GT-600 (Mobile Action Technology, Taiwan) data loggers enabled precise tracking of the dog's forays which were viewed on Google Earth engine (Figure 6.2).

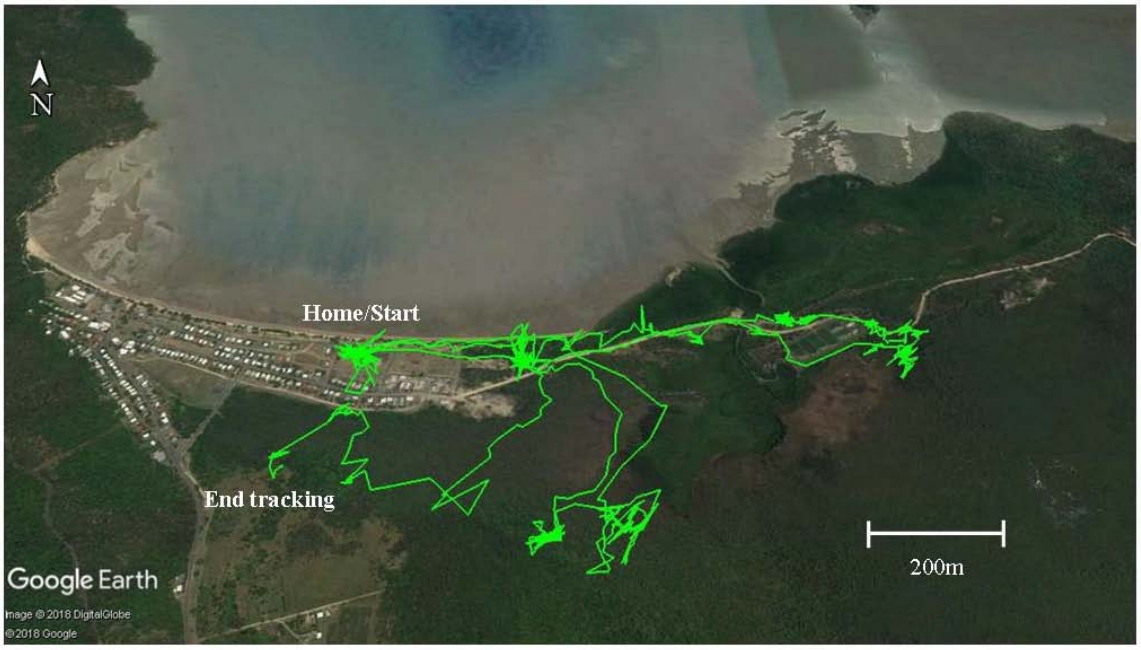

Figure 6.2 Track map from i-gotU GT-600 (Mobile Action Technology, Taiwan) data logger for female domestic dog CD01 over 96 hours in Yarrabah showing forays away from urban boundaries as viewed on Google Earth engine.

Following the download of data showing a particularly long foray where all five dogs from the same household had visited a site for a whole day and then revisited for several days afterward, the first author investigated the site and found the remains of a pig carcass. It was impossible to determine how the pig had died due to decomposition and scavenging. Thus, it may have been shot and left by a local hunter or killed by dingoes. It was most unlikely that the dogs killed the pig as no high-speed chase was recorded prior to the dogs' extended time at the site. The young male dingo (TD12) was also tracked in this area at a later date.

A map of the community showing waypoints recorded for both dogs and dingoes, along with home site for dogs, is shown in figure 6.3. Many waypoints around the dogs' homes have been removed to reduce crowding of those locations and to enable clearer viewing of dingo waypoints. Importantly, dingo home ranges almost completely overlapped that of the domestic dogs and dingoes spent a substantial amount of their time (98%) in areas used by dogs.

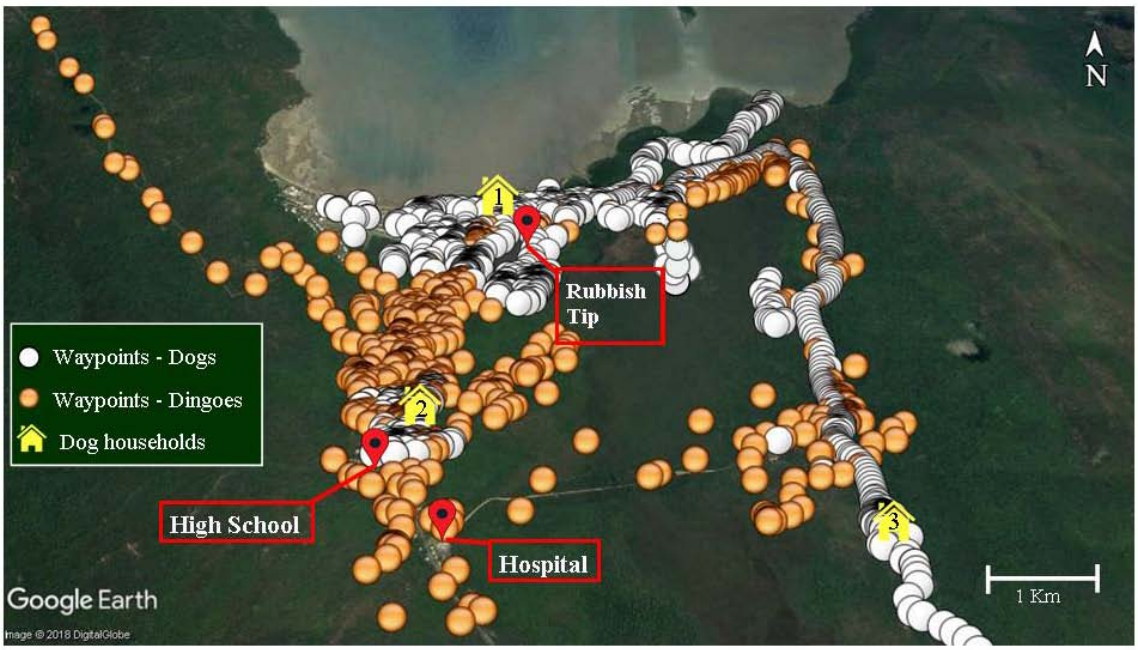

Figure 6.3 Waypoints for all tracked dingoes and dogs plus key locations in and around Yarrabah.

The camera traps situated at the trapping site for TD11 (Fig. 1) and the rubbish tip (Fig. 3) captured images of dingoes and dogs, plus horses (*Equus caballus*) and people. Although there was spatial overlap in resource use, the recorded waypoints along with captures by the trap cameras showed no overlap in the times study dogs and dingoes utilised the same areas. There was no discernable temporal pattern seen by dogs or dingoes utilising a specific habitat. Dogs, dingoes, horses and people were all seen both day and night and domestic dogs were often seen travelling with people.

6.6. DISCUSSION

My assessment of potentially zoonotic helminth parasites indicated widespread infections in dingoes and dogs from an Indigenous community in the Wet Tropics. The dog hookworm *A. caninum* was the most prevalent parasite in both Yarrabah dogs and dingoes in the region, infecting 91.8% and 100%, respectively. The high prevalence of *A. caninum* was similar to the most recent findings by Slapeta et al. (Šlapeta et al., 2015) on infections in free-roaming dogs in Yarrabah (100%). Reports from other parts of Australia indicate moderate to low prevalence (Gillespie and Bradbury, 2017; Wallner, 1999). Studies indicate that enteric infection with *A. caninum* is a leading cause of human eosinophilic enteritis in north eastern Australia (Prociv and Croese, 1990, 1996).

Although *A. ceylanicum* was not identified in the dogs in this community a previous study did find soil samples positive for *A. ceylanicum* in the area (chapter5) (Smout et al., 2017b). Unfortunately, the faecal samples of the two dingoes trapped within the community were not investigated with further molecular tools; however these animals were positive for strongyle-type eggs and *A. ceylanicum* was found in four other dingoes in this study and has been found previously in dingoes and community dogs in Far North Queensland (chapters 3 and 5) (Smout et al., 2017b; Smout et al., 2013). This parasite is of particular concern as it can cause a patent infection in humans (Koehler et al., 2013).

A further five helminths were found comprising three nematodes and two cestodes. *Trichuris vulpis* was detected in 48.2% of dogs and 5.1% of dingoes. This canine whipworm has been reported as common in domestic dogs in urban environments of Australia (Dunsmore and Shaw, 1990; Kelly, 1977) but appears to be uncommon in dingoes. Intermittent shedding of this parasite in faeces, along with methodology used for detection, may be the reason a lower prevalence was detected in faecal examination (31.3%) versus necropsy (78.6%) in dogs. An ongoing debate exists regarding the zoonotic potential of *T. vulpis* and its potential to cause visceral larva migrans in humans although cases of presumed *T. vulpis* infection in humans have been described (Traversa, 2011) along with studies using molecular techniques to speciate infection in humans (Areekul et al., 2010; George et al., 2016)

Previous reports show a lower prevalence of *T. canis* in other dog populations, both domestic and wild, compared with the present study of 29.4% and 41% overall in dogs and dingoes respectively. Wallner (1999) reported a prevalence of 12% in Townsville pound dogs and Coman (1972) recorded a 13.5% prevalence in wild dogs in Victoria. Human infection of *T. canis*, can result in fever, malaise, abdominal pain, wheezing and asthma and in extreme cases of the ocular form, total blindness (Feldman and Parker, 1992; Overgaauw and van Knapen, 2000).

Canine heartworm, *D. immitis*, was found in ten necropsied dingoes (37%) and eight (66.7%) of the trapped dingoes. The two dingoes trapped in the Yarrabah community both returned negative antigen test results and all domestic dog necropsies were free of infection. The absence of this parasite in community dogs may be a consequence of the ongoing use of ivermectin for the treatment of mange and heartworm (chapter 4) (Smout, 2015). Speare (2000) found an initial prevalence of 87% of heartworm in dogs over one year of age in Yarrabah. Following a 12 month ivermectin program the prevalence of heartworm had fallen to just 14%. The recent findings of Smout et al. (2016) (chapter 4), of a prevalence of 72.7% in dingoes in the region, suggests that this disease is as a continuing threat. Therefore, it is important that the results of this study do not lead to complacency in the community.

The tapeworm *Spirometra erinacei*, detected in dingoes (46.1%) is the second report of *S. erinacei* in dingoes in North Queensland, and suggests that the tapeworm is now established in dingoes in the area and likely reflects their diet and a suitable habitat for intermediate hosts (Brown and Copeman, 2003). *Dipylidium caninum* was detected in 71.4% of dogs and 3.7% of dingoes on necropsy. No infection was seen in faecal samples collected from either dogs or dingoes. Difficulty in finding proglottids in faeces may be the reason along with the time elapsed between when scats were deposited and collected. Proglottids are frequently motile and may have moved away from the sample before it was collected. Symptoms of dipylidiasis infection in infant humans have been reported but are generally rare and described as mild and nonspecific (Chappell et al., 1990).

The absence of *Echinococcus granulosus* in my study could be due to several reasons including the test methodologies used which are considered poor at recovering cestode

eggs from faecal samples although it should be noted that this parasite was also absent from necropsied animals. A previous study has also reported the absence of this parasite in domestic dogs in the Yarrabah community (Speare, 2000). Generally dingoes have a higher mortality rate near urban areas due to culling by local vertebrate pest control officers which leads to disruptions in social organisation (pack size, average age and hunting experience) causing dingoes to rely on smaller prey such as rats and bandicoots (*Permales nasuta*, *Isoodon macrourus*) (Morrant et al., 2017a; 2017b). Thus, their diet contains fewer macropods and transmission rates of *E. granulosus* are reduced (Jenkins et al., 2008).

The large tapeworm *Taenia hydatigena*, which is reported as common in wild dogs in Australia (Kelly, 1977), was also not found in any dingoes or dogs in my study. These results further suggest that ruminant livestock may not be an important component of the diet of dingoes in the Cairns area, as found by Morrant et al. (2017a; 2017b) who, in a concurrent study, analysed diet samples collected from the dingoes necropsied here along with others in the region and found only one out of 269 samples (0.004%) contained domestic bovine and one contained goat. A pocket of high cystic echinococcosis infection in cattle has been found previously on the Atherton Tableland, approximately 50kms inland from Cairns and at an elevation of 800 m (Banks et al., 2006).

Although the present study did not investigate ectoparasites due to captured dogs being housed together at the council pound before euthanasia, many of the dogs necropsied showed signs of chronic skin diseases due to *S. scabiei* and/or *D. canis* mite infection. Fleming et al (2001), reported sarcoptic mange as a widespread disease in dingo populations throughout Australia; however, no skin lesions were seen on any of the dingoes (n=39) in this study. The absence of infection in my study could be an indication that sarcoptic mange may be rare in dingoes in the Wet Tropics.

The risk of transmission of parasite infections is likely to vary both temporally and spatially depending on host behaviours and transmission pathways. My results show that dingo home-ranges almost completely overlapped those of domestic dogs although at no time were dingoes and dogs recorded together supporting previous findings that dogs avoid dingoes (Butler et al., 2014). During the study period a community member witnessing two large (25 -30 kg) male domestic dogs killed by three dingoes. The dogs

had been chasing two young foals when they were attacked by the dingoes. The encounter took less than five minutes, whereupon the dingoes returned into the surrounding bush. The first author viewed the dog carcasses finding both animals had deep lacerations to their throats and numerous other puncture wounds. Similar reports of aggression and predation by dingoes on domestic dogs are also common in other areas of the Wet Tropics and elsewhere in Australia, particularly on the boundary of rural and sub-urban settlements (Burger and Knowles, 1976; Butler et al., 2014).

As dingoes, and dogs, appeared to avoid direct contact, transmission of disease would generally occur indirectly through faecally-contaminated areas that are mutually used for resources such as food. The tropical climate of the region promotes parasite survival in the environment particularly for soil-transmitted helminth infections and hence allows for elevated parasite transmission and burdens throughout the year (Møller, 1998). Further investigations using specialised techniques and concentrating on parasites such as *Strongyloides stercoralis* should be undertaken in the region.

Previous studies tracking the home ranges of domestic dogs from Aboriginal communities have reported average home ranges of 9.7 km^2 (927 ha) for wandering dogs (Meek, 1999). Durr and Ward (2014) stated that dogs which roamed furthest were of most relevance for infectious disease transmission, because they could potentially vector disease over greater distances. Sparkes et al. (2014) found that male dogs tended to range over significantly larger areas than females. The dogs in my study had an average home range of 2.3 ±0.59 km^2 (230 ha) thus falling between the wandering and sedentary categories used by Meek (1999).

The two dingoes trapped within the community (TD11 and TD12) had smaller home ranges than those exhibited by the other trapped dingoes (Morrant et al., 2017a). This may be because they largely utilized anthropogenic resources not available to those dingoes outside the community setting. The young male dingo (TD12) was tracked to the local rubbish tip on numerous occasions suggesting that he may have been scavenging food from the area. Five of the collared dogs also frequented the rubbish tip

on an almost daily basis. This overlap of home ranges and indeed dietary niches increases the risk of disease transmission.

While there is a background risk of indirect transmission of parasites over the entire area where dingo and dog home ranges overlap, the primary risk is likely to be at locations where dog and dingo activity is concentrated. Mitigating strategies should include exclusion fencing of the rubbish tip, effective disposal of animal carcasses, public education about local zoonotic diseases and their prevention, and regular chemoprophylactic therapy of community dogs. Further studies utilising spatial statistical models would be invaluable to refine home-range and behavioural characteristics.

6.7. CONCLUSIONS

In summary, there is an elevated risk of transmission of canine parasitic diseases of zoonotic importance given the elevated parasite burdens in dogs and dingoes, and also on the substantial overlap of spatial habitat use and food resources of dingoes and domestic dogs, even within busy community areas. Hot spots of transmission between dingoes, dogs and also humans are likely to be sources of anthropogenic-derived food such as the rubbish tip, animal carcasses and the high school sporting grounds.

Further investigations on transmission risks for canine infectious diseases in other Indigenous communities are necessary to identify appropriate mitigation strategies. In order for animal disease control programs to have a sustainable outcome it is vital that extensive consultations with local environmental health officers and animal management workers are undertaken throughout the process.

6.8 ACKNOWLEDGMENTS

The authors would like to thank the Commonwealth Science and Industrial Research Organisation (CSIRO) Atherton, Cairns Council, Damian Morrant, Alfred Gray Jnr, Stephen Canendo, Shanna Mossman and Herman Sexton for assistance with sample collection and dingo trapping. This study was funded by an Australian Research Council Linkage Project (LP0882628), the Australian Rainforest Foundation, Skyrail Rainforest Foundation and CSIRO. All protocols were reviewed and approved by James Cook University Human and Animal Ethics Committees (Approval nos. H4264, A1495 and A1546).

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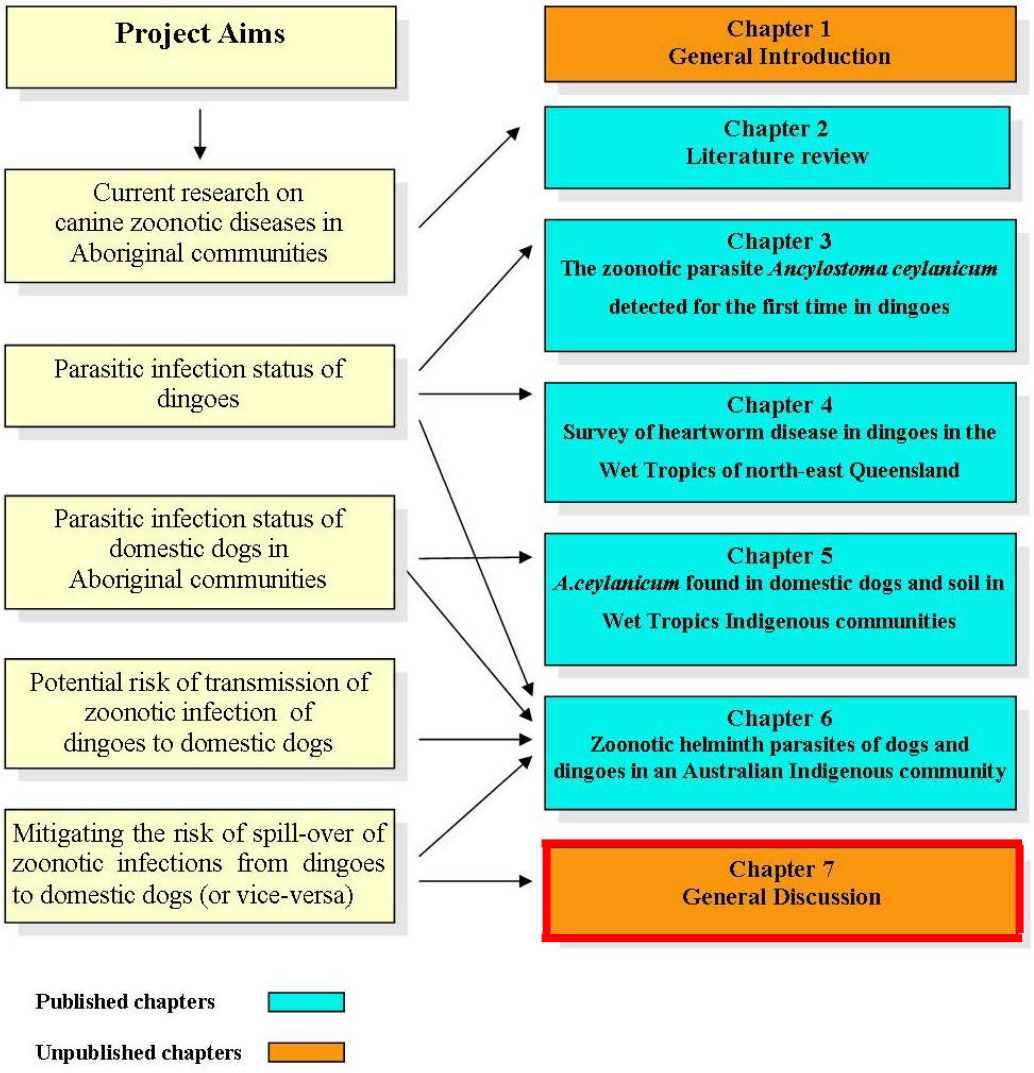
CHAPTER 7.

General Discussions

This final chapter summarises the outcomes of the previous chapters and again addresses how these match the specific thesis aims. I also further address aim (5) suggesting mitigation methods to reduce the public health risk of canine zoonotic diseases and recommend management strategies for dogs and their diseases in Indigenous communities in the Wet Tropics. Directions for future research have also been outlined.



GENERAL DISCUSSION AND CONCLUDING COMMENTS.

7.1 INTRODUCTION

This thesis is a compilation of several multidisciplinary studies undertaken to determine and characterise the ecological and epidemiological aspects of zoonotic helminth infections in dingoes and dogs in and around Indigenous communities in the Wet Tropics. A combination of factors including climate, living conditions, limited management of domestic dog health, presence of free-roaming dogs and close proximity of dingoes, places large proportions of community populations at risk of exposure to several debilitating zoonotic diseases. Through the publication of all of these studies, in peer-reviewed journals, a substantial and important contribution has been made to the limited body of knowledge of canine zoonotic diseases present in, often over-researched, Indigenous communities. Dogs remain today as an integral part of Indigenous community culture and the health and treatment of dogs is fundamentally linked to the health and contentment of people within that community. Collaboration with local, experienced environmental health workers enables workable and sustainable animal and health management practices which ultimately benefit those people in the community.

At present some emerging and re-emerging wildlife-derived zoonotic parasites and diseases are not at the forefront of public concern. Much more emphasis, and government funding, is placed on conditions such as obesity and smoking related illnesses. Whilst these conditions are of enormous societal concern, planning for the effective management of emerging wildlife diseases will also benefit global ecosystem integrity and human health and well being (Polley, 2005). Pathogen evolution is driven by anthropogenic environmental changes and a comprehensive approach to the interface of people, domestic animals, wildlife and pathogens is needed, along with identifying the driving mechanisms that predicate each new emerging infectious disease (EID) emergence and predictive modelling of how these drivers shape or promote future EID emergence potential and/or risk (Daszak et al., 2013).

7.2 INTRODUCTION CHAPTER

Chapter 1 outlined the general aims of this thesis and the objectives that needed to be addressed to allow an understanding of the risk of canine zoonotic infections in Indigenous communities. The following discussion encompasses the outcomes of the research described in the thesis and how these match the general aims outlined in the introduction. Future research priorities will also be addressed along with recommendations for improved control and management of dogs and their diseases in Indigenous communities in the Wet Tropics.

7.3 CANINE ZOONOTIC DISEASES IN AUSTRALIAN INDIGENOUS COMMUNITIES

Chapter 2 addressed aims point (1), current status of research and presented a review of the literature regarding canine zoonotic diseases in Australian Indigenous communities. This revealed that in over 40 years of community research only 19 peer-reviewed articles met the criteria for critical appraisal and had been published. The review also revealed that the small number of comparative studies conducted in communities almost never included people and dogs from the same household. There is a lack of high-quality comparative studies to determine whether dogs are contributing to human disease by transmitting zoonoses. Whilst funding constraints make it difficult to invest time in building relationships and employing local health care and animal management workers when undertaking a study, the benefits of local knowledge are vital to ensure an accurate sample of the community as a whole and thus improving the quality, extent and impact of research results.

In chapters 3-6, I covered aims (2-5) by identifying the parasitic infection burden of dingoes and community dogs and the pathways, mechanisms and risk of transmission of infections to humans (particularly in Indigenous communities). I was able to identify several important zoonotic helminth parasites that had the potential to be debilitating to humans. By identifying these sources of potential harm and the causal pathways through which that harm may eventuate I established that the zoonotic potential of these

parasites should not be underestimated. Indigenous communities are at particular risk because of the limited management of domestic dog health and the presence of free-roaming community dogs that, in the case of *A. ceylanicum, A. caninum, T. canis* and *T. vulpis,* can be exposed to parasite eggs and larvae in soil contaminated by dingoes. Together with the warm, moist conditions of the tropics this provides an ideal scenario for the success of both soil-transmitted helminth infections and other infectious parasitic elements. These warm, moist conditions are also ideal for vector mosquitoes of *D. immitis*.

7.4 LIMITATIONS OF THIS THESIS.

Whilst every opportunity was taken to study the parasitic infections of dingoes and community dogs throughout my research it is with regret that I was not able to perform any investigation of human hookworm infections (e.g. cutaneous larva migrans) or pulmonary dirofilariasis in the residents of the communities studied. Although James Cook University human ethics committee and National Ethics Application Form (NEAF) applications were successful and accepted for a human study of the Mossman community, funding was rescinded and no longer available to me to complete this study.

My plan was to arrange for human faecal samples to be collected from patients with symptoms of gastrointestinal disease by Mossman Gorge Health Care Clinic and sent to Sullivan Nicolaides Pathology for testing as per normal procedure. Those samples (anonymous) which are microscopically positive for strongyle eggs would be sent to me to undergo further molecular biological analysis to identify the types of nematode parasites present, including *Ancylostoma ceylanicum*. In the event that I did positively identify *A. ceylanicum* I would notify the clinic and they, in turn, would notify the patient and request, on a purely voluntary basis, that they collect a faecal sample from their dog/s if dogs are present at their home. These samples would also have been sent to me for molecular analysis in order to determine the zoonotic risk of the parasite in the community.

Further research is necessary to investigate canine zoonotic diseases in the Wet Tropics. Many zoonotic diseases were not covered in this thesis and organisms that still need to

be studied due to their potential to be harmful to human health in Australian Aboriginal communities include the following: *Ancylostoma ceylanicum*, *Strongyloides stercoralis*, *Campylobacter*, Zoonotic *Salmonella*, *Streptococcal* spp., *Staphlococcal* spp., *Leptospira interrogans*, *Echinococcus granulosus*, *Dipylidium caninum*, *Spirometra erinacei*, *Cryptosporidium* and *Cystoisospora canis*.

7.5 THE RISK OF DINGO AND WILD DOG POPULATIONS TO AUSTRALIAN BIOSECURITY.

During the term of my PhD I co-authored a paper reviewing Australia's health security practices which suggests pre-emptive approaches to reduce the risk of outbreaks, invasion/spread and establishment, may be more valuable than the more reactive model that currently exists (Murray et al., 2012). Research into the influences on parasite flow from wildlife to people, including climate change, human intrusion into wildlife habitats and other potential causes of ecosystem disruption could further drive management capacity, particularly in the regions where threats arise, resulting in a model that is applicable both in Australia and in other regions of the world that value and therefore aim to improve their strategies for maintaining health security.

Australia is one of the few countries in the world today that remains free of the 'traditional' rabies virus. In 1997 rabies was detected on the Indonesian island of Flores, just 300 kilometres off the Australian north coast. Flores is linked by a chain of islands to Timor and West Papua. From there, direct access to New Guinea is possible. A further threat of the disease entering New Guinea is its presence on the island of Sulawesi which provides an even more suitable, more inhabited, island 'stepping-stone' avenue to the East. Elevating levels of natural disasters occurring in the region will lead to displacement and relocation of many people, their pets and associated diseases. Two years ago the rabies disease made its way west from Flores to Bali (Clifton, 2010). Approximately 20,000 dog bites a year are now reported in Balinese clinics. If the disease becomes established in Papua, Australia will be extremely vulnerable due to lack of early warning capability in Papua New Guinea (PNG) and movement through the Torres Strait. Even though the distance between PNG and the Australian mainland is

approximately 150 km, the northernmost inhabited Australian islands, Saibai and Boigu Islands, are only four kilometres from the PNG coast.

Rabies is a disease that has been proven difficult to eradicate elsewhere in the world. With Australia's wild dog and feral pig populations, and the country's geography, particularly in the Cape York area, eradication here would prove doubly difficult. Due to the sometimes long incubation period of the disease, up to six months in some cases, dogs may be transported quite a distance before any signs of the disease are noted.

Ineffective rabies control responses in Flores and Bali illustrates that a number of pre-requisites are required to mount a rapid and effective response to a rabies outbreak. Australia currently lacks many of these pre-requisites (Scott-Orr, 2010). Through post border surveillance, dog management programs and improved response capability it may be possible to mitigate the risk of rabies entering Australia. Dog management programs are already semi-established and accepted in the Torres Strait region. Given the political situation in PNG, pre-border risk reduction may prove ineffective however; expenditure of funds beyond our borders to build capacity would certainly improve risk management. By increasing the attention on the drivers of disease emergence, an overall strategy for disease anticipation, prevention, control and response can be devised.

7.6 MITIGATION MEASURES

Australia has seen dramatic changes in rural landscapes due to urbanisation over the past 100 years. While leading to a decline in biodiversity of some species others, such as the dingo, are attracted to these new peri-urban and urban habitats due to the availability of an abundant food supply and the presence of structures in which to shelter (Mackenstedt et al., 2015). Mitigation measures to reduce risk of disease transmission should include exclusion fencing of rubbish tips, public education of local zoonotic diseases along with instruction on living safely near wild dingoes. Information should be available for hunters regarding the correct disposal of animal carcasses and regular chemoprophylactic therapy of community dogs should be undertaken. Some of these points have been expanded upon below.

7.6.1 DOG HEALTH PROGRAMS

Healthy living conditions in Indigenous communities are fundamentally linked to the health and management of domestic animals, particularly dogs, within that community. Poor dog health and overpopulation has implications for both animal welfare and public health (Bradbury and Corlette, 2006). Over the past 30 plus years many dog health programs have been initiated in Indigenous communities involving various combinations of population and parasite control. One unofficial program was run by Jack Shield, a veterinarian with the Queensland Department of Primary Industries in Yarrabah from 1989 to 1994 (Speare, 2007).

Previously, a local veterinary clinic had offered mobile pet desexing services to the Yarrabah community. Often these services were well received, when well organised, and provided an excellent resource to tackle the ongoing concerns of dog overpopulation in the community. Unfortunately, due to rising costs and council concern over waste disposal, even though the veterinarian performing the surgeries was removing and disposing of all waste, this program was cancelled.

Whilst I was undergoing field work in the community I investigated the feasibility of utilising the Royal Society for the prevention of cruelty to animals Queensland's (RSPCAQld) new, state of the art, mobile desexing unit. The Portable Animal Welfare Service, or PAWS as it is affectionately named, is a mobile surgery designed for desexing cats and kittens, although we were assured it could also be used for dogs. Unfortunately, at a cost exceeding $14,000 for 10 days (including travel) it was out of reach for the community.

Animal Management in Rural and Remote Indigenous Communities (AMRRIC) is a Northern Territory non-profit association that is currently federally funded by the Department of Families, Housing, Community Services and Indigenous Affairs (FaHCSIA). AMRRIC has developed a best practice model for implementing dog health programs in Indigenous Communities. The basis of the program and the key to its success in the Northern Territory are due to several key components including, historical understanding, community engagement and local employment and training. In order for the AMRRIC model to work in Queensland and Torres Strait Island Indigenous communities it will require intentional government commitment and

funding. Unfortunately in 2015, reduced government funding in Yarrabah saw the forced redundancy of several local council Indigenous staff, including the ranger and an animal management worker.

The best and most sustainable solution that I found for pet desexing in north Queensland was a program offered by a local Cairns organisation. Animal Welfare Cairns is a small opportunity shop run by volunteers who raise money, from selling donated merchandise, to partially subsidise veterinary costs of desexing or microchipping pets. Government concession card holders, or low income earners, could obtain a voucher which would entitle them to vastly discounted desexing prices for their pet and the vets would be reimbursed the difference from this organisation. Approximately 2500 vouchers were distributed last year alone which equated to around $250,000 in subsidised veterinary fees. This information was made available through a community newsletter (Appendix 7). The Yarrabah community's biggest problem now is getting the dogs to the closest veterinary clinics offering this service. Talk, regarding a small air-conditioned van to shuttle pets back and forth, was in process upon the completion of my field work in the community.

7.6.2 DOG CARE DAYS

In collaboration with Yarrabah animal management, Queensland Health and the RSPCA, I conducted two dog health days in the community over two years.

The aims of the dog care day were:

- To provide free flea, tick, mange and worm treatment to dogs in the community.
- To provide information about the parasites infecting dogs.
- To provide information about new dog laws and registration in the community.

A large portable marquee was set up in the community to enable people and their animals to attend. Educational posters, microscopes and live specimens were also available to be viewed. The excellent turn out by the community in our second year can be partially attributed to having the marquee available at two sites, so that it was easily accessible. I provided veterinary consultation to 97 dogs and one cat. This was a great

improvement on the previous dog health day held by the same team of collaborators the year before where only 37 dogs were seen at the one locality. Better advertising in the community and promotion of the day was also undertaken in its second year.

As a result of these dog health days, and my ongoing presence in the community raising people's awareness of parasites, many people were interested in acquiring anthelmintics for their dogs. By reducing the parasite burden of dogs in the population the health and breeding capacity of the animal is enhanced thus, whenever parasite prevention or treatment is introduced to a community subsequent population control procedures must also be undertaken. Information regarding discount desexing of pets through Animal Welfare Cairns was made available to residents and ongoing euthanasia of stray dogs in the Yarrabah community was also offered as a service to the Yarrabah Aboriginal Council over a period of four years during my fieldwork.

7.7 FUTURE RECOMMENDATIONS FOR IMPROVED CONTROL AND MANAGEMENT OF DOGS AND THEIR DISEASES IN INDIGENOUS COMMUNITIES IN THE WET TROPICS AND NORTHERN AUSTRALIA

Although there has been much applied research on dingo and wild dog issues in other areas of Australia, there has been very little work undertaken in the Wet Tropics. While ecological, epidemiology and parasitology studies will help understand the nature and scope of the wild dog and dingo problem, the key to delivering applicable results, transferable to other regions, is the inclusion of extensive consultations with local environmental health officers and animal management workers to understand human values and attitudes relating to dogs and their diseases and the needs of the community. Following my research methods will enable effective wild dog management strategies to be developed based on a sound knowledge of wild-dog diseases and an understanding of the needs and demands of communities in the Wet Tropics of northern Australia. These strategies may then be developed further for the Torres Strait Island region where biosecurity risks of canid disease transmission from neighbouring PNG are growing. .

Over recent years the Yarrabah community has been trying to establish methods for effective dog and horse management. More recently, the local council has been

anticipating introducing new dog laws and dog registration to the community. Many meetings were held where we discussed the introduction of these new laws and the best way to police them. It was determined that further infrastructure was necessary before these laws could be implemented. Vast improvements were needed at the local dog pound as drainage was a major problem and a secure pound facility that met all welfare requirements was required for horses. Following the study including dingo and dog movements around the Yarrabah community (Chapter 6), the council requested that I provide some guidelines for how to implement these new laws, and subsequent dog and horse management methods, which I developed and adapted for Yarrabah from successful models used by other Australian councils (Appendices 8 and 9).

A coordinated and multifaceted approach is necessary for the future development of dog health and management strategies in north Queensland Indigenous communities. Councils must endeavour to employ, and maintain, local, well trained staff that are dedicated to animal management within the community and are knowledgeable about dealing with wildlife in the surrounding region. These people should include rangers, animal management workers and environmental health officers. Councils should also support veterinarians who have established good relationships with people in the community and made a considerable effort to provide a mobile desexing service rather than blocking these efforts and without providing suitable solutions.

Pet care and zoonoses awareness should be incorporated into school curriculums to encourage responsible pet ownership from an early age. Community input should be encouraged when developing these programs to enable capacity building at community level in order to increase responsibility and ownership of a dog management solution. Only then can culturally significant matters be dealt with along with other management issues.

My results demonstrate that dingoes carry a wide range of potentially zoonotic helminth parasites. Infection transmission to livestock or people is one of the potential threats of uncontrolled dingo populations. While attempting to establish a disease-free wild population is not a feasible option, understanding the ecology, movements and origins of dingoes is an essential basis for managing the threats they pose. Infectious disease is a normal feature of most animals and may even benefit the host population by regulating growth (Anderson and May, 1978). Also, in light of ever increasing

anthelmintic resistance in dogs, untreated dingoes may serve as an important refugium of unexposed parasites which may help to dilute out anthelmintic resistant strains (Pers. comms. E. Jenkins). Appendix 10 comprises some simple tips and advice that I wrote during my PhD fieldwork and distributed to people living in areas of Nth Qld where dingoes/wild dogs may be present to ensure the safety of not only families, domestic pets and livestock, but also for the safety and wellbeing of Australia's top terrestrial predator, the dingo.

7.8 CONCLUSION

Previously the link between dog health and human health in Indigenous communities has been lacking in scientific data support. By using a "One Health" approach and incorporating multiple disciplines such as veterinary parasitology and epidemiology with ecological techniques such as animal trapping and tracking and integrating the results, I was able to gain a better understanding of the nature of the dingo problem in the Wet Tropics. This project has determined the potential risk of spill-over of helminth infections from dingoes to dogs (and vice-versa) and the people in the community and provided important information to health care workers to enable them to address and manage identified risk practices within the community. The project has, for the first time, enabled animal managers to understand the extent of the dingo helminth infection risk, and its differing perspectives across the Wet Tropics landscape therefore; suitable strategic responses can now be devised for different communities and regions across North Queensland.

By firstly establishing the prevalence of pathogens and current status of infection in dingoes in northern Queensland I was able to determine the pathways and mechanisms which lead to the potential risk of transmission of infection among dingoes, wildlife, domestic animals and humans. I then used many different means to increase the public's awareness of the links between infectious diseases in wildlife, domestic animals and people. These included publications of all studies in peer-reviewed journals, dog health days, media interviews, conference presentations, information pamphlets, community newsletter, reports and recommendations to Aboriginal council and assistance with educational program development within the Yarrabah community. These steps enabled

me to meet the overall aim of this PhD project which was to investigate the potential risk of spill over of infection from dingoes to domestic dogs (or vice-versa) and people, and develop recommendations to mitigate this risk and for improved control and management of dogs and their diseases in Indigenous communities in the Wet Tropics of far north Queensland.

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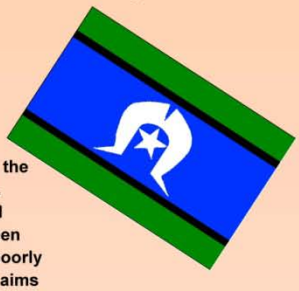
APPENDICES

APPENDIX 1 HEALTHY WILDLIFE, HEALTHY PEOPLE POSTER, NETHERLANDS 2010

Do populations of wild dogs threaten human health in Indigenous communities of the Wet Tropics?

Dr Felicity A Smout BSc BVSc (Hons) James Cook University, Townsville, Australia

European Wildlife Diseases Association
"Healthy wildlife, healthy people"
Vlieland, Netherlands, 2010



Background:

Wild dogs pose a potential threat to biodiversity conservation, the health of domestic animals and humans in the Wet Tropics. Indigenous communities are at particular risk due to limited management of domestic dog health. Currently the link between domestic dog and human health in Indigenous communities is poorly understood. By using a multidisciplinary approach this project aims to better understand the role of wild dogs in disease dynamics between domestic dogs and people in Indigenous communities, focusing on the Wet Tropics of Queensland.

Echinococcus granulosus

Dingo

Sarcoptes scabiei

Pilot studies have shown *E. granulosus* is present in wild dogs. This nematode can cause hydatidosis in humans and therefore is a possible 'spill-over' disease of significant concern.

Domestic dog

Spill-over vs Spill-back of Diseases

Domestic dog

Pilot studies have revealed no scabies infestation of wild dogs. This is a common disease in community dogs which poses a real threat for 'spill-back' of disease.

Methods:

Camera traps will be set to study wild dog presence and behaviour around communities and scats will be collected for disease analysis. Domestic dogs in the community will be given a veterinary health check and samples (faeces, blood, skin scrapings) will be taken for further disease analysis.

Additional samples will be collected from wild dogs trapped in control operations in the area. These dogs will provide more informative samples for disease analysis and parasite prevalence. Environmental samples will be collected from public recreational areas. Analysis of samples will be undertaken using conventional and molecular diagnostic tools to determine potential disease transmission.

Objectives:

Dog control and health management models will be developed with remote communities to improve human health, biosecurity measures and dingo conservation. These will be transferable to other regions, particularly to remote Indigenous communities in northern Australia.

The project will also encourage the capacity of communities to improve owners' dog management. By integrating ecological, veterinary and human health and social science into a systems approach, more targeted and cost effective control programs for wild dogs and their diseases will be developed in partnership with communities and government agencies. This project has the potential for significant impact because of its "one health" approach.

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APPENDIX 2 POSTER, ASP CONFERENCE, TASMANIA 2012

Do populations of wild dogs threaten human health in Indigenous communities of the Wet Tropics?

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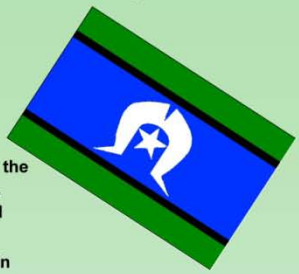
The Australian Society for Parasitology Inc.
Launceston, Tasmania. 2012



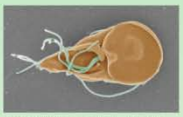
Background:

Wild dogs pose a potential threat to biodiversity conservation, the health of domestic animals and humans in the Wet Tropics. Indigenous communities are at particular risk due to limited management of domestic dog health and movements.

Currently the link between domestic dog and human health in Indigenous communities is poorly understood. By using a multidisciplinary approach this project aims to better understand the role of wild dogs in disease dynamics between domestic dogs and people in Indigenous communities, focusing on the Wet Tropics of Queensland.

Ancylostoma ceylanicum

Dingo



Giardia duodenalis

Pilot studies have shown *A. ceylanicum* is present in wild dogs. This nematode can cause a patent infection and disease in humans including blood loss, intestinal pain and cognitive impairment. It is therefore a possible 'spill-over' disease of significant concern.

Domestic dog

Spill-over vs Spill-back of Diseases

Domestic dog

Pilot studies have revealed zoonotic *Giardia* is present in community dogs. *Giardia* infection may be a significant public health issue in the community and may pose a real threat for 'spill-back' of disease.

Methods:

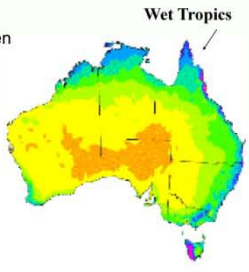
Camera traps and GPS collars are being used to study wild dog presence and behaviour around communities and scats have been collected for disease analysis. Some domestic dogs in the community will be given a veterinary health check and fitted with GPS collars to monitor their movement. Samples (faeces, blood, skin scrapings) will be taken for further disease analysis.

Additional samples have been collected from wild dogs trapped in control operations in the area. These dogs will provide more informative samples for disease analysis and parasite prevalence. Environmental samples have been collected from public recreational areas. Analysis of samples is currently being undertaken using conventional and molecular diagnostic tools to determine potential disease transmission.

Objectives:

Dog control and health management models will be developed with remote communities to improve human health, biosecurity measures and dingo conservation. These will be transferable to other regions, particularly to remote Indigenous communities in northern Australia.

The project will also encourage the capacity of communities to improve owners' dog management. By integrating ecological, veterinary and human health and social science into a systems approach, more targeted and cost effective control programs for wild dogs and their diseases will be developed in partnership with communities and government agencies. This project has the potential for significant impact because of its "one health" approach.



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APPENDIX 3 WAAVP PRIZE POSTER, PERTH 2013

First report of *Ancylostoma ceylanicum* in wild canids.

Felicity A. Smout[1], R.C. Andrew Thompson[2], Lee F. Skerratt[1]
[1]James Cook University, Queensland. [2]Murdoch University, Western Australia.

Ancylostoma ceylanicum male bursa — Photo: Felicity Smout

Introduction:

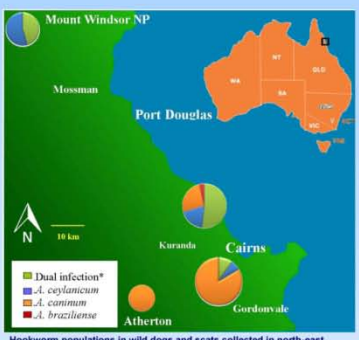
The parasitic nematode ***Ancylostoma ceylanicum*** is common in dogs, cats and humans throughout Asia and has previously been discovered in domestic dogs in Australia.

A. ceylanicum can cause a **patent infection in humans**. Studies have revealed that this parasite can cause severe abdominal discomfort and diarrhoea, as well as cognitive impairment, and should be considered to be of significant **zoonotic** importance.

Here, we look at the geographical distribution of hookworm species in the Cairns region of northern Australia and report for the first time *A. ceylanicum* in wild canids, specifically in the **dingo (*Canis lupus dingo*).**

Methods:

The study area was restricted to localities within the Wet Tropics World Heritage Area in north-east Queensland, Australia. Eighty-nine wild dog scats were collected over a 12 month period during 2010/11. Twenty-six wild dog carcasses were supplied by Cairns Council animal control officers and local landholders following routine control measures from 2007. Samples then underwent morphological and molecular identification.
No wild dogs were killed specifically for this study.



Hookworm populations in wild dogs and scats collected in north-east Queensland, Australia. (Circle size reflects the number of positive samples collected in the surrounding area; the segments indicate the composition of the population according to species, as represented in Table 1).
***Dual infection indicates infection with both *A. ceylanicum* and *A. caninum*.**

Results:

Table 1. Location and prevalence of hookworm species from wild dog scats and wild dog necropsies.

Parasite	Location	Number of positive samples (% prevalence)	
		Scats*/n	Necropsy†/n
Ancylostoma ceylanicum	MtWindsor NP	11/11 (100)	-
	Northern Cairns	17/25 (68)	2/2 (100)
	Southern Cairns	4/13 (30.8)	1/17 (5.9)
	Atherton	-	0/7 (0)
Ancylostoma caninum	MtWindsor NP	5/11 (45.5)	-
	Northern Cairns	19/25 (76)	2/2 (100)
	Southern Cairns	11/13 (84.6)	17/17 (100)
	Atherton	-	7/7 (100)
Ancylostoma braziliense	MtWindsor NP	0/11 (0)	-
	Northern Cairns	1/25 (4)	0/2 (0)
	Southern Cairns	0/13 (0)	0/17 (0)
	Atherton	-	0/7 (0)
Dual infections (*A. ceylanicum* and *A. caninum*)	MtWindsor NP	5/11 (45.5)	-
	Northern Cairns	12/25 (48)	2/2 (100)
	Southern Cairns	2/13 (15.4)	1/17 (5.9)
	Atherton	-	0/7 (0)

*PCR, † Morphological identification.

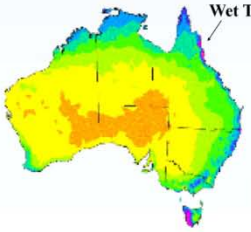
Discussion:

This study reports, for the first time, not only the presence of the hookworm *A. ceylanicum*, in wild canids and the first occurrence of this parasite in Far North Queensland, it also illustrates that it is the dominant hookworm species of wild canids in Mt Windsor National Park. Wild dogs used in this study were generally 'problem' animals killed in council control operations. They were frequently found to be utilising areas close to people and farms and therefore may have been in close contact with domestic dogs in which *A. caninum* is the dominant hookworm infection. There may be a spill-over of infection from domestic dogs that influences the hookworm species present in the wild dog population. In contrast, the majority of wild dog scat samples were found further afield in National Parks and rural areas, away from human habitats and in areas dominated by rainforest.

Dingo (*Canis lupus dingo*) — Photo courtesy Nic Papalia

Conclusion:

The zoonotic potential of this parasite should not be underestimated. Indigenous communities are at particular risk because of the limited management of domestic dog health and the presence of free-roaming community dogs that can be exposed to parasite eggs and larvae in soil contaminated by wild dogs. Together with the warm, moist conditions of the tropics this provides an ideal scenario for the success of soil-transmitted helminth infections.



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IJP International Journal for Parasitology Parasites And Wildlife

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APPENDIX 4 - SUMMARY OF ARTICLES INCLUDED IN CHAPTER 2 REVIEW.

Study, objectives and study design	Sampling method, size and ethical considerations.	Key findings
Welch & Dobson 1974 To outline the prevalence of antibodies to *D. immitis* in Aboriginal and Caucasian Australians in relation to *D. immitis* in dogs. Cross-sectional survey.	381 human participants. Including, random selection, 54 Caucasian subjects with clinical eosinophilia and five with parasitologically diagnosed *D. immitis* dirofilariasis as pulmonary coin lesions and a single ocular infection. 545 dog samples – selection method not stated. No ethical considerations mentioned.	There was a corresponding high prevalence of antibody and elevated antibody titres in the human populations in tropical regions where infection rates in dogs were high and associated with exposure to vector mosquitoes for long periods of the year. Aboriginal sera showed a higher frequency of anti-*D. immitis* antibodies at greater titres than similarly selected Caucasian sera. However, acute and chronic reactions to *D. immitis* have only been demonstrated among Caucasian Queenslanders. The authors suggest that Aboriginal Queenslanders develop a greater protective immunity to *D. immitis* than Caucasians because of their greater contact with the parasite and its vectors.
Welch *et al* 1979 Epidemiology of *D. immitis* and *T. canis* in Australia Cross-sectional survey	1150 dog blood samples for *D. immitis* 653 dog faecal samples for *T. canis* selection method not stated. Human blood samples were also collected from each of the study areas. Sample number not stated. No ethics mentioned. Thanks given to community councils for permission to work in Aboriginal Settlements.	Dogs of all breeds appeared equally susceptible to *D. immitis* with infection being more common in older male dogs. Found heartworm infection to be most common in dogs living in areas with close proximity to permanent bodies of water. *T. canis* infection was present in about 75% of dogs from all areas studied except in Central Australia where lower infection was seen. The prevalence of serum antibody to Dirofilaria antigens in man was proportional to the incidence of respective canine infections at each location.

Study, objectives and study design	Sampling method, size and ethical considerations.	Key findings
Kaminski & Green 1977 Reports the findings of a variant of *M. canis* causing tinea capitis in Aboriginal children from Maningrida. Cross-sectional survey	Purposive sampling of 83 children during clinics. Sampled both normal and abnormal scalps. 10 dog samples – selection method not stated. No ethics mentioned. Acknowledge the children, NT health and Aboriginal staff.	Found 11 children, four cats and two dogs with variant 'Manangrida' type *M. canis*. State that the cats and dogs at the settlement have been shown to be reservoirs of this fungus, which has caused ringworm of the scalp and body of Aboriginal children. No strains of typical *M. canis* were isolated at Maningrida.
Schnagl et al 1978 Report findings of Coronavirus-like particles in a survey of people in WA. Cross-sectional survey	15 dog faecal samples – selection method not stated. 760 faecal samples from children and 43 adults with and without gastroenteritis. No ethics mentioned. Acknowledged help of community and child health services.	Coronavirus-like particles were found in: 258/582 Aboriginal children, 36/178 non-Aboriginal children, 24/31 Aboriginal adults, 7/12 non-Aboriginal adults and 9/15 dogs. Infections were equally prevalent in those with or without symptoms of diarrhoea. The proportion of children who excreted the particles increased with age. The particles were indistinguishable between those found in humans and those found in dogs.
Schnagl & Holmes 1978 Report the findings of Coronavirus-like particles in dogs from Aboriginal communities. Cross-sectional survey	58 dog faecal samples – selection method not stated. Human samples also collected – selection method and sample size not mentioned. No ethics mentioned. Acknowledged help of community and child health services.	Coronavirus-like particles were found in: 24/58 dogs. These particles were indistinguishable in size and morphology from those found in a large number of human stool specimens also collected in the same areas at the same times. Due to few reports of canine enteric corona-viruses it is difficult to gauge how widespread and important they are, not only as a health risk to dogs, but also to humans.

Study, objectives and study design	Sampling method, size and ethical considerations.	Key findings
Meloni et al 1988 [letter] Report the findings *Giardia* spp. from Fitzroy Crossing. Cross-sectional survey	71 child faecal samples (age 1-6), 10 dog faecal samples. – selection method not stated. No ethics mentioned. Acknowledged help for collection of samples.	The prevalence of giardial infection was 32% in children. This high prevalence may be an underestimate as only single stool samples were collected and a direct smear was used to examine faecal material. All 10 dogs returned negative results for *Giardia*.
Hopkins et al 1997 Determine the potential of zoonotic transmission, by characterisation of *Giardia* isolates Cross-sectional survey	13 human faecal samples (mostly children under 6 years old) 9 dog faecal and intestinal samples of culled animals. No ethics mentioned. Acknowledged community health nurses	Dog isolates revealed 4 different groups. Groups 1 and 2 contained all of the human and 3 dog isolates, whereas groups 3 and 4 consisted entirely of *Giardia* samples recovered from dogs. The findings show that dogs can harbour all 4 genetic groups of *Giardia* isolates. It is possible that some *Giardia* genotypes observed in different hosts may represent discrete genetic subgroups capable of exhibiting some level of host adaptation therefore zoonotic transmission of *Giardia* infections between humans and dogs does not occur frequently in these communities.
Lee & Hampson 1994 Characterise previously published genetically distinct intestinal spirochaetes. Determine relatedness of isolates from different sources and look for epidemiological links. Cross-sectional survey	49 Aboriginal children 1 dog isolate that lived in the same community as the children – selection method not stated. Informed consent of children and parents from original paper when samples collected, however not stated in this paper.	Canine isolate was closely related to those of Aboriginal children who lived with the dog. Should be considered as a possible reservoir for spirochaetal infection.

Study, objectives and study design	Sampling method, size and ethical considerations.	Key findings
Lee & Hampson 1996 Determine prevalence of cultivable intestinal spirochaetes in Australian dogs and to assess the association with diarrhoea. Cross-sectional survey	76 dogs (Fitzroy crossing Aboriginal community, WA) 54 dogs (Perth refuge) 38 dogs (Veterinary hospital) 35 dogs (Jervis bay Aboriginal community, NSW) – selection method not stated. No ethical considerations mentioned.	18.7% of samples were positive for spirochaetes, refuge (24.1%) but no significant difference was found between locations. Stool samples were categorised: normal, abnormal and watery. Abnormal and watery samples were significantly more likely to contain spirochaetes than normal samples however these may simply be commensals flushed out by increased bowel movements. In contrast to Aboriginal communities, children in Perth were rarely colonised by intestinal spirochaetes, but city dogs had similar rates of colonisation to those in Aboriginal communities. These lower rates in urban children may be related to more hygienic facilities in the city.
Meloni et al 1993 Parasite survey to determine the prevalence of *Giardia* and other intestinal parasites in children, dogs and cats from Aboriginal communities in the Kimberley. Cross- sectional survey	385 human faecal samples (some repeat) Describes compliance rate: 1mth-3yrs (80%) 4-5yrs (50%) 6 or more (20%) Fresh dog faeces collected and small intestines from culled dogs. Selection method and sample size not mentioned. No ethics mentioned. Acknowledged community health nurses	This study has shown that children from Aboriginal communities in the west Kimberley region of WA, particularly in the age group one to five years, are commonly infected with intestinal parasites. The high prevalence rates of *Giardia* and other enteric parasites in this survey are indicative of poor living conditions and low levels of hygiene. The high prevalence of hookworm and *Giardia* infection in dogs is of potential zoonotic significance for humans in these communities.

Study, objectives and study design	Sampling method, size and ethical considerations.	Key findings
Jenkins & Andrew 1993 Report the number and species of helminth parasites present in dogs from an Aboriginal community in NSW. Cross-sectional survey	15 dogs necropsied. Convenience sample, dog culling created an opportunity. No ethics mentioned. Acknowledged community council and peoples support.	Several parasites of public health concern were recovered from the dogs including *Echinococcus granulosus* and *Ancylostoma caninum*. Dog faeces were abundant in areas commonly used by children for recreation. The authors suggested that an education program along with surveillance and treatment of dogs should be included in all community health programmes for Aboriginal people, because control of parasites is likely to be of direct benefit to the general health of the community.
Thompson et al 1993 Report the prevalence of parasites in cats and dogs from five Kimberley communities. Cross-sectional survey	188 dogs necropsied – selection method not stated. No ethics mentioned. Acknowledged community health nurses and council workers.	Several parasites of public health concern were recovered from the dogs including *Giardia* and *Ancylostoma caninum*. The authors state that this survey clearly demonstrates that dogs and cats constitute an important public health problem in Aboriginal communities. Children are already disadvantaged, with respect to nutritional status and poor hygiene, and attempts should be made to control the additional dangers of contracting infectious diseases form their pets.

Study, objectives and study design	Sampling method, size and ethical considerations.	Key findings
Walton et al 1999 Microsattellite typing method developed to explore whether *Sarcoptes scabiei* mites of dog and human origin in overlapping and geographically isolated populations are genetically different. Cross-sectional survey	16 humans from 15 households. Houses for study were selected through cases with uncomplicated clinical scabies or crusted scabies. 17 Dogs with mange from 12 households. Researcher and participant ethics explained.	Multilocus analysis shows that genotypes of dog-derived and human-derived scabies cluster by host species rather than by geographic location. Highly significant variability between all 3 loci for 4 crusted scabies patients in the same community but different households. Genotypes for mites from 3 puppies and 1 baby did not overlap at 2 loci. Results suggest that some genetic exchange between mite subpopulations found within a host family may be occurring. Current authors note: Reanalysis of the data using more appropriate methods showed that dog to human transmission occurred multiple times and was an important component of the epidemiology of human scabies.(Morrison, 2005)
Walton et al 2004[11] Extended 15 microsatellite marker system to study gene flow between sympatric host associated populations of scabies in NT Cross-sectional survey	14 human mites from NT communities. 9 dog mites from NT communities. – selection method not stated. No ethical considerations mentioned.	Using Cytochrome Oxidase subunit I sequence analysis phylogenetically separated the mites into three groups: A: humans - Panama B: humans - NT C: humans & dogs – USA and NT Authors state that microsatellite typing then performed on 15 microsatellite loci show that mites are different and this supports the previous 1999 study. No microsatellite typing was conducted on any of the NT dog isolates from the group C mixed human and dog isolates and authors suggest that group C is a result of a rare genetic exchange event.

Study, objectives and study design	Sampling method, size and ethical considerations.	Key findings
Palmer et al 2007 Establish prevalence, species distribution and risk factors associated with hookworm infection in dogs and cats in Australia. Cross-sectional survey	Canine faecal samples: 568 from refuges 766 from Vet clinics (without gastro) 57 from Aboriginal communities Grouped according to climatic zones (tropical, arid or temperate). Bulk of locations were from an arid zone Convenience sampling No ethical considerations mentioned.	This study detected *Ancylostoma ceylanicum* for the first time in Australia in 10.9% of the dogs found positive for hookworm. It was determined that dogs from refuges, dogs originating from a tropical climatic zone, dogs aged one year or less, and those dogs which had not received anthelmintics were significantly more likely to be parasitized.
Parkar et al 2007 Develop a reliable PCR method for characterization of Blastocysts and compare with invitro culture for the diagnosis of *Blastocystis*. Cross-sectional survey	10 dog faecal samples from central desert Aboriginal community – selection method not stated. Other human and animal samples. No ethical considerations mentioned.	The study reports that all isolates of *Blastocystis* isolated from primates and their human handlers at the Perth Zoo were placed within subtypes 1 or 5, as were 3 dogs and 1 human isolate from Thailand, and 1 dog isolate from Australia. A single *Blastocystis* isolate from a Thai human was shown to be 100% similar to an isolate from a dog living in the same community. Author states that this is the first study to provide molecular-based evidence supporting the zoonotic potential of *Blastocystis* in dogs in a natural setting.

Study, objectives and study design	Sampling method, size and ethical considerations.	Key findings
Hii et al 2011 Investigate the prevalence of spotted-fever group organisms (*Rickettsia felis*) in dogs using PCR assays. Cross-sectional survey	130 dog blood samples from Maningrida Aboriginal community Project approved by University of Qld animal ethics committee.	First study to report the presence of *R. felis* infection in indigenous community dogs in the NT. Positive prevalence was 2.3% was lower than those reported in pound dogs in SE QLD (9%). This was attributed to the transportation of blood using FTA cards and different DNA extraction technique which may have partially affected the prevalence detected in indigenous community dogs. There is no mention of pathogenicity of this parasite to dogs or humans. The authors state that further study is needed to determine the pathogenesis and pathogenic potential of *R. felis* infections in dogs.
Schrieber et al 2014 Report the findings of an identical strain of *Streptococcus dysgalactiae* subsp. *equisimilis* in a child and a dog. Cross-sectional survey	Opportunistic sample from a child when presented at clinic with pyodermal lesions. at the same time dogs were being sampled for streptococci. Researcher and participant ethics explained. Informed consent of children and parents.	Pharyngeal swabs collected from child and dog, cultured, morphologically and molecularly identified to reveal identical strain of SDSE demonstrating cross-species transmission can occur between humans and dogs. The author states that because the SDSE strain was not causing clinical disease in either child or dog, no comment could be made about pathogenicity of the strain in either host and further studies were required.

Study, objectives and study design	Sampling method, size and ethical considerations.	Key findings
Shield et al 2015 Combination of 7 parasite surveys to determine the prevalence of *Giardia* and other intestinal parasites in children from Arnhem Land. Cross- sectional survey	314 human participants July 1994 – October 1996. Dog numbers not stated. Ethical approval for this study was obtained from the Human Research Ethics Committee of the NT Department of Health and Menzies School of Health Research	Seven surveys for intestinal parasites were conducted by a quantitative formol-ether method on faecal samples. Serological testing was conducted for ***Strongyloides stercoralis*** and ***Toxocara canis*** IgG by enzyme-linked immunosorbent assays. Faecal testing indicated a very high prevalence of intestinal parasites, especially in schoolchildren. The decrease in percentage positive for hookworm over the two years was likely due to the albendazole deworming programme, and recent evidence indicates that the prevalence of hookworm is now low. However there was no sustained decrease in percentage positive for the other parasite species.

APPENDIX 5. CROWE CRITICAL APPRAISAL TOOL (CCAT) RESULTS.

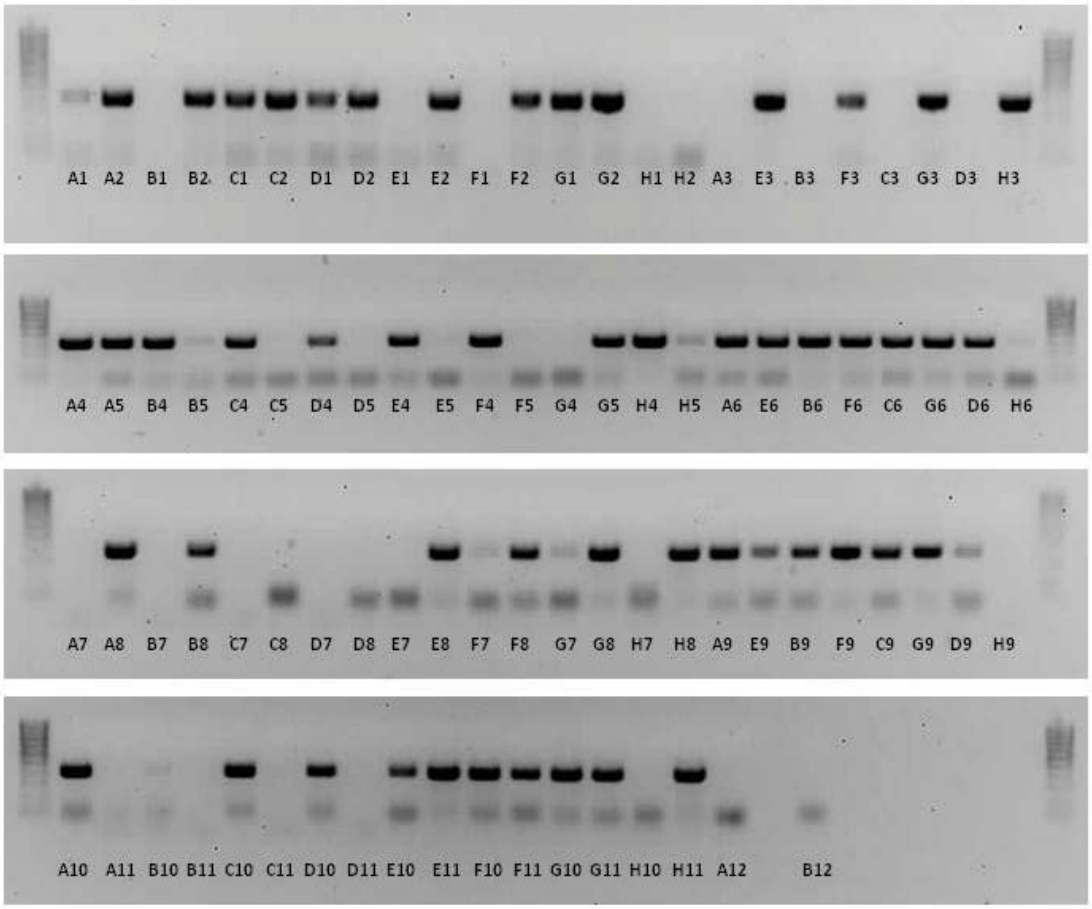
		Categories (1-8)*								
Author	**Year**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Total**
Welch & Dobson	**1974**	3	4	0	3	3	0	3	3.5	19.5
Kaminski & Green	**1977**	3	4	0	1.5	3	0	3.5	2.5	17.5
Schnagl et al	**1978**	3	3	0	2	2.5	0	4	4	18.5
Schnagl & Holmes	**1978**	2	3	0	0.5	1.5	0.5	3	3.5	14
Welch et al	**1979**	4	4.5	0	1.5	2.5	1	4.5	4	22
Meloni et al	**1988**	3	3.5	0	1.5	1.5	0	2.5	3.5	15.5
Meloni et al	**1993**	3.5	4	1	2	3	0	3	2	18.5
Jenkins & Andrew	**1993**	3.5	4.5	0	2	3	1	2.5	2.5	19
Thompson et al	**1993**	3	3.5	0	1.5	2.5	0	3	3	16.5
Lee & Hampson	**1994**	4	4.5	0	3	2.5	0	3	3.5	20.5
Lee & Hampson	**1996**	3.5	4	0	2	2	0	3	3.5	18
Hopkins et al	**1997**	4.5	5	1.5	2.5	4.5	0	4	5	27
Walton et al	**1999**	2.5	4.5	1	1.5	4.5	4	3	2	23
Walton et al	**2004**	3	4.5	1	0.5	3	0	4	3.5	19.5
Palmer et al	**2007**	3.5	5	0.5	1.5	3	0	3.5	4	21
Parkar et al	**2007**	3	3	0.5	1.5	4	0	3.5	4	19.5
Hii et al	**2011**	3	4	0	4	3	4	2	3	23
Schrieber et al	**2014**	4	5	1	2	4	5	3	4	28
Shield et al	**2015**	4	4	0	3	3	4	5	3	26

*Categories (1-8)

1. Preamble (Title, Abstract and text overall).
2. Introduction (Background and Objective).
3. Design (Research design, measure, bias).
4. Sampling (method, size, protocol).
5. Data collection (method, protocol).
6. Ethical matters (participant ethics and researcher ethics).
7. Results (analysis, integration, interpretation, outcome).
8. Discussion (interpretation, generalisation, conclusion).

APPENDIX 6. PHOTOS OF HOOKWORM AGAROSE GEL ELECTROPHORESES WITH DILUTIONS FROM LAB BOOK.

16/03/2012 Hookworm YBF 36-50, MUF 01-06, TD01,02,04,05 and dilutions



	1	2	3	4	5	6	7	8	9	10	11	12
A	YBF31	YBF39	YBF47	MUF05	YBF31	YBF39	YBF47	MUF05	YBF31	YBF39	YBF47	-VE POST
B	YBF32	YBF40	YBF48	MUF06	YBF32	YBF40	YBF48	MUF06	YBF32	YBF40	YBF48	
C	YBF33	YBF41	YBF49	YBF33	TD01	YBF41	YBF49	TD01	YBF33	YBF41	YBF49	
D	YBF34	YBF42	YBF50	YBF34	TD02	YBF42	YBF50	TD02	YBF34	YBF42	YBF50	
E	YBF35	YBF43	MUF01	YBF35	TD04	YBF43	MUF01	TD04	YBF35	YBF43	MUF01	NEAT
F	YBF36	YBF44	MUF02	YBF36	TD05	YBF44	MUF02	TD05	YBF36	YBF44	MUF02	1 IN 2
G	YBF37	YBF45	MUF03	-VE PRE	YBF37	YBF45	MUF03	MUF05	YBF37	YBF45	MUF03	1 IN 4
H	YBF38	YBF46	MUF04	+VE Duo	YBF38	YBF46	MUF04	MUF06	YBF38	YBF46	MUF04	

APPENDIX 7: YARRABAH COMMUNITY NEWSLETTER

Animal Antics

Hello everyone, my name is **Dr Felicity Smout** and I am a veterinarian and researcher currently completing a PhD with James Cook University on diseases in dingoes in Nth Qld and dogs in Indigenous communities. Many of you may already know me as I've been working in Yarrabah for the past 3 years, helping out with Dog Care Days and offering discounted veterinary services such as vaccinations, worming and mange treatments along with advice to owners on the care and wellbeing of their pets. I can often be seen working down at the Ranger Station. I also go out on house calls to Djenghi, Bukki and Reeves Creek as well as in the mission.

Part of my research involves trapping dingoes and fitting them with a GPS collar so I can record their movements around the community. Last November we trapped a female tan dingo and called her Xena. She has been spotted on the range several times since then, so if you see her we'd love to hear about it down at the Ranger Station. Remember, she is wild though so please don't approach her. I also have small trackers on a few of the local domestic dogs to see what they get up to when we're not looking. Remote camera traps out in the bush also record other animal's movements.

Discounted Desexing Available

Many local vets support the Cairns Animal Welfare discount desexing program aimed at people suffering financial hardship who otherwise can't afford costs like desexing. You can choose to take your pet to any vet clinic in Cairns and still receive the Animal Welfare discount price. Some of the benefits of desexing dogs and cats include reduced risk of diseases such as cancer of reproductive organs. Pets are less prone to wander and fight so fewer "cheeky" dogs. No more unwanted puppies or kittens and pets often live longer and healthier lives.

Here are a few local vets that you can ring right now to make an appointment to have your pet desexed at the Animal Welfare discounted prices below.

Gordonvale Vet Surgery - **Phone :**(07) 4032 9988

Centenary Park Veterinary Clinic - **Phone :**(07) 4045 5555

Earlville Veterinary Surgery - **Phone :**(07) 4054 1755

Cats – Female spey - $85, Male castration - $60

Dogs – Female spey 3 months old or less - $110, Up to 20kg - $125, Over 20kg - $135

Dogs – Male castration up to 20kg - $85, More than 20kg - $100, Over 30kg - $135.

APPENDIX 8: DOG REPORT AND RECOMMENDATIONS FOR YARRABAH ABORIGINAL COMMUNITY.

In order to establish good communication between council officers and members of the community on this matter, I feel it is imperative that information regarding the new council by-laws, relating to domestic dogs in the community, be widely released to allow the public plenty of time to understand how it will affect them.

Pamphlets explaining dog registration, costs involved, requirements for animal welfare etc., should be distributed to each household along with information on the benefits of desexing animals (see below). Posters should be placed around shops and schools to further explain these new laws. This should be an ongoing campaign to ensure no member of the community is caught unawares.

Members of the community should be given every opportunity to volunteer their animal to be re-homed if suitable or euthanased if not.

Registration discounts could apply to people receiving welfare payments as well as those who have had their animals desexed. Animals could be micro-chipped when they are registered and desexed to ensure ownership is known.

People who require more than the allotted number of dogs per premises should be made to explain their need for the extra animal/s and pay additional fees.

Community council housing, eg. fences, will have to be repaired and maintained in good condition to ensure the containment of all animals.

Following the introduction of these laws animals that are found wandering on the street should be seized by the ranger and taken to the dog pound. All avenues should be exhausted in order to try and locate and notify the owner. This may include placing photos of the animal around the shops and schools etc. The owner would then need to pay costs to release the dog from the pound and register the animal if unregistered or may choose to have the animal re-homed. If no owner can be located in the specified time (eg. two weeks) the animal will then be available to be re-homed if suitable or euthanased.

Pound facilities will need to be repaired and maintained to ensure they are suitable and meet all animal welfare requirements (at present this is not so).

I am happy to advise and assist in all of the above matters.

Dr Felicity Smout (BVSc)

Benefits of Desexing

There are many reasons why pet owners should desex their pets. As well as helping to stop pet overpopulation, the following are some of the other benefits associated with desexing cats and dogs.

Health

- Reduced risk of getting cancer or other diseases of the reproductive organs, such as testicular cancer, prostate cancer/disorders in males, and cystic ovaries, ovarian tumours, acute uterine infections and breast cancer in females, and also other diseases like mange, mammary cancer and peri-anal tumours.
- Females can suffer from physical and nutritional exhaustion if continually breeding.
- Pets generally live longer and healthier lives.

Behavioural

- Pets are less prone to wander, fight, and are less likely to get lost or injured.
- Reduces territorial behaviour such as urinating indoors.
- Less likely to suffer from anti-social behaviours. They become more affectionate and become better companions.
- Eliminates "heat" cycles in females and their efforts to get outside in search for a mate.
- Eliminates male dogs' urge to "mount" people's legs.

Cost

- Reduces the cost to the community of having to care for unwanted puppies and kittens in pounds and shelters.
- No additional food or vet bills for the offspring.
- No need to find homes for unwanted or unexpected litters of puppies or kittens.
- Save money from expensive surgeries from car accidents or fights, which are less likely to occur if your pet doesn't roam around.
- Dumping puppies and kittens is an ethical cost, as well as being illegal and inhumane.
- The price of desexing is more affordable to those in financial need.

Animal Welfare is a local Cairns charity which raises funds to help improve the welfare of animals in the Far North Queensland area and has a discount desexing program (half price) available for people suffering financial hardship who otherwise can’t afford essential pet ownership costs like desexing.

This locally organised campaign is supported by all the vets in Cairns, meaning you can choose to take your pet to any vet clinic in Cairns and still receive the Animal Welfare discount price. Just make sure you organise this in advance.

Yarrabah council could provide air-conditioned transport to ferry these animals back and forth to veterinary clinics which would ensure many dogs were desexed in a short period of time.

APPENDIX 9: HORSE REPORT AND RECOMMENDATIONS FOR YARRABAH ABORIGINAL COMMUNITY.

In order to establish good communication between council officers and members of the community on this matter, I feel it is imperative that information regarding the new council by-laws, relating to horses in the community, be widely released to allow the public plenty of time to understand how it will affect them.

Pamphlets explaining these new laws and requirements for animal welfare etc., should be distributed to each household. Posters should be placed around shops and schools to further explain these new laws. This should be an ongoing campaign to ensure no member of the community is caught unawares.

Members of the community should be given every opportunity to volunteer their horse to be sold and receive the benefits of that sale before new laws are enforced. This sale/s should take place outside the Yarrabah community (eg. Mareeba saleyards), to ensure a more public arena, which would result in receiving a better price for their animal.

Following the introduction of these laws, horses that are found wandering on the street should be seized by the ranger and taken to the horse pound at Balamba. All avenues should be exhausted in order to try and locate and notify the owner. This may include placing photos of the animal around the shops and schools etc. The owner would then need to pay costs to release the horse from the pound and show that they have a paddock which meets with council requirements (see below) to maintain the horse. The owner may choose to have the animal re-homed or sold. If no owner can be located in the specified time (eg. two weeks) the animal will then be available to be re-homed or sold with all benefits going to council.

Pound facilities will need to be repaired and maintained to ensure they are suitable and meet all animal welfare requirements (at present this is not so). Basic shelters will need to be erected along with reliable water source (not leaking) and feed (round bales of hay in correct feeders to stop wastage). Secure fencing, gates and padlocks will be necessary along with signage showing clearly that this area is the horse pound and trespassers will be prosecuted. Video surveillance could be set up using covert cameras such as those I used to study dingoes in the area. Security and police patrols could be maintained to ensure compliance of the public. Public liability insurance of this area should also be maintained to protect council.

As the situation stands currently, horses are roaming the community in full knowledge of the council, if an accident were to occur where a person was injured or even killed by a horse, Yarrabah council would be held highly accountable.

I am happy to advise and assist in all of the above matters.

Requirements for keeping a horse

- The minimum paddock size should be 2000m2 (half an acre) per horse with Yarrabah council approval of shelter, fencing, reliable water source and feed. Infant animals are not counted until the animals are 12 months of age or weaned.
- Stallions from two years of age should be kept in a Yarrabah council approved stallion enclosure.
- All owners need to apply for a permit to keep a horse with full details including full photographs of both sides and head of the horse showing all markings clearly.

Enclosure requirements

Enclosures must be maintained in a clean and tidy state and constructed to:

- Prevent the animal from escaping
- Not be within 10 meters of a neighbouring dwelling
- Not be within 10 meters of any premises operated for the purposes of the manufacture, preparation, storage or sale of food for human consumption.
- Enclosures for stallions must consist of double fencing around the perimeter of the area where the stallion is kept.

General requirements

The owner must ensure that keeping the horse/s does NOT result in:

- Animal faeces build up
- Breeding or harbouring of flies or vermin
- Damage to property
- Harm to human health or safety or personal injury
- Nuisance
- Significant disturbance, inconvenience or annoyance to a person's enjoyment of their place of residence.

Please note that the points above are my recommendation only and can be changed if you feel a more suitable requirement should be undertaken as long as it does not interfere with the animal's welfare.

APPENDIX 10: EDUCATION FOR PEOPLE LIVING IN REGIONS WHERE DINGOES FREQUENT.

1) Ensure you are not attracting dingoes to your home. Don't throw food scraps or used cooking oil out into your yard. Ensure proper disposal of rubbish, fencing of your yard, not leaving food out for your pet animals and not allowing your animals to roam freely. Sensor lights work well; even garden solar lights (around $1 each) will deter dingoes from approaching a house. Keep vegetation clear of the house so there is an area of open space between surrounding vegetation and the house.

2) Recently there have been some dingo sightings around the horse paddocks and I just wanted to try to allay any fears you may have for your horses. Dingoes are very efficient hunters and generally prey on smaller mammals such as bandicoots and wallabies. They are extremely unlikely to approach any of the horses and risk a kick when there is much easier prey around. Dingoes are also very territorial and may be viewed as actually protecting the horses by keeping domestic dogs away. Domestic dogs are far more likely to chase and bite horses as a game. They don't have to kill for their food and so have far more energy to waste on this type of behaviour.

3) People concerned about protecting breeding livestock from dingoes (and other predators) should seriously consider using livestock guardian animals. Dogs, donkeys and alpacas can all be used. This is a hugely under-utilised defence system against predator attacks. This is 24 hours, 7 days a week, defence. As a vet I have worked with well trained guardian dogs and have found that they are amazingly dedicated animals with their number one priority being protection of the livestock.

4) Utilisation of remote cameras and targeted trapping of a problem animal is highly recommended if there is a dingo that is causing problems. The use of 1080 and strychnine baiting is indiscriminate and does not achieve this and as a result, often leads to increased problems by killing the alpha male and/or female of the pack and leaving behind young who are less able to hunt wildlife effectively on their own and so may go for the easier option eg. someone's chickens. Also by trapping at the problem area, not out in the bush where there hasn't been a problem, you are more likely to trap the problem animal.

Two simple rules to keep everyone safe –

- Never approach a dingo
- Never offer food to a dingo.