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**Evaluation of One Health intervention
strategies against zoonotic canine
hookworms and threadworms in remote
Aboriginal and Torres Strait Islander
communities in Northern Australia**

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Doctor of Philosophy

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degree of Doctor of Philosophy

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Abstract

Dogs occupy many important roles in remote Australian Aboriginal and Torres Strait Islander communities, having practical, cultural, or spiritual significance. Due to the remoteness of these communities and often limited funding, access to veterinary services may be sporadic to non-existent. As such, animal health is frequently poorer compared to urban settings and infections with parasites may become more prevalent. Zoonotic soil-transmitted helminths (STHs) such as hookworms and threadworms are parasites capable of causing severe illness in dogs and people. This represents an important One Health challenge requiring evidence of its scope along with effective, cost-effective and culturally responsive interventions. However, the evidence needed to address this issue is sporadic or non-existent. The aims of this thesis are to address these knowledge gaps by providing evidence of the true prevalence of STHs in these settings, evaluating animal health program impacts, and assessing the efficacy, effectiveness and cost-effectiveness of parasite treatment programs.

Firstly, a systematic review and meta-analysis identifies the parasite species, host species and locations involved in STH infections in remote Aboriginal and Torres Strait Islander communities. Concerningly, this evidence shows continued endemicity in these settings featuring prevalences of 18.9% (95% CI 15.8-22.1) for *Strongyloides stercoralis* and 77.3% (95% CI 63.7-91.0) for *Ancylostoma caninum* infections in dogs and humans despite concerted human treatment program efforts over the last one hundred years.

An evaluation of a long-term annual animal health program in West Arnhem Land demonstrates that improvements to body and skin condition of dogs is possible alongside reductions in the numbers of dogs per household. Furthermore, the number of dogs per household represents a significant risk factor for canine hookworm infection, with dogs living in a household with three or more dogs having 7.01 times (95% CI 1.99 to 24.69) the odds of being *A. caninum* positive compared to dogs from a household with less than three dogs, indicating that sustained animal health programs can have

broad effects within a One Health framework. However, both canine hookworm and threadworm were found in high prevalence of 86.9% (95% CI 80.4 to 91.8) and 14.4% (95% CI 9.6 to 20.9) respectively, indicating that annual treatment programs are not effective in reducing prevalence and that evidence-based treatment programs are needed.

A comparative field trial assessed the short-term efficacy and medium-term effectiveness of three endoparasite and three ectoparasite treatment programs, finding that oral off-label ivermectin was the most efficacious and effective with a cure rate of 89.7% (95% CI 73.6-96.4) compared to spot-on moxidectin and oral oxbendazole for canine hookworm. However, ivermectin was not suitable for ectoparasite treatments, while oral afoxolaner and imidacloprid/flumethrin collars were effective.

Finally, a cost-effectiveness analysis using a local government payer perspective assessed the cost per dog free of canine hookworm and ectoparasite infection for each treatment over a four-year time horizon. Off-label ivermectin was the most cost-effective treatment for canine hookworm with an annual cost of \$22.85 per dog treated, while imidacloprid/flumethrin collars were the most cost-effective option for ectoparasites in this setting. Sensitivity analyses supported these results, and prevalence modelling indicated that community elimination of canine hookworm may be possible with ongoing three-monthly ivermectin treatment.

This thesis highlights the importance of cultural responsiveness, community leadership, meaningful engagement, and sustainable animal health programs. Sustained regular animal health program visits balancing available resources and community needs are recommended, as are thorough record keeping, program evaluation, and the use of effective and cost-effective parasite treatments such as ivermectin. Working within a One Health framework, remote community animal programs stand to greatly improve the wellbeing of dogs as well as Aboriginal and Torres Strait Islander peoples.

Declaration

This is to certify that the work described in this thesis is original work conducted by the author between February 2019 and November 2024. Due acknowledgement has been made in the text where other materials have been used or occasional assistance received. The thesis is less than 100,000 words in length, exclusive of tables, figures, references, and appendices. No part of this thesis has been submitted for any other degree or diploma.

Cameron Raw

Date: 02/11/2024

Thesis disruption statement

Field work was an essential component of this thesis, with aims targeted at delivering tangible outcomes and policy advice for remote Aboriginal and Torres Strait Islander communities. I experienced significant disruptions to planned field work due to the effects of the COVID-19 pandemic, however.

With field work commencing in mid-2019 in three remote Aboriginal communities in Arnhem Land in the Northern Territory, original plans were to continue prevalence surveys in these communities and to progress to field efficacy and cost-effectiveness studies in 2020. However, in March of 2020 Melbourne was placed in COVID-19 lockdown for 43 days, while the Northern Lands Council in the Northern Territory enforced an immediate ban on all non-essential travel to remote Aboriginal communities. This ban continued until June, with two-week quarantine restrictions continuing to be imposed on any arrivals to the Northern Territory. A further 111 days of lockdown were imposed in Melbourne between July and October, along with further restrictions on any travel from Melbourne to the Northern Territory.

These ongoing restrictions and lockdowns represented barriers to conducting the fieldwork necessary to address the aims of this thesis but were crucial to protecting the health of Aboriginal and Torres Strait Islander people in remote communities. A major reassessment of research project planning was necessary in May of 2020 due to these ongoing disruptions. After consulting with my supervisory panel, it was concluded that ongoing research in Arnhem Land would be suspended until such time that remote community health could be ensured and restrictions on travel eased. As such, alternative research arrangements would need to be made, representing a major change to the research conducted for this thesis.

In place of field work in Arnhem Land, a new project was proposed in consultation with community members in the Torres Strait Islands which would allow efficacy and cost-effectiveness field trials to proceed. However, the state of Queensland which governs

the Torres Strait Islands did not open its borders to other states until February 2021. Field work commenced in March 2021, with a six-month field trial initiated. At the planned six-month follow-up point in September, Melbourne was in its sixth COVID-19 lockdown of 77 days, meaning that I could not conduct field work at this crucial point, and rapid changes were necessary to recruit a veterinarian from Tasmania to complete sampling in Torres Strait Islander communities.

COVID-19 lockdowns and travel restrictions had profound impacts on the original research plan for this thesis and required a new research project to be developed in communities with whom I did not have an established relationship. This process required creativity, cultural humility, and flexibility, which was often required at very short notice. Nonetheless, all thesis aims were addressed and practical outcomes and policy advice were delivered for remote Aboriginal and Torres Strait Islander communities.

Preface

Publication status of research forming part of this thesis

Chapter 2: published by PLoS Neglected Tropical Diseases on October 24, 2022

Raw C, Traub RJ, Zendejas-Heredia PA, Stevenson M, Wiethoelter A (2022) A systematic review and meta-analysis of human and zoonotic dog soil-transmitted helminth infections in Australian Indigenous communities. PLoS Negl Trop Dis 16 (10) DOI: [10.1371/journal.pntd.0010895](https://doi.org/10.1371/journal.pntd.0010895)

Author	Contributions
Cameron Raw	Conceptualisation, data curation, formal analysis, investigation, methodology, project administration, validation, visualisation, writing – original draft preparation, writing – review and editing
Patsy A Zendejas-Heredia	Validation and writing – review and editing
Mark Stevenson	Formal analysis, supervision, writing – review and editing
Rebecca J Traub	Conceptualisation, formal analysis, methodology, project administration, supervision, writing – review and editing
Anke Wiethoelter	Conceptualisation, data curation, formal analysis, methodology, project administration, supervision, writing – review and editing

Chapter 3: prepared for submission to the Australian Veterinary Journal

Raw C, Tudor E, Traub RJ, Wiethoelter A. Impact of sustained dog health programs and risk factors for canine soil-transmitted helminth infection in remote Aboriginal communities in West Arnhem Land

Author	Contributions
Cameron Raw	Conceptualisation, data curation, formal analysis, investigation, methodology, project administration, validation, visualisation, writing – original draft preparation, writing – review and editing
Elizabeth Tudor	Conceptualisation, investigation, project administration, supervision, writing – review and editing
Rebecca J Traub	Conceptualisation, methodology, supervision, writing – review and editing
Anke Wiethoelter	Conceptualisation, formal analysis, methodology, project administration, supervision, writing – review and editing

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Raw C, Traub RJ, Wiethoelter A (2024) A comparative field efficacy trial of three treatment programs against endo- and ectoparasites in naturally infected dogs. Front Vet Sci 11:1460452 DOI: [10.3389/fvets.2024.1460452](https://doi.org/10.3389/fvets.2024.1460452)

Author	Contributions
Cameron Raw	Conceptualisation, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, validation, visualisation, writing – original draft preparation, writing – review and editing
Rebecca J Traub	Conceptualisation, funding acquisition, methodology, project administration, supervision, writing – review and editing
Anke Wiethoelter	Conceptualisation, formal analysis, methodology, project administration, supervision, validation, writing – review and editing

Chapter 5: in revision following peer review by EcoHealth

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Author	Contributions
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Anke Wiethoelter	Conceptualisation, project administration, supervision, writing – review and editing
Rebecca J Traub	Conceptualisation, funding acquisition, project administration, supervision, writing – review and editing
Virginia Wiseman	Conceptualisation, supervision, writing – review and editing
Caroline Watts	Conceptualisation, data curation, formal analysis, methodology, supervision, validation, writing – review and editing

Conference presentations and seminars arising from work in this thesis

Raw C (2020) Dogs, worms and long days in the home study. Australian Society for Parasitology Conference Online

Raw C (2022) Poor efficacy of single-dose oxibendazole against canine hookworm in a field setting. International Congress of Parasitology (ICOPA), World Federation of Parasitologists, Copenhagen, Denmark

Raw C (2023) One Health applications in Indigenous communities. Recent Advances in Emergency Animal Diseases Annual Symposium, Australian Centre for Disease Preparedness, Geelong

Raw C (2024) One Health perspectives in Aboriginal and Torres Strait Islander communities. Applying One Health Principles to Wildlife Management Symposium, Taronga Conservation Society, Sydney

Raw C (2024) A One Health intervention for the control of zoonotic soil-transmitted helminths in remote Australian Indigenous communities. Biomolecular Horizons, Melbourne

Raw C (2024) Evaluation of One Health intervention strategies against zoonotic canine hookworms and threadworms in remote Aboriginal and Torres Strait Islander communities in Northern Australia. PhD completion seminar. Melbourne Veterinary School, University of Melbourne

Raw C (2024) Cost-effectiveness of antiparasitic interventions for the treatment of *Ancylostoma caninum* hookworms, fleas and ticks in dogs in remote Torres Strait Islander communities. International Symposium on Veterinary Epidemiology and Economics, Sydney

Raw C (2024) A comparative field efficacy trial of three treatment programs against endo-and ectoparasites in naturally infected dogs. International Symposium on Veterinary Epidemiology and Economics, Sydney

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List of abbreviations

AFO	Afoxolaner chew treatment group
AMRRIC	Animal Management in Rural and Remote Indigenous Communities
AP	Apparent prevalence
APVMA	Australian Pesticides and Veterinary Medicines Authority
CFF	Centrifugal faecal flotation technique
CI	Confidence interval
<i>cox1</i>	Cyclooxygenase 1 gene
cPCR	Conventional polymerase chain reaction
CR	Cure rate
DAG	Directed acyclic graph
DNA	Deoxyribonucleic acid
DS	Direct faecal smear
EHV	Equine herpes virus
ELISA	Enzyme-linked Immunosorbent assay
EPG	Eggs per gram of faeces
ERR	Egg reduction rate
FE	Formol-ether concentration method
FLU	Flumethrin/imidacloprid collar treatment group
GB	GenBank accession number
HIV	Human immunodeficiency virus
HTLV-1	Human T-Lymphotropic Virus Type 1
HVR	Hypervariable region
ICAM	International Companion Animal Management Coalition
IFAT	Indirect fluorescent antibody technique
ITS-1	Internal transcribed spacer region 1
IVM	Off-label oral ivermectin treatment group
L3	Third-stage larva
MDA	Mass drug administration
MOX	Moxidectin/imidacloprid spot-on treatment group
MpAd	Morphological identification of adult worms
NGO	Non-government organisation
NTD	Neglected tropical disease
OR	Odds ratio
OXI	Oxibendazole/praziquantel tablet treatment group
PCR	Polymerase chain reaction

PNG	Papua New Guinea
PRISMA	Preferred Reporting Items for Systematic reviews and Meta-Analyses
qPCR	Quantitative polymerase chain reaction
RNA	Ribonucleic acid
SD	Standard deviation
SE	Enzyme-linked immunosorbent assay serology
SSF	Saturated salt faecal flotation
STH	Soil-transmitted helminth
TP	True prevalence
WAAVP	World Association for the Advancement of Veterinary Parasitology

Chapter 1: Literature review

1.1 Introduction

Dogs fulfil a range of diverse and important roles in Australian Aboriginal and Torres Strait Islander communities. They may be companions, nighttime guards against unwanted people, animals or spirits, hunting partners or even members of the intricate kinship system (Smith and Litchfield 2009; Constable et al. 2010; Kennedy et al. 2021a). In contrast to some media reports citing large numbers of 'stray' dogs, most dogs in remote Aboriginal and Torres Strait Islander communities are owned and cared for, and the human-animal bond is strong (Constable et al. 2010; Gosford 2010; Hayes 2017). Nonetheless, it is evident to non-Indigenous visitors to communities, animal health practitioners and Aboriginal and Torres Strait Islander residents alike that the inequity in health outcomes described between Indigenous and non-Indigenous people is also apparent between dogs in Indigenous communities and urban counterparts (Constable et al. 2008; National Indigenous Australians Agency 2020; Bennett and Archer-Lean 2023). The cause of this disparity is multifactorial but may in part be explained by a difference in historical interactions between canids and Aboriginal and Torres Strait Islander people.

Dingoes (*Canis lupus dingo*), according to fossil records, have been present in Australia for at least the last 3450 years, although the exact timing and cause of introduction from Asia remains uncertain (Fillios and Taçon 2016). Subsequently, dingoes were widely adopted into Aboriginal and Torres Strait Islander communities as companions and hunting partners, spreading rapidly across the landmass of Australia and becoming an important part of cultural custom and everyday life (Wilks and Williamson 1998; Smith and Litchfield 2009; Constable et al. 2010; Shipman 2021). Dingoes were commonly taken as puppies for adoption into communities and were generally able to maintain independence from humans by hunting, with many returning to a wild existence once they reached maturity (Constable et al. 2010). Dingoes breed only once per year with an average litter size of four pups, most do not breed in their first year of life, and mating behaviours are reduced during drought conditions, leaving little risk of overpopulation and little requirement for action on the part of Aboriginal and Torres Strait Islander

people to maintain a sustainable dingo population (Catling et al. 1992; Bennett and Archer-Lean 2023).

Colonisation of Australia from 1788 brought with it the domestic dog (*Canis lupus familiaris*), which were not only adopted into Aboriginal and Torres Strait Islander communities as dingoes were before them, but also hybridised with dingoes (Newsome and Corbett 1982; Senior et al. 2006). While Western taxonomic systems separate dingoes from domestic dogs, Aboriginal and Torres Strait Islander culture frequently does not distinguish between the two in terms of description or interactions between people and canids (Bennett and Archer-Lean 2023). However, in contrast to dingoes, dogs and many dog-dingo hybrids are much more reliant on humans for provision of food and care and are capable of breeding twice per year with a greater number of puppies per litter (Catling et al. 1992; Smout et al. 2017a). The independent existence and sense of autonomy of dingoes has transferred to the management of dogs, where dogs are mostly free to roam, play, hunt, scavenge and mate throughout communities rather than being kept confined at their owners' property (Kennedy et al. 2018). Dogs may even travel between communities of their own volition (Dürr and Ward 2014; Molloy et al. 2017). The greater reproductive potential of dogs combined with unrestricted mating access and an inclination to remain close to humans can lead to considerable overpopulation in Aboriginal and Torres Strait Islander communities, many of which have little to no access to regular veterinary services (Wilks and Williamson 1998; Smout et al. 2017a). Poorer health outcomes associated with overpopulation include malnutrition from a lack of availability of food, injuries from dog fights and greater risk of infectious disease spread. Infectious diseases which may become widespread in these community dogs can include viral infections, bacterial agents, and range of parasitic organisms including roundworms, hookworms, threadworms, tapeworms, protozoa and ectoparasites. Many of these infections have zoonotic potential (Smout et al. 2017a; Cumming et al. 2022). While these consequences are acknowledged by Aboriginal and Torres Strait Islander community members, without regular access to veterinary services little can be achieved to combat these issues. As a result, community tensions can escalate, community disempowerment may develop

and in severe circumstances animal abuse may occur (Smout et al. 2017a; Kennedy et al. 2021a).

Veterinary visits can occur quarterly in some of the more serviced communities, to biannually or annually in others, with some receiving more sporadic services. Obstacles and challenges to veterinary service provision in Aboriginal and Torres Strait Islander communities can include limited funding of owners or local government organisations, logistical challenges related to remoteness or climatic patterns, or lack of availability of veterinary or paraveterinary personnel (Phelan et al. 2007). Some human health professionals assert that animal health programs have questionable public health benefits and that advocacy for their funding jeopardises the sustainability of Aboriginal health programs which are already underfunded (Currie 1995; Australian Human Rights Commission 2017; Smout et al. 2017a). Assessments of Australian Government efforts to address inequities in health between Indigenous and non-Indigenous people is reported annually in the Closing the Gap Report (National Indigenous Australians Agency 2020). To date, many efforts to close the gap have failed and are not on track to be achieved by the target of 2031. In many health metrics gaps have widened, with deficient or inappropriate resourcing being identified as a key contributor (Angell et al. 2017). While under resourcing of several Aboriginal and Torres Strait Islander health programs is evident in government reporting, animal health programs need not come at the cost of human health and may instead contribute to improved human health (National Indigenous Australians Agency 2020).

Aboriginal and Torres Strait Islander people suffer from disproportionately high rates of neglected tropical diseases (NTDs) compared to their non-Indigenous counterparts (Kline et al. 2013). One area of importance to both animal and human health is soil-transmitted helminth (STH) infections. Examples of such parasites are hookworms (including those of the genus *Ancylostoma* and *Necator*) and threadworms (including those of the genus *Strongyloides*), both of which can occur as a free-living stage in the soil when expelled in animal or human faeces. These genera are endemic throughout Oceania and can cause a range of disease manifestations from a mild gastrointestinal upset or anaemia to death in severely burdened animals or humans (Bradbury and Traub

2016; Page et al. 2016). It is uncertain whether STHs were present in Australia prior to colonisation, although contact with Macassan traders from modern-day Sulawesi in Indonesia may have been a source of original infection (Bradbury and Traub 2016). Several factors may favour high prevalence and zoonotic transmission of STH infections in remote Aboriginal and Torres Strait Islander community environments, including hot and humid climates in the tropical or subtropical North of Australia and the potential for high levels of environmental contamination due to large dog and cat populations. Resource and health-literacy inequalities faced in these remote Aboriginal and Torres Strait Islander communities along with overcrowding may also favour environmental contamination, and zoonotic and anthroponotic spread (Melody et al. 2016).

With clear health inequities between Indigenous and non-Indigenous people as well as between dogs in Indigenous communities and their urban equivalents, effective and culturally responsive interventions are essential for both populations. While independent approaches in each species may be impactful, infectious agents capable of infecting both human and canine hosts such as zoonotic STHs call for broader considerations. A One Health framework acknowledging the interconnectedness of human, animal and environmental health highlights the potential for zoonotic transmission, but also the strengths that may come about from integrated One Health interventions. However, if these interventions are to be adopted, evidence is first needed on the prevalence of zoonotic STH species likely to be of concern in remote Aboriginal and Torres Strait Islander communities to establish them as a priority in need of intervention. Prospective interventions must then be evaluated to ensure that they are effective in achieving their proposed aims, whether that be in reducing prevalence, reducing risks of infection or providing other forms of health care. If interventions are to be adopted in resource-limited settings such as those found in remote Aboriginal and Torres Strait Islander communities, economic evaluations are necessary to ensure that they are cost-effective and sustainable. While many infectious diseases may be prevalent in either dogs or people in remote Aboriginal and Torres Strait Islander communities, one of the most important groups capable of infecting both species are STHs, specifically hookworms and threadworms.

1.2 Hookworms

1.2.1 Overview

Hookworms infected nearly half a billion people globally as of 2010 according to a global burden of disease report, with an estimated average prevalence in the Oceania region of 47.9% owing largely to a 60.6% prevalence in Papua New Guinea (Pullan et al. 2014). First described in humans in Australia in 1889, hookworms have been found in humans, dogs and cats in all states and territories (Palmer et al. 2007; Palmer et al. 2008; Bradbury and Traub 2016).

Hookworm eradication efforts in the human population in Australia were at their most intensive during the 1919-1924 Australian Hookworm Campaign (Sweet 1924). While this control program was largely successful, its efforts focussed on eradicating infection in non-Indigenous populations and as a result hookworm remains endemic in many northern Aboriginal and Torres Strait Islander communities where housing, infrastructure, sanitation and healthcare inequalities still exist (Sweet 1924; Holt et al. 2010; Bradbury and Traub 2016; Melody et al. 2016).

In Australia, seven hookworm species of importance to human and animal health have been reported and are included in Table 1.1, though species identification and zoonotic capacity in some reports were based on egg or larval morphology and would require sequencing to confirm. In this literature review chapter, the parasite species identified in published literature are presented as reported despite potential misclassification due to the diagnostic methods used. *Ancylostoma duodenale* and *Necator americanus* infect humans, while *Ancylostoma caninum*, *Ancylostoma braziliense*, *Uncinaria stenocephala* and *Ancylostoma ceylanicum* infect dogs and produce cutaneous larva migrans in humans (Traub et al. 2021). *Ancylostoma ceylanicum* and *A. braziliense* also infect cats, with *A. caninum* and *U. stenocephala* infections seen in rare cases. In addition, *A. ceylanicum* is also capable of producing patent, egg-shedding infections in humans, and recent evidence suggests that *A. caninum* may also be capable of this, albeit rarely (Traub et al. 2008; Traub 2013; Inpankaew et al. 2014a; Ngcamphalala et al. 2020).

Ancylostoma tubaeforme infects cats only and has limited known zoonotic potential, with little skin penetration observed (Traversa 2012).

Table 1.1 Hookworm species infecting dogs, cats and humans in Australia

Hookworm species	Natural host(s)	Infection in humans
<i>Ancylostoma duodenale</i> ^a	Humans	Patent infection, humans primary host
<i>Necator americanus</i> ^b	Humans	Patent infection, humans primary host
<i>Ancylostoma ceylanicum</i> ^c	Humans, dogs, cats	Cutaneous larva migrans, patent infection, significant zoonosis
<i>Ancylostoma caninum</i> ^d	Dogs, cats	Cutaneous larva migrans, limited evidence of patent infection, eosinophilic enteritis
<i>Ancylostoma braziliense</i> ^e	Dogs, cats	Chronic cutaneous larva migrans or 'creeping eruptions', no evidence of patent infection
<i>Uncinaria stenocephala</i> ^f	Dogs, cats	No evidence of patent infection, cutaneous larva migrans
<i>Ancylostoma tubaeforme</i> ^g	Cats	No evidence of zoonotic transmission

^a Reynoldson et al. 1997; ^b Koehler et al. 2013; ^c Smout et al. 2013, Koehler et al. 2013; ^d Prociw and Croese 1990, Palmer et al. 2007; ^e Smout et al. 2013; ^f Palmer et al. 2007; ^g Palmer et al. 2007

Global distributions patterns for these parasites largely reflect the warm and moist environments that they thrive in during the free-living stage of their life cycle (Loukas et al. 2016). *Uncinaria stenocephala* is an exception, which favours more temperate climates and is thus less likely to be present in the more northern regions of Australia where most of the remote or very remote Aboriginal and Torres Strait Islander population live (Beugnet et al. 2018). This review focuses on zoonotic hookworms more likely to be found in dogs in remote Aboriginal and Torres Strait Islander communities, those being *A. caninum*, *A. braziliense* and *A. ceylanicum*.

1.2.2 Hookworm biology

Infection with *Ancylostoma* spp. in the definitive host occurs with the ensheathed third larval (L3) stage of the parasite present in the environment. Larvae may enter the body by cutaneous penetration of the host or, in the case of *A. caninum* and possibly *A. duodenale*, through the ingestion of contaminated soil or substrates (Bradbury and Traub 2016; Loukas et al. 2016). Following oral ingestion, larvae develop into adults in the small intestine (Landmann and Prociw 2003). Following cutaneous infection, larvae migrate via blood vessels or lymphatics to the heart and then the lungs through pulmonary arterioles. They then pass into the pulmonary alveoli and ascend the bronchial tree to the pharynx, where they are swallowed. Upon reaching the small intestine, larvae develop into adults which live in the intestinal lumen and attach to the intestinal wall and feed on blood (Bradbury and Traub 2016). Mature adults in the small intestine reproduce and eggs are passed in the faeces, which hatch in the environment after one to two days releasing rhabditiform L1 larvae. With favourable moist and warm conditions in the faeces or soil, L1 larvae undergo development through two moults to become the infective L3 stage, which can occur in five to ten days. Maximal larval survival rates occur between 20-30°C with maximum larval development between 28-32°C (Brooker et al. 2006). At humidity levels below 50% *Ancylostoma* eggs do not embryonate or hatch, whereas larval development is optimal between 80-85% humidity (Tandon et al. 1998). L3 larvae can persist under favourable conditions in the environment for three to four weeks before infecting their next host, completing the life cycle in a total of approximately six weeks (Beugnet et al. 2018).

Alterations to this life cycle can occur, leading to differences in prepatent periods or modes of transmission. Following penetration of the skin, larvae of *A. caninum* and *A. duodenale* may also migrate to organs other than the lungs including striated muscle and become encysted and lay dormant for months or years (Marsh and Lakritz 2023). They can later move to the gut and develop into adult worms, or in the case of a pregnant bitch or human, they can migrate to mammary tissues and be transmitted to neonates via milk. Larvae may also be ingested by and encyst in the tissues of paratenic

hosts such as rats or mice and remain infective when they are then eaten by a dog or cat (Beugnet et al. 2018).

1.2.3 Risk factors for infection

The warm and moist environmental conditions of Australia's tropical and subtropical regions have been highlighted as risk factors in both animal and human surveys of hookworm (Loukas et al. 2016). In a national survey, dogs and cats from tropical climates were found to be 5.6 and 8.4 times more likely to be positive for hookworm respectively, compared to those living in non-tropical climates (Palmer et al. 2008). In the Final Report of the Australian Hookworm Campaign in humans, Sweet described an increase in detected hookworm prevalence north of 32 degrees south latitude and in areas with greater than 40 inches (1016 mm) precipitation annually. Six surveyed districts with an average annual precipitation of 95.5 inches (2425 mm) had an average hookworm prevalence of 21.65%, in comparison to an average prevalence of 0.9% in six districts with an average of 30.3 inches (770 mm) annual precipitation (Sweet 1924). A study of faecal prevalence, distribution and risk factors associated with canine STHs contaminating urban parks found that 88.9% of dog parks in tropical regions were contaminated with canine STHs, compared to 30.8% and 36.8% in sub-tropical and temperate climate regions, respectively (Massetti et al. 2022).

Studies in nearby countries with climate patterns similar to that of Northern Australia have reported several factors associated with increased risk of *A. ceylanicum* infection in humans. Individuals in Malaysia who reported close contact with dogs and cats were shown to be 2.9 times more likely to be infected with hookworms than those who did not have close contact with dogs and cats (Ngui et al. 2012). The same study also reported that those walking barefoot were 5.6 times more likely to be infected with hookworm than those who wore shoes. A study in China reported high prevalence of *A. ceylanicum* in dogs and cats to be a risk factor for human infection (Chen et al. 2012). Walking barefoot and contact with dog faeces-contaminated areas are also implicated as potential causes in a series of 38 cases of human eosinophilic enteritis in Townsville,

Queensland, one of which was confirmed via adult worm morphology to be caused by *A. caninum* (Prociv and Croese 1990). Close contact with dogs and cats and the areas that they defaecate, as well as walking barefoot are daily occurrences in many remote Aboriginal and Torres Strait Islander communities. This may provide abundant opportunities for zoonotic transmission of *Ancylostoma* spp. infections in this setting, though risk factors for infection as well as prevalence in relevant host species remains unclear based on current literature.

1.2.4 Clinical manifestations

Initial clinical signs in dogs and cats are associated with the penetration of the skin by infective larvae which secrete collagenase, allowing breakdown of the basement membrane of the skin. Penetrative and migratory lesions may become inflamed, itchy, scaly or lead to secondary bacterial infection (Traub et al. 2021). Larval migration through the lungs and bronchial tree can cause coughing, signs of pneumonia and mild pulmonary haemorrhage presenting as epistaxis. Further clinical signs are caused by adult hookworms in the small intestine, with damage caused to the intestinal wall resulting in congestive haemorrhagic enteritis, which may present as diarrhoea with or without haemorrhage. Chronic weight loss and anaemia caused by L4 larvae and immature adults may ensue in higher worm burdens and can be particularly devastating to very young animals who may have been infected via the transmammary route (Beugnet et al. 2018). The degree of blood loss varies between *Ancylostoma* species in the acute phase of infection, with *A. caninum* leading to the most pronounced blood loss, followed by *A. ceylanicum* and *A. braziliense* (Miller 1968).

Human infection with *A. ceylanicum* has been demonstrated in both experimental and natural settings with adult worms isolated from the jejunum, which parallels human-specific hookworms such as *A. duodenale* and *N. americanus*. *Ancylostoma ceylanicum* has also been detected in the ileum, caecum and colon (Traub 2013). High burdens of infection may lead to anaemia as seen in cats and dogs. In some cases, human *A. ceylanicum* infection may present in a similar manner to *A. caninum*. Infections with *A.*

caninum can exhibit subclinical to severe clinical features, including abdominal pain, diarrhoea with haemorrhage, blood eosinophilia and eosinophilic enterocolitis. These clinical features have been found to be the result of only a single adult worm deemed to be *A. caninum* in all examined cases, though the species were not confirmed via sequencing (Prociv and Croese 1990; Walker et al. 1995). *Ancylostoma caninum*, unlike *A. ceylanicum*, appears only to attach to the more distal intestinal mucosa in the ileum, caecum and colon.

Upon cutaneous penetration, all species of hookworm larvae can cause pathology in the skin in humans (Traub et al. 2021). This can vary in severity from subclinical to a papular pruritic rash known as cutaneous larva migrans or, colloquially, ‘ground itch’ (Bowman et al. 2010). *Ancylostoma braziliense* has also been implicated in what is often a more pronounced dermatological manifestation known as creeping eruptions, which present as tortuous, raised pruritic tracts following the path of larval migration through the skin (Bradbury and Traub 2016; Traub et al. 2021). This has been thought to be a result of dog- and cat-adapted hookworms lacking the enzymes necessary to break through the basement membrane in human skin, thereby trapping them in superficial skin layers. There are no known cases of *A. braziliense* reaching or establishing patent infection in the human gut.

1.2.5 Diagnostic methods

Most early studies of hookworm prevalence in both animals and humans in Australia have relied upon coproscopic techniques for detecting hookworm infection and did not proceed to identify species involved (Sweet 1924). Identification and numeration of eggs in faecal specimens requires careful sample collection, preparation and storage to ensure that eggs are not damaged or allowed to hatch before coproscopic evaluation. Coproscopic evaluation of the intensity of hookworm infection has largely been performed via two techniques in which eggs can be counted – floatation and the Kato-Katz technique, with the former being found to be superior in terms of diagnostic sensitivity, egg per gram counted and detection of low egg burdens (Inpankaew et al.

2014b). Eggs of different hookworm species are indistinguishable microscopically, and therefore zoonotic infections may have been misattributed to anthrophilic hookworm species (Bowman et al. 2010). Similarly, non-patent and thus non-egg-shedding human infections with *A. caninum* are likely to have been missed due to the difficulty of diagnosis in those cases. Only through colonoscopy or careful examination of resected bowel to reveal adult worms have some of these elusive infections been definitively diagnosed (Prociv and Croese 1990; Croese et al. 1994; Walker et al. 1995; Croese et al. 1996). Some misidentification based on isolation of adult worms has also occurred, with the first apparent identification of *A. ceylanicum* in Australia a result of erroneous morphological classification of what was in time determined to be *A. braziliense* (Traub et al. 2007). Misclassification and difficulty in species identification of eggs has led to the widespread adoption of molecular techniques in identifying hookworm infections in both animals and people. However, previously published reports using diagnostic methodologies inappropriate for species-level identification remain unchallenged and present a sizeable knowledge gap and source of confusion.

Conventional and, to a greater degree, quantitative polymerase chain reactions (qPCR) have allowed rapid throughput of large numbers of samples and identification of multiple species of hookworms in parallel. These molecular methods also demonstrate greater diagnostic sensitivity for detection of hookworms in faecal samples when compared to coproscopic methods, particularly in cases shedding low numbers of eggs (Cools et al. 2019; Stracke et al. 2019). In high burden of infection, both qPCR and coproscopic methods demonstrate a diagnostic sensitivity of over 90% (Knopp et al. 2014; Cools et al. 2019). The high analytical sensitivity of qPCR in its ability to amplify small amounts of DNA make it useful in determining effectiveness of treatment programs (Papaiakovou et al. 2019). Quantitative PCR methods are also being used to determine the intensity of infection where detection thresholds are a direct result of the amount of target DNA present in the sample and have been found to correlate with egg counts per gram of faeces (Hii et al. 2018).

1.2.6 Zoonotic hookworm in animals in Aboriginal and Torres Strait Islander communities

In an Australian national study of gastrointestinal parasites of dogs and cats, the highest prevalences of hookworm in dogs were found in the Northern Territory (17.5%), Queensland (10.2%) and Western Australia (8.1%) (Palmer et al. 2007). Notably, samples sourced from dogs in Aboriginal and Torres Strait Islander communities had the highest prevalence of hookworm infection (14%) compared to those sourced from animal shelters (11.4%) or veterinary clinics (3%) (Palmer et al. 2007).

To date, *A. ceylanicum* has been detected via sequencing in canines in the Aboriginal communities of Mossman and Yarrabah in Queensland (Smout et al. 2017b). Furthermore, environmental contamination with hookworms has been detected in Mossman, Yarrabah and Lake Placid where soil samples have been found to be positive for *A. ceylanicum* and *A. caninum* (Smout et al. 2017b). *Ancylostoma caninum* has also been reported in canines in Aboriginal communities in northern Western Australia, northern Queensland, northern and central Northern Territory, and in northern and coastal New South Wales using a variety of coproscopic and molecular methods (Meloni et al. 1993; Jenkins and Andrew 1993; Palmer et al. 2007; Šlapeta et al. 2015; Smout et al. 2017b; Smout et al. 2018; Rusdi et al. 2018). Even on the outskirts of Aboriginal and Torres Strait Islander community settings the shared utilisation of resources such as food scraps and rubbish tips by dogs, wild dogs and dingoes provides opportunity for transfer of infection between these populations (Smith and Litchfield 2009; Bombara et al. 2017; Gabriele-Rivet et al. 2019).

1.2.7 Zoonotic hookworm in humans in Aboriginal and Torres Strait Islander communities

Evidence of human infections with *A. ceylanicum* in Aboriginal and Torres Strait Islander communities is limited. Two cases of human infection with *A. ceylanicum* confirmed via sequencing were reported in Perth and the Pilbara region of Western Australia (Koehler et al. 2013). The data from these samples were anonymised, however, and do not indicate if the infected individuals lived in Aboriginal communities. While *A. ceylanicum*

has not been directly documented in Papua New Guinea (PNG), case reports of returned Dutch defence force personnel and Japanese travellers using morphological and molecular methods, respectively, have demonstrated *A. ceylanicum* infections which were likely to have originated from PNG (Anten and Zuidema 1964; Yoshikawa et al. 2018). Given the similar climate in the Torres Strait Islands, the ongoing trading which occurs between PNG and these islands and the demonstrated capacity for disease spread via these routes, *A. ceylanicum* infection in Torres Strait Islander communities appears likely (Lawrence and Lawrence 2004; Bainomugisa et al. 2019).

Contrasting the limited reports of human *A. ceylanicum* infections, a series of cases reported eosinophilic enteritis associated with *A. caninum* identified via adult worm morphology in humans in Townsville, Bundaberg, Nambour, Brisbane, Ipswich and Lismore, although these cases were not described as coming from Aboriginal communities (Walker et al. 1995). Human cases of *A. braziliense* in Australia have not yet been reported in the scientific literature. Given the risk factors of a climate favourable to STH development, close contact of humans with dogs and cats and barefoot walking, it seems likely that northern Australian Aboriginal and Torres Strait Islander communities would have prevalence of zoonotic hookworms in humans similar to nearby countries. Published data to support this inference is lacking at present, however, and requires further investigation.

1.2.8 Treatment and control

Ancylostoma spp. are usually sensitive to benzimidazoles and macrocyclic lactones, which have formed the basis of most mass drug administration (MDA) control programs in humans and animals (Thompson et al. 2001; Gates and Nolan 2009; Jia et al. 2012; Kline et al. 2013). Other treatment classes used to treat hookworm infections have included pyrimidines, though these have had reported treatment failure (Reynoldson et al. 1997), and octadepsipeptides, although only the latter will treat migrating larvae (Beugnet et al. 2018).

Metrics commonly used to assess treatment efficacy for hookworms include cure rates (CR) and egg reduction rates (ERR) (Moser et al. 2017). Cure rates are calculated as a percentage in which the number of individuals positive for a parasite pre-treatment and negative post-treatment is divided by the total number of dogs positive for the parasite pre-treatment. The ERR is also calculated as a percentage in which the post-treatment count of eggs per gram of faeces is subtracted from the pre-treatment count and divided by the pre-treatment count (Geurden et al. 2022). A systematic review and meta-analysis of 56 randomised controlled studies concerning the efficacy of current drugs for the treatment of human STH infections found that single-dose albendazole had a higher CR and ERR against human hookworm infections (79.5% CR, 89.6% ERR) when compared to mebendazole (32.5% CR, 61.0% ERR), levamisole (10.3% CR, 61.8% ERR) and pyrantel pamoate (49.8% CR, 71.9% ERR) (Moser et al. 2017). Another systematic review and meta-analysis had similar findings for single-dosing regimens, but also included drug combinations such as single-dose albendazole-ivermectin or mebendazole-ivermectin (Clarke et al. 2019). Both combinations demonstrated a significantly higher efficacy against other important STHs such as *Strongyloides* and may show promise in regard to widening the scope of treatment strategies to other NTDs affecting Aboriginal and Torres Strait Islander communities (Heukelbach et al. 2004).

Ongoing human hookworm control programs have taken many forms at international, national and community levels. Some programs consist solely of regular anthelmintic administration as either blanket or targeted group treatments, while others also incorporate improvements to sanitation, education and treatment of associated anaemia (Bradbury and Traub 2016). The latter additions provide benefits not only to hookworm control but contribute to economies of scope as they also mitigate risk factors for several other NTDs. Published reports of efficacious and effective treatment and control programs for canine hookworms in remote Aboriginal and Torres Strait Islander are currently lacking and require investigation.

1.3 Threadworms

1.3.1 Overview

Strongyloides, commonly known as threadworms, is a large genus of over fifty species, most of which show substantial host specificity (Page et al. 2016; Barratt et al. 2019). Human infections are most commonly associated with *Strongyloides stercoralis*, but infections with *Strongyloides fuelleborni fuelleborni* have been documented in Southeast Asia and Africa and *S. fuelleborni kellyi* in Papua New Guinea (Olsen et al. 2009). As of 2017 an estimated 8.1% of people or 613.9 million people worldwide were infected with *S. stercoralis*, with disease presentations ranging from subclinical to severe and life-threatening (Bisoffi et al. 2013; Taylor et al. 2014; Buonfrate et al. 2020). *Strongyloides stercoralis* also infects dogs, while infections with *S. stercoralis*, *S. felis*, *S. planiceps* and *S. tumefaciens* have been documented in cats (Thamsborg et al. 2017; Wolcan et al. 2019). The validity of the determination of *S. tumefaciens* and *S. felis* as distinct species has been called into question in a review by Wolcan *et al.* of *Strongyloides* species capable of infecting cats. This argument asserts that the similar colonic nodule pathology, evidence of *S. stercoralis* being sequenced from colonic nodules, along with the limited and often incomplete morphological observations performed to establish these species do not provide enough evidence to definitively classify these as distinct species, particularly in the case of *S. tumefaciens* (Wolcan et al. 2019). To date, only *S. stercoralis* and *S. felis* have been reported in Australia, though the detection of *S. felis* was based on larval identification, hence molecular confirmation of this species is still needed (Speare and Tinsley 1987; Page et al. 2016; Thamsborg et al. 2017; Wolcan et al. 2019).

1.3.2 *Strongyloides* biology

The parasitic element of the *S. stercoralis* lifecycle begins with infection of the host with third-stage filariform larvae from contaminated environments. As with hookworm *Strongyloides* infect their hosts by cutaneous penetration or through oral ingestion of filariform larvae (Grove 1989). Larvae undergo migration via lymphatics or blood vessels

to reach the lungs, ascend the bronchial tree and are coughed up and swallowed. Larvae are also capable of undergoing visceral or connective tissue migration to reach the small intestine (Grove 1989). Once in the small intestine larvae moult twice to become adults, all of which are female. Adult females live in the submucosa of the small intestine and produce up to 40 eggs per day by means of parthenogenesis (Grove 1996). Rhabditiform larvae hatch from these eggs before being passed in stool. In the environment, larvae feed on bacteria and undergo several moults and develop to become either infective third-stage filariform larvae, thus completing the cycle, or free-living adult worms. Adult worms may be male or female and can reproduce sexually, however cycles of sexual reproduction cannot occur indefinitely in any *Strongyloides* species and is limited to one generation in *S. stercoralis* (Yamada et al. 1991; Ross et al. 2017). This may be critical to prevention and control strategies for this parasite as it implies that environmental contamination is finite in its potential duration. Larval offspring of sexual reproduction then develop to become infective third-stage filariform larvae, beginning their parasitic stage. Current literature has reported populations of *Strongyloides* spp. in China reproducing solely via parthenogenesis, while in other populations sexual reproduction is more common (Bradbury et al. 2021). Proposed explanations for these differences include host immune factors and climatic variation (Bradbury et al. 2021). Transmammary infection to puppies can occur if the bitch is infected late in gestation or during lactation (Thamsborg et al. 2017). Several species of flies have also been deemed capable of acting as vectors for *Strongyloides* spp. and their distribution may affect *Strongyloides* infection risks (Getachew et al. 2007).

The lifecycle of *S. stercoralis* is unique in that larvae hatching from eggs within the small intestine can develop to become infective L3 larvae, penetrate the intestinal wall, undergo visceral migration back to the lungs, and complete their life cycle within the same host. This mode of autoinfection can lead to chronic, life-long infections without the need for repeated external exposure (Grove 1989; Leighton 1990; Grove 1996).

The immune status of the host plays a role in reducing the proliferation of *S. stercoralis* autoinfective larvae with a window between infection and development of an immune response of approximately three months (Grove 1989). Larval development can become

unrestricted in immunocompromised individuals by means of immunosuppressive disease or administration of immunosuppressive drugs such as corticosteroids or chemotherapy (Grove 1996). This manifestation, referred to as hyperinfection, results from hundreds of adults producing thousands of autoinfective larvae which emerge synchronously and undergo visceral migration. In more complicated cases, termed disseminated strongyloidosis, these larvae migrate through the body freely to all organs often leading to multiple organ failure (Genta et al. 1986; Olsen et al. 2009). When penetrating the intestinal wall and migrating through tissues autoinfective larvae can also transport enteric bacteria which can propagate serious infections in multiple sites throughout the body (Grove 1989; Grove 1996). There is evidence that corticosteroids may stimulate the production of autoinfective larvae by accelerating development, and administration of these compounds has been implicated in over 60% of fatal cases (Genta 1992; Page et al. 2016). Hyperinfection occurs in approximately 1.5 to 2.5% of strongyloidosis cases in humans and while it can occur in immunocompetent people and animals, this is uncommon (Vadlamudi et al. 2006).

1.3.3 Risk factors for infection

Environmental conditions favourable to *Strongyloides* spp., like those of hookworms, are the warm and moist conditions of tropical or subtropical regions (Buonfrate et al. 2020). The restricted nature of their free-living stage means that they cannot live in the environment indefinitely, however. Free-living adult worms can survive in the environment for up to 10 days (Page and Speare 2016). Larvae of *S. stercoralis* can live for several weeks in moist soil and temperatures of 23-28°C, however cases of transmission have been reported occurring in climates with temperatures as low as 4°C or as high as 40°C (Speare 1989; Beknazarova et al. 2016a). Most of these reported cases in unusual climates occurred in poorer sanitary conditions which appears more important, particularly considering *Strongyloides'* ability to maintain chronic infection within the same host, thus reducing the reliance on free-living worms to spread infection (Beknazarova et al. 2016a).

Viral diseases, particularly those which compromise immune function, have been theorised to be a factor in the dynamics of *Strongyloides* infection and disease progression (Page et al. 2016). Human T-Lymphotropic Virus Type 1 (HTLV-1) infection has been the focus of several studies of comorbidities in Aboriginal communities due to its reported high prevalence of 36.8% in Aboriginal adults (Einsiedel et al. 2021). While HTLV-1 positive status does not appear to be significantly associated with an increased risk of serologically diagnosed strongyloidosis, it is a risk factor for hyperinfection and hospital admission for complicated strongyloidosis (Einsiedel and Fernandes 2008; Einsiedel et al. 2008; Einsiedel and Woodman 2010; Einsiedel et al. 2014; Einsiedel et al. 2016a; Einsiedel et al. 2016b). Somewhat surprisingly, a study of human immunodeficiency virus (HIV) infected individuals with strongyloidosis found that advanced HIV disease, as determined by low CD4⁺ cell counts, was not correlated with hyperinfection. Data suggested that increased CD4⁺ cell counts instead favoured direct development of infective larvae, whilst lower counts favoured the indirect free-living reproductive pathway (Viney et al. 2004; Olsen et al. 2009).

To date there have been conflicting conclusions drawn on the zoonotic relevance of *S. stercoralis* in dogs. While some molecular and epidemiological studies challenge the zoonotic potential of *S. stercoralis*, finding no correlation between the infection status of a dog and its owner, others have established the ability for human-to-dog host adaptation, making the converse adaptation a distinct possibility (Takano et al. 2009; Schär et al. 2014a; Thamsborg et al. 2017). Some reports contend that some strains are adapted to humans while others are adapted to dogs (Grove 1989), while others state that based on genotypic evidence it is likely that *S. stercoralis* originated in dogs and adapted to human hosts (Bradbury et al. 2021). A study in Cambodian villages isolated two distinct genetic clades in dogs; one found only in dogs and the other indistinguishable from *S. stercoralis* isolated in humans in the same area (Jaleta et al. 2017). Recent evidence revealed a human infection with what was thought to be a dog-only clade, suggesting the potential for interbreeding and genetic exchange between *S. stercoralis* populations (de Ree et al. 2024). This also highlights the substantial reservoir that dogs and cats may represent for zoonotic infections.

1.3.4 Clinical manifestations

Strongyloides stercoralis infection, while potentially serious and fatal, can be vague and non-specific in terms of clinical presentation in both dogs and humans. This can make diagnosis difficult as it may not be included in differential diagnosis lists initially or may be confused for other diseases with similar presentations. Clinical signs and symptoms of uncomplicated strongyloidosis may include gastrointestinal, cutaneous or respiratory signs (Beknazarova et al. 2016a; Chapman et al. 2024). The majority of strongyloidosis cases in dogs, cats and humans are asymptomatic, however, making control difficult in many cases (Page and Speare 2016; Thamsborg et al. 2017).

Initial clinical signs in dogs and humans, if evident, are related to infective larvae penetrating and migrating through the skin (Grove 1989). The only pathognomonic clinical feature of chronic strongyloidosis in humans is larva currens. This is described as a snaking, erythematous rash which progresses rapidly at 2-10cm per hour as larvae move through the skin, most commonly observed on the trunk or buttocks (Grove 1989). A cough or irritation of the throat may develop as the larvae reach the lungs and ascend the bronchial tree. Gastrointestinal signs develop as a result of the development of adult worms in the small intestine, with abdominal pain, nausea, anorexia, weight loss, diarrhoea or constipation known to occur (Beknazarova et al. 2016a).

Infections may be self-limiting and are often subclinical, though chronic infections can include a range of ongoing clinical manifestations depending on the number of autoinfective larvae and whether carriage of enteric bacteria has occurred (Grove 1989). Clinical signs associated with the gastrointestinal tract in humans and dogs may include ongoing diarrhoea, malnutrition, wasting, abdominal pain, ileus, melaena or haematochezia. A range of sequelae may develop as a result of transmitted enteric bacteria, including abscesses forming in the kidneys, liver or other organs which may also affect cardiac or nervous system function (Page and Speare 2016). Eosinophilia is present in 10-70% of chronic strongyloidosis cases but is unreliable as a diagnostic tool, particularly in hyperinfection where values may be normal despite large numbers of migrating autoinfective larvae (Fisher et al. 1993; Page and Speare 2016).

Mortality in cases of hyperinfection can be as high as 87% in humans, which is suggested in part to be because of often-delayed diagnosis (Olsen et al. 2009; Taylor et al. 2014). As in humans, dogs are more likely to succumb to hyperinfection if they are immunocompromised (Genta et al. 1986; Thamsborg et al. 2017; Buonfrate et al. 2017). Dogs experimentally infected with human strains of *S. stercoralis* have been found to have increased likelihood of developing hyperinfection and greater severity of disease. The same may be true of natural anthroponotic infection (Thamsborg et al. 2017).

1.3.5 Diagnostic methods

Most species of *Strongyloides* including *S. stercoralis* present a diagnostic challenge compared to many other soil-transmitted helminths as the diagnostic life cycle stage found in faeces is the larva rather than the egg. As such, diagnostic techniques designed to detect eggs will fail to detect this life stage, particularly those which rely on floatation of eggs in saturated solutions. While larvae can be viewed by direct faecal smear, this has poor sensitivity due to the low number of larvae shed in most infections unless hyperinfection is present (Page et al. 2016). The Kato-Katz method is a cheap, simple test, and the standard diagnostic method proposed by the World Health Organisation for soil-transmitted helminth detection but has poor diagnostic sensitivity in the detection of *Strongyloides* spp. infections (Page et al. 2016). As such, its use in surveys may contribute to the underestimation of *Strongyloides* prevalence worldwide (Taylor et al. 2014).

The Baermann technique, in which larvae can migrate out of faeces and concentrate in a collection funnel, can be used but may be challenging to scale in larger programs due to the large amount of faeces, equipment and time required (Knopp et al. 2014). Koga Agar plate culture allows for the development and concentration of larvae which can then be inspected microscopically for morphological classification or can be submitted for DNA extraction and molecular diagnostic analysis (Glinz et al. 2010; Schär et al. 2013). This method, however, relies on live larvae to be present in the sample which must be differentiated from hookworm larvae (Page and Speare 2016).

Conventional and quantitative PCR have been used more recently to detect *Strongyloides* spp. in stool and are becoming widely used in high-throughput survey methodologies in both human and animal studies (Mejia et al. 2013; Massetti et al. 2020). PCR targets are usually either the 18S rRNA small subunit, the 28S rRNA gene, the internal transcribed spacer region 1 (ITS-1), or the mitochondrial DNA cytochrome c oxidase subunit I (*cox1*) gene. PCR has shown differing results in terms of diagnostic sensitivity in different studies, with results ranging from 11.6% to 96.6% (Watts et al. 2016). Lower sensitivities may be due to low levels of larval shedding in faeces, often seen in chronic strongyloidosis, compounded by comparison to reference methods employing larval concentration techniques. Diagnostic specificity of this method is high, with results ranging from 85.7% to 100% (Verweij et al. 2009; Watts et al. 2016).

The products of *Strongyloides* spp. PCR assays may also be subjected to deep amplicon sequencing to allow differentiation of *Strongyloides* haplotypes. Hypervariable regions of the 18S ribosomal RNA allow genetic groupings to be determined based on single nucleotide polymorphisms (SNPs). Hypervariable region I (HVR-I) haplotypes I to XVII and hypervariable region IV (HVR-IV) haplotypes A to V have been reported thus far (Beknazarova et al. 2019; Barratt et al. 2019; Bradbury et al. 2021; de Ree et al. 2024). This method has revealed haplotypes found in several hosts, such as HVR-IV haplotype A which is associated with infective strains in humans and dogs and can consequently support the hypothesis of potential zoonotic or anthroponotic spread (Barratt et al. 2019; Bradbury et al. 2021). Sequencing analysis has also revealed infections with multiple haplotypes within the same host (Beknazarova et al. 2019). The greater variability found in the *cox1* gene allows for the generation of cluster dendrograms in which *Strongyloides* clades are revealed, providing further evidence of genetic groupings alongside HVR haplotypes (Barratt et al. 2019; Bradbury et al. 2021). As more haplotype sequencing information comes to light, a greater understanding of host-parasite interactions, global distribution and inter-host spread can be established.

One of the most widely used diagnostic tests in studies of human strongyloidosis in Australia has been enzyme-linked Immunosorbent assay (ELISA) serology which detects IgG antibody response to infection and therefore may not detect newly acquired

infections (Speare and Durrheim 2004). The value of serology in cases of strongyloidosis has been a source of discussion, and while some infections may be missed it appears to be useful in assisting with diagnosis and determining cure of infection via reduction in antibody levels following treatment (Speare and Durrheim 2004; Page et al. 2006; Page and Speare 2016; Buonfrate et al. 2017). Another benefit of serology in detection of strongyloidosis is that serum sample collection is often more acceptable to Aboriginal and Torres Strait Islander communities, as faecal sampling is a taboo undertaking for many residents. The usefulness of different diagnostic tests also varies depending on disease manifestation. More acute strongyloidosis may be very difficult to detect on stool microscopy, and easier to detect on serology as migrating larvae at the point of infection may have provoked an immune response. Conversely, in hyperinfection, larvae may be abundant on microscopy, however serology and eosinophilia may be negative in an immunocompromised individual (Page and Speare 2016).

1.3.6 Strongyloides spp. in animals in Aboriginal and Torres Strait Islander communities

There have been few studies of *Strongyloides* in animals in Australia to date, only a fraction of which are related to Aboriginal and Torres Strait Islander communities. Published studies do not name Aboriginal communities from which samples were sourced, but the region they describe includes known endemic areas of human strongyloidosis such as Arnhem Land in the Northern Territory and the Kimberley in Western Australia (Beknazarova et al. 2019; Beknazarova et al. 2020).

A survey of gastrointestinal parasites in the Northern Territory found *S. stercoralis* via sequencing in as many as 17.2% of samples, while a study of 464 urban and rural shelter dogs found a prevalence of only 0.2% via coproscopy (Palmer et al. 2008; Beknazarova et al. 2019). *Strongyloides felis* has been detected in 33.5% of cats in Townsville, which represent the only location of this parasite on mainland Australia, with no reports in Indigenous communities (Speare and Tinsley 1987). The species identification in this

study was made via larval identification however, and did not utilise sequencing to confirm a distinction from *S. stercoralis*.

1.3.7 *Strongyloides stercoralis* in humans in Aboriginal and Torres Strait Islander communities

Surveys of *Strongyloides* spp. prevalence have been inconsistent and shown varying results, possibly explained by the imperfect accuracy of chosen diagnostic techniques. Many surveys have not been published in peer-reviewed literature, but rather presented in conference proceedings or in reports to medical networks (Page et al. 2016). Many studies of *S. stercoralis* in Australia have focussed on Aboriginal communities in Northern Australia as locations of historic endemicity. Reports from these locations indicate prevalence from stool examination of up to 41% and seroprevalence up to 60% (Flannery and White 1993; Page et al. 2006). Even those studies which have not specifically focussed on Aboriginal communities have found higher rates of infection in Aboriginal populations. A 1993 study by Fisher, McCarry and Currie of strongyloidosis in the Northern Territory reviewed 2000 stool examination results covering a twelve-month period at the Royal Darwin Hospital. Of these, 68 patients tested positive to *S. stercoralis*, of which 64 (94%) were Aboriginal (Fisher et al. 1993). The prevalence of *S. stercoralis* in all known hosts in Aboriginal and Torres Strait Islander communities and the zoonotic potential of this parasite requires further information.

1.3.8 Treatment and control

Ivermectin is the treatment of choice for *Strongyloides* infections in both dogs and humans and has been shown to be more effective than other anthelmintics commonly used for STH eradication such as albendazole (Suputtamongkol et al. 2011; Hays et al. 2017; Paradies et al. 2019; Chapman et al. 2024). An unusual progression of chronic strongyloidosis in a human patient with concurrent HTLV-1 has been reported in which clinical disease and evidence of hyperinfection considerably worsened after initiation of ivermectin treatment, before gradual recovery (Choksi et al. 2016). Because of the

parthenogenic potential of female worms in the host, the intention of treatment must be complete eradication. Effectiveness of treatments against migrating larvae is generally lower, but unless hyperinfection is present, the number of migrating larvae is usually small (Mejia and Nutman 2012).

Given the efficacy and safety of ivermectin treatments, a 'treat and test' method is commonly used (Miller et al. 2018). In this approach treatment is given at the same time as a test is performed, therefore reducing the numbers of individuals lost to follow-up, which can be a critical factor in Aboriginal and Torres Strait Islander communities (Page et al. 2016). It is surprising that MDA programs using ivermectin in Aboriginal and Torres Strait Islander communities do not yet seem to have identified issues of anthelmintic resistance. This is theorised to be in part because of *Strongyloides'* parthenogenic reproduction limiting the spread of resistance genes (Taylor et al. 2014). The free-living stage of the lifecycle may also contribute by allowing a refugia of untreated worms to establish in the soil, thereby diluting resistance genes amongst this more genetically diverse population (Krolewiecki et al. 2013).

A systematic review by Miller *et al.* of barriers to control of strongyloidosis in Aboriginal and Torres Strait Islander communities identified five critical points for action. These included reporting protocols between health providers and communities; widespread testing of Aboriginal and Torres Strait Islander patients, especially those who are immunocompromised or in endemic areas; upskilling of medical professionals on the risk of strongyloidosis in Aboriginal and Torres Strait Islander communities; establishing of testing and treatment programs; and measuring prevalence at a community level, reporting this and acting on results (Miller et al. 2014). Community-directed education and health programs have reportedly been very effective as part of *Strongyloides* control programs, as well as empowering communities by reinforcing ownership of their own health outcomes (Miller et al. 2018). Important parts of these programs have included advocacy for the wearing of shoes, hand hygiene and education concerning lifecycles.

Given the low survival of free-living adults and larvae in the environment, even under favourable conditions, arguments have been raised for control strategies to focus on

reservoirs of infection (Beknazarova et al. 2017). A single larva in a host, it is reasoned, presents a much greater threat to a community's health than a single larva in the soil. However, mass drug administration approaches are usually reserved for human hosts and fail to consider the potential for zoonotic spread from dogs as reservoirs of infection (Beknazarova et al. 2017). Furthermore, mass drug administration trials have failed to eliminate infection on their own, despite high levels of participation, suggesting that treatment of only human hosts may not be the defining factor in control and elimination (Kearns et al. 2017; Hays et al. 2017). Environmental treatment with nematicides has also been proposed to control environmental transmission, although concerns of resistance arise in this case (Beknazarova et al. 2016b).

Strongyloides infection can lead to serious morbidity and mortality in Aboriginal and Torres Strait Islander communities as a consequence of hyperinfection and disseminated strongyloidosis, yet it is not on the Australian National Notifiable Disease List. This comes despite the variety of diagnostic tests available, relative ease of prevention and treatment without major side-effects and reasonable cost-effectiveness of these measures (Miller et al. 2014; Beknazarova et al. 2018). This, alongside the risk of missed diagnoses due to non-specific symptoms may mean that what little data is available in Australia may grossly underestimate infection prevalence in both dogs and people (Fisher et al. 1993; Page and Speare 2016; Gorgani-Firouzjaee et al. 2022).

1.4 Animal health programs in Aboriginal and Torres Strait Islander communities

Remoteness is a notable feature of many Aboriginal and Torres Strait Islander communities in the north of Australia. The Accessibility/Remoteness Index of Australia is utilised by the Australian Bureau of Statistics and defined as an objective measure of road distance to heavily populated urban centres with access to services (Glover and Tennant 2003). All of Arnhem Land in the Northern Territory as well as the Torres Strait Islands are classed as Very Remote under this system, highlighting the lack of accessibility of many services in these settings. For those living in remote Aboriginal and

Torres Strait Islander communities the nearest veterinary clinic may be a several-hour drive, a river crossing, or even a charter plane flight away. Inaccessibility of veterinary care may also be influenced by uncertainty between stakeholders over responsibility for animal health. In most Australian settings animal health is the direct responsibility of the owner. However, the free-roaming nature of dogs in Aboriginal and Torres Strait Islander communities draws animal health as well as its broader human health ramifications into a more public focus. Owners may also have limited income to contribute to animal health care (Markham and Biddle 2016). As such, remote community animal health programs operating within communities have been key to the accessibility of veterinary services and parasite control in these settings.

Animal health programs may operate with veterinarians providing services as individuals, as part of a clinic, in conjunction with educational institutions or as part of charity organisations such as Animal Management in Rural and Remote Indigenous Communities (AMRRIC) or Vets Beyond Borders. These services may be delivered as a local government employee, a contracted provider funded by local government or as a volunteer arrangement (Phelan et al. 2007). Most animal health programs focus on a core of surgical and/or chemical desexing, parasite control through treatment and preventative administration, and treatment of sick and injured animals (Wilks and Williamson 1998; Bradbury and Corlette 2006; Australian Veterinary Association 2012; Ma et al. 2020b). Community education on responsible pet ownership, animal diseases and zoonoses may also be included in these programs (Constable et al. 2011; Constable et al. 2013a; Constable et al. 2013b; Willis and Ross 2019).

Community engagement is highlighted as a key aspect of Aboriginal and Torres Strait Islander community animal health program in several published studies. Meaningful engagement has been noted as essential in terms of the efficiency of a program, ensuring that community needs are addressed and in contributing to the empowerment of communities in the health of their animals (Constable et al. 2011; Ma et al. 2020b; Kennedy et al. 2021a; Kennedy et al. 2021b).

1.4.1 Animal health program evaluation

Appropriate program evaluation is a key part of animal health programs in Aboriginal and Torres Strait Islander communities in refining practices as well as providing evidence of impact to communities and funding bodies. A scoping review of indicators used to evaluate dog population management revealed the use of 29 indicators under the categories of dog welfare, care provided to dogs, population density, risks to public health, public perception, rehoming of dogs, impacts of dogs on wildlife, and impact of dogs on livestock (Hiby et al. 2017). These categories are also used by the International Companion Animal Management Coalition in a document providing guidance on appropriate impact measures (International Companion Animal Management Coalition 2015).

Literature concerning the characterisation and evaluation of animal health programs in remote Aboriginal and Torres Strait Islander communities is limited, with only three reports of longitudinal program evaluations. The periods of time evaluated in these studies do not exceed four years, and varying measures of impact make comparison challenging. Evaluations covering this time frame may not reveal long-term impacts on animal health or account for the benefits of establishing ongoing relationship with communities (Phelan et al. 2007).

A study evaluating the impacts of a dog health program in the Northern Territory community of Wadeye over a 12-month period found that the proportion of desexed dogs increased from 17.6% to 44.3% over that time (Riley et al. 2020). The authors also found a 77% increase in the proportion of dogs with ideal body condition scores, and a 9% increase in the proportion of dogs with ideal skin scores. Another evaluation of a dog health program in thirteen Aboriginal communities in New South Wales between 2016 and 2019 found similar impacts with an increase of desexed dogs from 7.4% to 37.3%, though only one community was evaluated over the entire four-year period (Ma et al. 2020b). In contrast, an evaluation of a dog health program in Numbulwar in the Northern Territory between July 2004 and April 2006 reported no discernible trend in changes to the proportion of desexed animals throughout the evaluation, nor to the

number of dogs per house (Bradbury and Corlette 2006). Sporadic worsening of skin condition and higher incidence of scabies was also noted. While this was initially attributed to a transition from monthly to 3-monthly ivermectin treatments, further data revealed continuing fluctuations in line with seasonal variation indicating the substantial influence that seasonality can have in tropical settings and the necessity of longer-term evaluations.

Changes in incidence of people presenting to health clinics due to dog bites also featured in two of these evaluations, though reported impacts of dog health programs on this measure have differed. One study found no significant change in incidence over one year (Riley et al. 2020), while another found a significant reduction over four years in three communities (Ma et al. 2020b). Other reports of animal health programs have been published, though these report treatments administered or animals observed with no evaluation of program impact over time (Burleigh et al. 2015). The absence of long-term program evaluation in Australian communities, represents a knowledge gap in the impacts of animal health programs with enduring community relationships.

1.5 Health economics of hookworm and *Strongyloides* spp. control programs

Given that local governments or NGOs are most commonly responsible for the provision of parasite treatment programs for dogs in remote Aboriginal and Torres Strait Islander community settings, data on the cost-effectiveness of available treatments is of use. Local government income and thus expenditure budgets are primarily based on the number of properties from which rates can be collected. Areas designated as an Aboriginal community living area, however, are exempt for paying rates under the Northern Territory Local Government Act, meaning that very few properties in areas such as Arnhem Land are subject to rates collection (Northern Territory of Australia 2019). As such, local governments as well as NGOs may be reliant on sporadic grant schemes to support animal health and parasite treatment programs, meaning that

established cost-effective interventions are needed to have maximal impact with these limited funds.

Concerningly, no published economic evaluations exist for deworming programs in humans in Australia, let alone in Aboriginal and Torres Strait Islander community settings or among animals within those communities. A systematic review of the scope and quality of economic evaluations of Aboriginal and Torres Strait Islander health programs found fourteen studies eligible for inclusion, though none included evaluations of parasite treatment programs (Doran et al. 2022). The authors highlighted that while the included studies were of good quality, there is a dearth of economic evaluations in this setting which is essential in efforts to reduce health inequities for Aboriginal and Torres Strait Islander communities. However, economic evaluations from other countries provide insights into what may contribute to cost-effective approaches in Aboriginal and Torres Strait Islander communities.

Two major factors are shown to contribute to the cost-effectiveness of a treatment or control program for STHs; economies of scale, in which a greater number of treatments are given more efficiently thus reducing cost per treatment; and economies of scope, in which delivering multiple disease control activities together can increase efficiencies and reduce costs (Turner et al. 2016). Improvements to the cost-effectiveness of combined interventions may be seen even when the diseases or species being treated are vastly different. A study modelling the potential effects combining multiple neglected tropical disease programs in Madagascar found that providing treatments for wuchereriosos, schistosomosis, *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm infections could lead to substantial health, economic and education benefits, with a benefit-cost ratio of between 5:1 and 31:1 (De Neve et al. 2018). Similarly, in a study in Tanzania, an integrated health program delivered an MDA program for STHs including hookworms in humans as well as vaccination for rabies in dogs. This integrated approach resulted in significant time savings yet with similar coverage compared to each program delivered individually (Lankester et al. 2019). It also resulted in a 33% lower cost per deworming dose and a 16% lower cost per rabies vaccination.

Targeting of demographic groups may influence cost-effectiveness in human and animal interventions, both in terms of targeting groups with greater risk of infection or harnessing established distribution methods by integrating with other interventions. While school-age children have been the targeted group in many soil-transmitted helminth control programs internationally, questions have been raised as to the validity of this method and its ability to achieve the desired elimination of infection in populations (Turner et al. 2015). A study of this practice suggests that although transmission from children can be significant in many cases, the potential for adults to continue to act as reservoirs of infection is significant (Anderson et al. 2013). Evidence suggests that programs be assessed on a case-by-case basis as the parasites to be treated, prevalence in community, local engagement and other comorbidities will all play a role in determining the most appropriate course of action and which groups will be most cost-effective to target for treatment (Turner et al. 2015; Turner et al. 2021; Caesar delos Trinos et al. 2023). The dynamics of infection in different age groups of dogs and the cost-effectiveness of targeting different demographic groups are yet to be studied and currently do not have any known parallels to human research.

Another factor affecting the cost-effectiveness of STH treatment programs, particularly in long-term evaluations, is community coverage. Costs associated with transporting materials and personnel to remote areas may be substantial and may mean that interventions in remote locations are not cost-effective. While large numbers of people or animals can be treated quickly and inexpensively in densely populated areas, omitting remote areas from interventions raises issues of inequity (Turner et al. 2021).

Economic evaluations are valuable in decision making and policy development for any human or animal health program, but particularly in the resource-limited settings of remote Aboriginal and Torres Strait Islander communities. Whether payers are local governments, NGOs or owners in animal health programs, information is needed to guide decisions on cost-effective and appropriate treatments. Economic evaluations of treatment programs for STHs in remote Aboriginal and Torres Strait Islander communities represents an area requiring investigation given the lack of published data. Working with Aboriginal and Torres Strait Islander communities is crucial in these

evaluations and aids in establishing that treatment programs are not only cost-effective but also acceptable and feasible in these remote settings.

1.6 Thesis aims

The true prevalence of zoonotic STHs in Aboriginal and Torres Strait Islander communities remains unclear, and the lack of reporting of prevalence in all possible hosts makes an understanding of possible zoonotic transmission difficult. Previously published data using a range of diagnostic tests with imperfect accuracy also makes it difficult to establish a broader picture without meta-analysis. Further preventing an understanding of the risk and extent of potential zoonotic transmission is the dearth of published risk factors for zoonotic STH infection in dogs and analyses of the haplotypes of *Strongyloides* spp. infecting those dogs.

The scarcity of published reports on the impacts of animal health programs in these settings means that addressing zoonotic STH infections in dogs using evidence-based approaches remains elusive. Furthermore, evidence for the efficacy and effectiveness of treatments in these settings is lacking, meaning that there is little information to guide treatment and prophylaxis decisions by veterinarians, animal health workers or owners. Importantly, the absence of information on the cost-effectiveness of these strategies means that funding bodies such as local governments or NGOs cannot appropriately budget for such interventions.

Considering the significant knowledge gaps around these issues, the aims of this thesis are to:

1. Systematically review available literature, understand the locations, parasite and host species involved, and provide an estimate of the individual study and pooled true prevalence of STH infections in Australian Aboriginal and Torres Strait Islander communities
2. Characterise and examine the impacts of a long-term animal health program in three remote Aboriginal communities

3. Explore the prevalence, risk factors and genetic groupings of soil-transmitted helminth infections in dogs in three remote Aboriginal communities in Arnhem Land
4. Examine the short-term efficacy and medium-term effectiveness of two labelled antiparasitic treatment programs in comparison to the off-label usage of ivermectin in a Torres Strait Islander community setting
5. Determine the cost-effectiveness of three treatments for *Ancylostoma caninum* and three ectoparasite treatments for fleas and ticks under field conditions in a Torres Strait Islander community setting

Chapter 2: A systematic review and meta-analysis of human and zoonotic dog soil-transmitted helminth infections in Australian Indigenous communities

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2.1 Abstract

Soil-transmitted helminths (STH) infect 1.5 billion people and countless animals worldwide. In Australian Indigenous communities, STH infections have largely remained endemic despite control efforts, suggesting reservoirs of infection may exist. Dogs fulfil various important cultural, social and occupational roles in Australian Indigenous communities and are populous in these settings. Dogs may also harbour zoonotic STHs capable of producing morbidity and mortality in dogs and humans. This review provides an overview of human and zoonotic STH infections, identifies the Australian Indigenous locations affected and the parasite species and hosts involved. The meta-analysis provides estimates of individual study and pooled true prevalence of STH infections in Australian Indigenous communities and identifies knowledge gaps for further research on zoonotic or anthroponotic potential.

A systematic literature search identified 45 eligible studies documenting the presence of *Strongyloides stercoralis*, *Trichuris trichiura*, *Ancylostoma caninum*, *Ancylostoma duodenale*, *Ancylostoma ceylanicum*, undifferentiated hookworm, and *Ascaris lumbricoides*. Of these studies, 26 were also eligible for inclusion in meta-analysis to establish true prevalence in the light of imperfect diagnostic test sensitivity and specificity by Rogan-Gladen and Bayesian methods. These studies revealed pooled true prevalence estimates of 18.9% (95% CI 15.8-22.1) for human and canine *S. stercoralis* infections and 77.3% (95% CI 63.7-91.0) for canine *A. caninum* infections indicating continued endemicity, but considerably more heterogenous pooled estimates for canine *A. ceylanicum* infections, and *A. duodenale*, undifferentiated hookworm and *T. trichiura* in humans.

This review suggests that the prevalence of STHs in Australian Indigenous communities has likely been underestimated, principally based on imperfect diagnostic tests. Potential misclassification of hookworm species in humans and dogs due to outdated methodology, also obscures this picture. High-quality contemporary studies are required to establish current true prevalence of parasite species in all relevant hosts to

guide future policy development and control decisions under a culturally sound One Health framework.

2.2 Introduction

Soil-transmitted helminths (STHs) are Neglected Tropical Diseases (NTDs) and infect an estimated 1.5 billion people and countless animals worldwide (Jourdan et al. 2018). In Australian Indigenous communities, it is necessary to develop an understanding of not only the STHs of concern to humans, but also other hosts such as dogs, that may act as zoonotic reservoirs for STHs that can mature to adulthood in humans, as well as environmental factors which may contribute to transmission. The importance of the relationship between people and canines is clear in the great significance and diverse roles that dogs hold in these communities; as companions, hunting partners, spiritual guardians and members of the intricate kinship system (Constable et al. 2008; Smith and Litchfield 2009; Constable et al. 2010).

STHs relevant to humans in Australia include hookworms (including *Ancylostoma duodenale*, *Ancylostoma ceylanicum* and *Necator americanus*), threadworms (*Strongyloides stercoralis*), whipworms (*Trichuris trichiura*) and *Ascaris lumbricoides*. These genera are endemic throughout nearby Oceanic countries with hookworms, in particular, constituting overall prevalence of 48% among the 9.6 million humans (excluding Australia and New Zealand) residing in this region (Pullan et al. 2014; Bradbury and Traub 2016). Papua New Guinea, which has a climate similar to that of Northern Australia, is overrepresented in terms of hookworm and appears to drive this regional prevalence, with an estimated prevalence of 60.6% based on a Global Burden of Disease study by Pullan and colleagues in 2010 (Pullan et al. 2014).

Several zoonotic STHs of canines and felines are able to mature in human hosts including the hookworms *A. ceylanicum* and *A. caninum* (Traub et al. 2008; Inpankaew et al. 2014a; Bradbury and Traub 2016; Smout et al. 2017a). *A. ceylanicum* has been established as the second most common hookworm infecting humans in the Asia-Pacific region, and it is the only zoonotic hookworm known to cause patent, egg-shedding

infections in humans (Traub et al. 2008; Traub 2013; Inpankaew et al. 2014a; Bradbury et al. 2017). *A. caninum* has mostly been thought to be a parasite of dogs but can mature in humans forming non-patent infections (Prociv and Croese 1990; Croese et al. 1994; Walker et al. 1995). Recent evidence of egg-shedding infections in humans however, suggest that patent infections are possible (Ngcamphalala et al. 2020). Strongyloidiasis is also a potential zoonosis, with *S. stercoralis* comprising two distinct genetic clades, one restricted to dogs and another infecting humans, non-human primates, dogs and cats (Jaleta et al. 2017; Bradbury et al. 2021). Routine anthelmintic use in canine or human hosts along with sanitation may reduce environmental contamination with larvae, reducing infection pressure on humans or canines sharing the same environment (Takano et al. 2009; Schär et al. 2014a; Thamsborg et al. 2017; Jaleta et al. 2017).

2.2.1 Parasite biology

Several lifecycle and transmission features are common to STH species found in Australia, along with some important differences, with both helping to inform diagnosis, treatment and control strategies as part of a One Health approach for these important STH species. The most important commonality of all STHs is that infectivity to the next host is reliant on an essential period of development in the environment (usually soil) ranging from less than one to over 14 weeks, depending on environmental conditions such as temperature and humidity (Brooker et al. 2006; Bethony et al. 2006). Third stage filariform larvae of all hookworm species and *Strongyloides* can infect their host by percutaneous penetration of exposed skin in contact with contaminated matter. People who regularly walk barefoot outdoors, as regularly observed in remote Australian Indigenous communities, demonstrate higher risk of hookworm and *Strongyloides* infection (Miller et al. 2018). A Malaysian study found that humans who routinely walked barefoot outdoors were 5.6 (95% CI 2.9 – 10.7) times more likely to be infected with hookworms compared with those who routinely wore shoes (Ngui et al. 2012). *T. trichiura* and *A. lumbricoides* infect their human definitive host by ingestion of substrates contaminated with embryonated eggs. Third-stage filariform larvae of some

Ancylostoma spp. can also infect their hosts via ingestion of contaminated matter. Dogs may also become infected with *A. caninum* by the ingestion of paratenic hosts such as rats or mice (Beugnet et al. 2018).

Once infected, lifecycles and associated symptoms differ between STH genera. Adults of hookworms and *Strongyloides* spp. reside in the small intestines, and *Trichuris* spp. in the colon of dogs and humans, thus producing signs that range from subclinical to severe and include intestinal haemorrhage, anaemia, abdominal discomfort and diarrhoea (Bradbury and Traub 2016; Centers for Disease Control and Prevention 2016; Page et al. 2016; Beknazarova et al. 2016b; Stracke et al. 2020). Human infections with the zoonotic hookworm *A. caninum* have, in some cases, presented clinically with eosinophilia and acute eosinophilic enterocolitis, which has thus far been found to be the result of only a single pre-adult worm in all closely examined cases (Prociv and Croese 1990; Walker et al. 1995). While this suggests that patent infections are not possible in humans, past, as well as more recent evidence demonstrating egg-shedding, challenge this paradigm (Ngcamphalala et al. 2020; Traub et al. 2021). Hookworms and *Strongyloides* spp. can also produce dermatological symptoms that include raised itchy rashes, urticaria and pulmonary symptoms of coughing as a result of percutaneous and hepatopulmonary migration (Bowman et al. 2010). *Ascaris* may also stimulate coughing related to its hepatopulmonary migration following ingestion, but otherwise usually only causes mild abdominal discomfort in light burdens (Centers for Disease Control and Prevention 2019). The autoinfective cycle of *S. stercoralis* and its ability for massive synchronous larval emergence (hyperinfection) in immunocompromised or immunosuppressed patients can lead to mortality rates as high as 87% in both humans and dogs (Genta et al. 1986; Olsen et al. 2009; Taylor et al. 2014; Thamsborg et al. 2017; Buonfrate et al. 2017). This is important because while most *S. stercoralis* cases are self-limiting in dogs, the non-specific clinical presentation that may manifest in both dogs and humans, can potentially progress rapidly to a state of hyperinfection or disseminated strongyloidiasis, which constitute a poor prognosis (Olsen et al. 2009; Bisoffi et al. 2013; Taylor et al. 2014).

2.2.2 Environmental factors

Several factors may favour environmental contamination with eggs and larvae thereby facilitating infection in remote Indigenous community settings. The hot and humid climate of the tropical or subtropical North of Australia favour development and survival of infective stages of STHs in the soil, with dogs from tropical climates found to be 5.6 (95% CI 3.3 – 9.5) times more likely to be infected with hookworms compared to dogs from non-tropical climate zones in Australia (Palmer et al. 2007). Another study found a greater proportion of Australian dog parks in tropical regions (91.7%) to be contaminated with STHs compared with those in temperate (39.5%) and subtropical regions (33.3%) (Traub et al. 2021; Massetti et al. 2022). The same study found 44.5%, 4.8% and 0.9% of dog parks in tropical, subtropical and temperate regions to be contaminated with *A. caninum*, respectively (Massetti et al. 2022). In the final report of the Australian Hookworm Campaign 1919-1924, Sweet described an increase in detected hookworm prevalence north of 32 degrees south latitude and in areas with greater than 1016 mm precipitation annually (Sweet 1924).

2.2.3 Host and socio-economic factors

Large populations of owned and stray dog and cat populations in Indigenous communities contribute to large burdens of environmental contamination with STH eggs or larvae. Close and frequent contact between hosts in shared environments increases risk of zoonotic exchange in these settings, as shown in other countries such as Malaysia in which close contact with dogs or cats resulted in a 2.9 times increase in the risk of hookworm infection (Nguui et al. 2012). These risks and the importance of the human-animal bond in Australian Indigenous communities necessitates a culturally relevant One Health understanding of these infections (Riley et al. 2021; Traub et al. 2021).

Indigenous Australians suffer from disproportionately high rates of NTDs compared to their non-Indigenous counterparts (Kline et al. 2013; Gordon et al. 2017). Historically, around the time of the Australian Hookworm Campaign in the 1920s, Indigenous populations were found to have higher prevalence of both hookworms (62% vs 15%)

and *Strongyloides* (0.8% vs 0.03%) compared with their non-Indigenous counterparts (Bradbury and Traub 2016; Page et al. 2016). More recently, efforts towards control and prevention of these parasites in Indigenous communities have been largely ineffective, inconsistent or completely lacking (Miller et al. 2014; Bradbury and Traub 2016). Resource and health-literacy inequalities faced in these remote communities along with overcrowding also contribute to greater risk in several disease categories including STH infections (Melody et al. 2016). At a time where government efforts to close the health inequality gap between Indigenous and non-Indigenous Australians is not on track to be achieved by the target of 2031, understanding sources of infection and risks of transmission are particularly important (National Indigenous Australians Agency 2020).

In order to understand the risk of transmission and identify targets for prevention and control, it is important first to define a clear, One-Health-focussed picture of parasite distribution in human, canine and feline host species which share common environments. With this background, the aim of this manuscript is to systematically review the literature and provide an overview of research conducted in this area, understand the locations, parasite and host species involved, provide an estimate of the individual study and pooled true prevalence of STH infections in Australian Indigenous communities and to identify knowledge gaps which may shed further light on the potential for zoonotic or anthroponotic spread. In this way a more holistic, One Health perspective may be attained rather than focussing control efforts at any one location, parasite or host in isolation. Such information has the potential to guide effective and culturally relevant policy and practices, drive community involvement and deliver preventive and curative solutions for problems plaguing Australian Indigenous communities for decades.

2.3 Methods

2.3.1 Search protocol

The literature search was focussed on soil-transmitted helminth infections (outcome) in humans and zoonotic STH infections in canines and felines in Australian Indigenous communities (population). In March 2020, veterinary, medical and public health databases were searched including PubMed, Embase, Directory of Open Access Journals, Web of Science, CAB Abstracts, Scopus, Medline, Biosis, APAIS Health, CINAHL, EBM Reviews and Google Scholar. Search terms included a combination of parasite species or disease manifestations (i.e. “Ancylostoma” OR “Ancylostomiasis” ...), AND location (i.e. “Australian Aboriginal community” OR “Aboriginal community” ...) AND study population (i.e. “Dog” OR “Human” ...) as detailed in Table 2.1. Reference lists of eligible articles and other works of frequent authors were also searched, and further articles included if eligible. The protocol was registered with the international prospective register of systematic reviews (PROSPERO), in accordance with PRISMA guidelines. Due to PROSPERO database constraints, human (PROSPERO CRD42020166266) and animal (PROSPERO CRD42020165388) protocols were registered separately with mutual references in each.

Table 2.1 Systematic review search terms

Domain	Search terms ^a
Parasite/disease	<i>Ancylostoma</i> , " <i>Ancylostoma duodenale</i> ", " <i>Ancylostoma ceylanicum</i> ", " <i>Ancylostoma caninum</i> ", ancylostomiasis, <i>Ascaris</i> , " <i>Ascaris lumbricoides</i> ", ascariasis, helminthiasis, Helminth, Hookworm, "Hookworm infection", "Intestinal helminth", <i>Necator</i> , " <i>Necator americanus</i> ", Nematode, "Soil-transmitted helminthiasis", "Soil-transmitted helminth", <i>Strongyloides</i> , " <i>Strongyloides stercoralis</i> ", strongyloidiasis, Threadworm, <i>Trichuris</i> , " <i>Trichuris trichiura</i> ", trichuriasis, Worms, Parasites
Location	"Australian Aboriginal community", "Aboriginal community", "Indigenous Australian", "Indigenous community"
Population	Dog, Dingo, Canine, Canis, Cat, Feline, Felis, Human, Adult, Child, Children, Infant

^a Search terms combined using Boolean logic, with row terms combined using 'or' statements and 'and' statements used to combine terms between rows

2.3.2 Exclusion criteria

Full search result lists and exclusion assessments were stored in a proprietary spreadsheet (Microsoft Excel v. 1908, Microsoft Corporation, Redlands, California) and study citation details and digital copies of published papers managed using Mendeley Desktop (v. 1.19.8, Elsevier). After removing duplicates, studies were excluded if the work was not conducted in Australian Aboriginal or Torres Strait Islander communities; these included review articles, editorials, commentaries, letters, conference proceedings, or abstracts that did not contain data on the presence of soil-transmitted helminth species *A. duodenale*, *A. caninum*, *A. ceylanicum*, *A. lumbricoides*, *Necator americanus*, *S. stercoralis* or *T. trichiura*; study data were not from canine, feline or human hosts; data from another study was repeated or if a diagnostic methodology was not reported. When two or more studies containing repeated data were compared, those with a smaller subset were excluded. Studies using a case-control methodology were only included if STH infection was not a defined inclusion criterion for cases.

Studies were excluded from quantitative meta-analysis if they contained pooled results from Indigenous community and non-Indigenous community locations that could not be disaggregated; they did not report a quantitative measure of disease frequency such as prevalence or incidence or did not provide sufficient data to allow prevalence or incidence to be calculated; or if they did not provide sufficient detail of diagnostic methodology to allow sensitivity and specificity data to be linked. If uncertainties arose surrounding the inclusion or exclusion of a study, this was discussed amongst the research team and a consensus was formed.

2.3.3 Data extraction

The following data were extracted from each eligible study (Appendix 1): title, authors, publication year, study objectives, study site(s), study periods, study design, host species, sample type, sample size, parasite species, diagnostic technique, number of positive samples, factors affecting prevalence or incidence and the presence of an ethics statement. In cases of intervention studies, pre-treatment prevalence or incidence was included in order to be more comparable to observational studies.

The presence of bias in the reported data was assessed for all studies following the method described by Hoy (Hoy et al. 2012). In brief, this method involves assessing studies across the domains of selection, nonresponse, measurement and analysis leading to an overall summary estimate of study bias. This method was selected for its relevance to studies of prevalence and ease of use, and was modified to consider Indigenous human, dog or cat populations rather than the national population. Ten percent of studies were extracted and critically appraised in duplicate by two research team members including the primary author to check for agreement. The remainder of the studies were then extracted and assessed by the primary author and incorporated into Appendix 1.

2.3.4 Data analyses

Prevalence data were extracted from the eligible studies and expressed as apparent prevalence (AP), i.e., the reported number of test-positive individuals divided by the total number of individuals tested. Due to the use of several different diagnostic techniques, each of them with imperfect sensitivity and specificity, variations in AP may arise by means of differences in diagnostic test accuracy. The AP of each study was therefore reanalysed as true prevalence (TP) taking into account imperfect test diagnostic sensitivities and specificities in order to allow comparisons of prevalence across studies. Sensitivity and specificity data for diagnostic tests used in the included studies were extracted from peer-reviewed articles which calculated these measures. Where multiple estimates of sensitivity and specificity for a given diagnostic test were found in the literature, an average of these measures was taken. Where no sensitivity and specificity data could be found in peer-reviewed literature, expert opinion was sought from two veterinary parasitologists, with averages taken from these opinions. *S. stercoralis* serologic tests can vary widely in sensitivity and specificity based on cut-offs, study populations, reference methods and test methods (Watts et al. 2016). Five of the ten studies included for meta-analysis stated the use of IgG ELISA (Page et al. 2006; Einsiedel et al. 2014; Hays et al. 2015; Kearns et al. 2017). A further four studies (Einsiedel and Woodman 2010; Einsiedel et al. 2013; Einsiedel et al. 2016a; Einsiedel et al. 2016b) published by common authors did not refer to serological methods used but utilised the same study population and adjacent study timeframes, and thus are assumed to have used the same IgG ELISA assay. Another study (Miller et al. 2018) did not specifically state the serological method used to diagnose *S. stercoralis* exposure but referenced a study in which indirect immunofluorescence test (IFAT) was utilised. Given that these methods have very similar reported sensitivities and specificities in the source studies referenced in Table 2.2, the average diagnostic parameters were used in calculations of TP. Sensitivity and specificity estimates and their sources are detailed in Table 2.2.

Table 2.2 Sensitivity and specificity data for tests used in studies included for meta-analysis

Parasite and diagnostic test	Sensitivity (%)	Specificity (%)	Source
Hookworm			
Conventional PCR (cPCR)	84.7	87.6	(Massetti et al. 2020; Fleitas et al. 2021)
Direct faecal smear (DS)	16.3	100 ^a	(Khurana and Sethi 2017)
Formol-ether concentration (FE)	49.9	100	(Glinz et al. 2010; Fenta et al. 2020)
Morphology of adult worms (MpAd)	13.8	100	(Harriott et al. 2019)
Saturated salt flotation (SSF)	37.9	100 ^a	(Massetti et al. 2022)
<i>Trichuris trichiura</i>			
Direct faecal smear (DS)	14.9	100 ^a	(Khurana and Sethi 2017)
Formol-ether concentration (FE)	63	100	(Glinz et al. 2010; Fenta et al. 2020)
Saturated salt flotation (SSF)	63.9	96.4	(Adolph et al. 2017)
<i>Strongyloides spp.</i>			
Direct faecal smear (DS)	30	100 ^a	(Nielsen and Mojon 1987; Siddiqui and Berk 2001; Shield and Page 2008)
ELISA serology (SE)	82.4	92.5	(Requena-Méndez et al. 2013; Bisoffi et al. 2014; Kalantari et al. 2020)
Formol-ether concentration (FE)	34	100	(Shield and Page 2008)
qPCR	88.9	92.7	(Schär et al. 2013)
Zinc sulphate flotation (SSF)	9.3	100 ^a	(Schär et al. 2014b)

^a Assumed specificity when no data available from peer-reviewed sources. Based on very low likelihood of false positives occurring via these diagnostic techniques

TP estimates were calculated using the method described by Rogan and Gladen (Rogan and Gladen 1978) implemented in the contributed epiR package (v 2.0.39, Stevenson et al., 2021) in R version 4.1.2 (R Core Team, 2021). In studies where the AP was less than $(1 - \text{diagnostic test specificity})$ the Rogan Gladen estimate of TP was less than zero (Speybroeck et al. 2013). Similarly, if AP was greater than the diagnostic test sensitivity the Rogan Gladen estimate of TP was greater than unity. For these studies a Bayesian approach was used to estimate TP as described by Messam *et al.* (Messam et al. 2008) Here, estimates of the distributional form of diagnostic sensitivity, specificity and TP were used as priors and a Markov chain Monte Carlo approach used to combine these prior estimates with the empirical data to return a posterior estimate of TP and its 95% credible interval (Ng-Nguyen et al. 2017). Distributions for sensitivity and specificity were taken from confidence intervals from the sources listed in Table 2.2 or were estimated within a 95% confidence interval by expert opinion if no published data were available. Estimates of the 'true' number of positive samples as a proportion of the total number of samples tested for each study (calculated using the Rogan-Gladen or Bayesian approach described above) allowed us to calculate a summary estimate of the true prevalence of soil-transmitted helminths across all of the studies included in the systematic review using the contributed metafor (v 3.0-2, Viechtbauer, 2021) package in R. True prevalence estimates and their 95% confidence intervals were plotted as a forest plot using the contributed forestplot (v 2.0.1, Gordon and Lumley, 2021) package in R.

The easting and northing location of the centroid of the area covered by each of the studies included in this review were plotted as a symbol map with colour and shape used to indicate host species. Geospatial analyses and mapping were carried out using the contributed ggplot2 (v 3.3.5, Wickham *et al.*, 2021) package in R. Mapping shapefile data were obtained from GADM database of Global Administrative Areas (v 2.8, GADM, 2018). Location details for Indigenous communities were obtained from the Australian Government National Indigenous Australians Agency (National Indigenous Australians Agency 2021).

2.4 Results

The protocol for identifying, screening and excluding studies along with the numbers of studies excluded based on the criteria detailed in the methods section is shown in Figure 2.1. There were 327 studies initially identified after duplicates were removed. Following initial screening and full-text assessment for eligibility, 45 studies were eligible for qualitative synthesis. Of those, 26 fitted the criteria for inclusion in the meta-analysis.

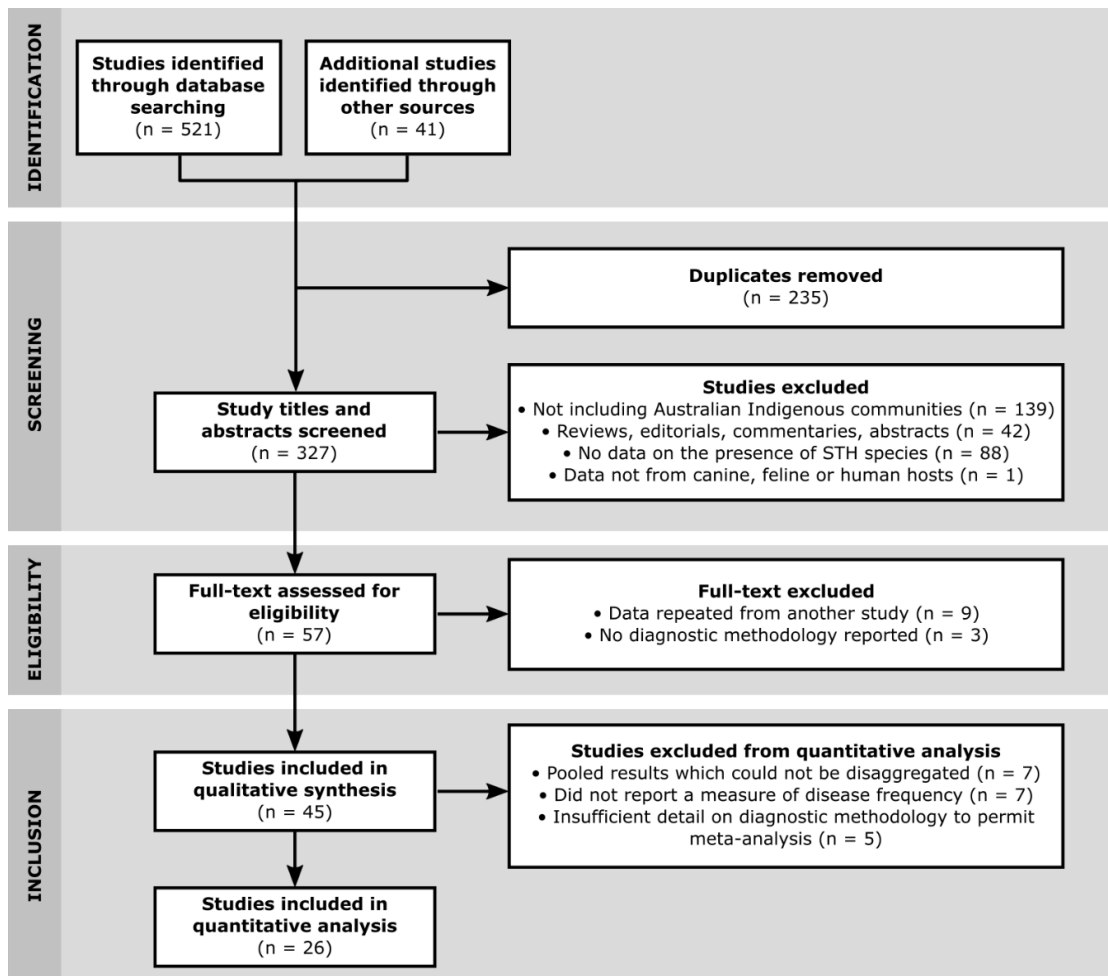


Figure 2.1 PRISMA flow diagram of the search, screening for eligibility and inclusion in qualitative and quantitative synthesis

The distribution of included studies by decade of publication is shown in Figure 2.2. Studies included in both quantitative and qualitative synthesis are included in this figure. While studies were identified for inclusion from 1921 to 2020, no studies were identified

in the period from 1925 to 1969. Most included studies were published in the 1990s (31%) and 2010s (42%) with only 11% of studies published in the intervening decade.

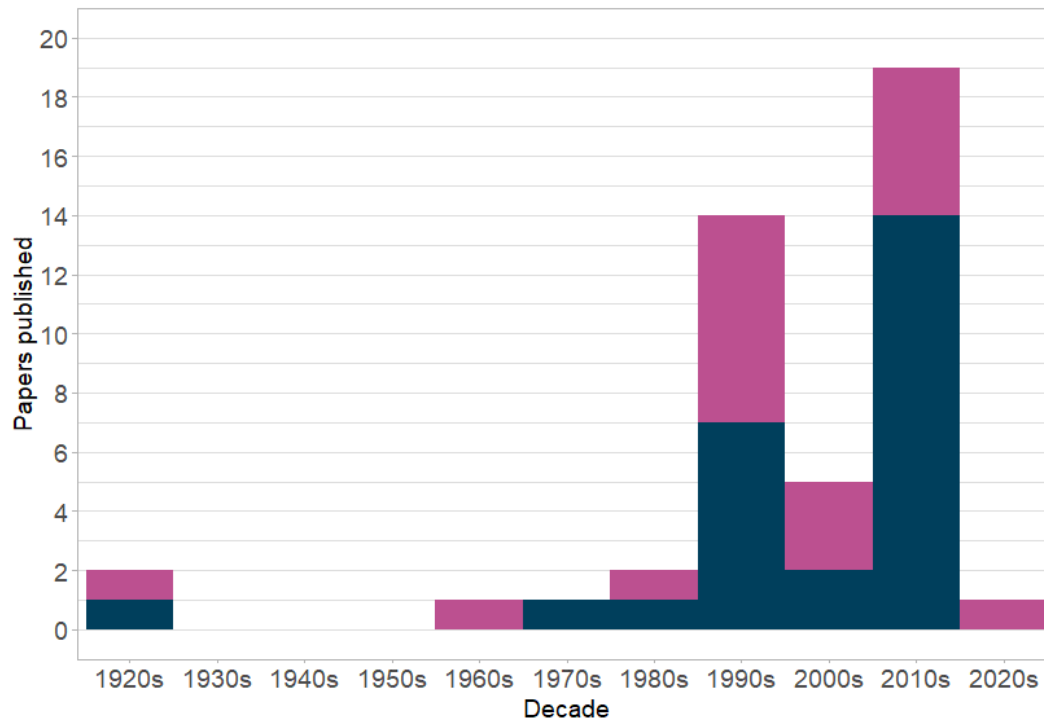


Figure 2.2 Studies included in quantitative and qualitative synthesis published per decade

The geospatial distribution of STH infection data from the included studies is shown in Figure 2.3. These studies reported the presence of STH infections in Indigenous communities in the Northern Territory (21 of 45 studies; 47%), Western Australia (15 of 45 studies; 33%), Queensland (14 of 45 studies; 31%) and New South Wales (4 of 45 studies; 9%). While some studies reported data on locations in multiple states or territories, no studies meeting inclusion criteria reported data on STH infections in South Australia, Victoria, Tasmania or the Australian Capital Territory. Some of the most studied locations of the included studies include the Kimberley (12 of 45 studies; 27%), Far North Queensland (7 of 45 studies; 16%), Arnhem Land (7 of 45 studies; 16%), and Alice Springs and surrounds (6 of 45 studies; 13%).

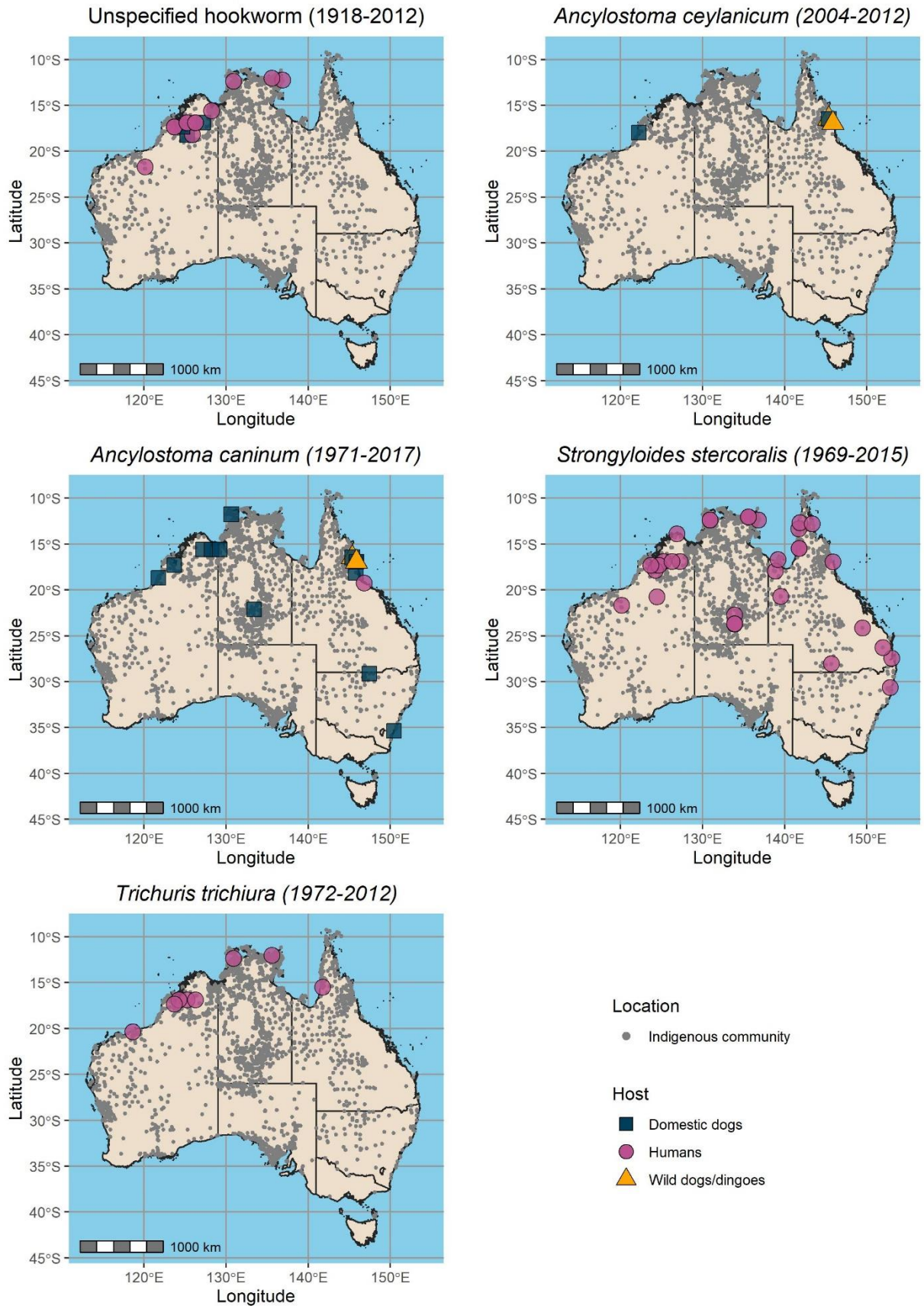


Figure 2.3 Detected STH species and sampling date spans in domestic dog, wild dog, dingo and human hosts in relation to Australian Indigenous community locations

Where hookworms were identified by the presence of ova only, these studies were designated as 'hookworm', regardless of whether species were designated in the study as this differentiation is not possible by microscopy. *Ascaris lumbricoides* has been omitted from mapping as only one case was reported in a 1992 study from the Kimberley region in North-West Western Australia (Gunzburg et al. 1992).

Indigenous communities were not exclusive in all studies, with seven studies reporting data from Indigenous and non-Indigenous community locations which could not be disaggregated. Eleven studies did not designate a specific Indigenous community location, either for the purpose of community privacy and anonymity or because state-wide or nationwide Indigenous community data were aggregated. Five studies featured sampling from either Alice Springs or Darwin hospitals, both of which service large areas and would include data from many Indigenous communities within a large radius of each hospital.

Most included studies were surveys (25 of 45; 56%), with the remainder of studies being case-control studies (9 of 45; 20%), cohort studies (7 of 45; 16%) and case reports (4 of 45; 9%). Sample sizes in the included studies had a median of 91.5 samples and a range of 2 to 9956.

Only 52% of studies specified that ethical approval was sought, none of which were published prior to 1997. All included studies since 2010 reported human or animal ethics approval.

Human samples were the most common host sample type analysed in the included studies (37 of 45; 82%), followed by domestic dogs (11 of 45; 24%) and wild dogs/dingoes (3 of 45; 7%). For the purposes of this review the term 'domestic dog' refers to dogs living in an Indigenous community who may have been owned by an individual, a family, or a community more generally. Wild dogs and dingoes may be dogs living wild existences, dog/dingo hybrids or pure dingoes, though distinctions were not made in the included studies. While felines were included in the search protocol, no studies were identified which fitted the inclusion criteria.

Faecal samples were the most commonly featured sample type collected from hosts in the included studies (32 of 45; 71%), followed by blood (11 of 45; 24%), dog or wild dog/dingo intestinal content from necropsy specimens (5 of 45; 11%), surgical biopsies of sections of intestine in humans (3 of 45; 7%), soil from in and around Indigenous communities (1 of 45; 2%), and sputum (1 of 45; 2%).

A wide range of diagnostic tests were used to detect STH infections with serology (SE) most commonly used, though only in *S. stercoralis* studies (12 of 45; 27%), followed by direct smear or wet mount microscopy (DS) (9 of 45; 20%), saturated salt flotations (SSF) (8 of 45; 18%), conventional PCR (cPCR) (5 of 45; 11%), formol-ether concentration method microscopy (FE) (3 of 45; 7%), morphological identification of adult worms from necropsy specimens in dogs or surgical biopsies in humans (MpAd) (3 of 45; 7%), and quantitative PCR (qPCR) (1 of 45; 2%).

Data on the presence of *S. stercoralis* was reported in the greatest proportion of studies (28 of 45; 62%), followed by undifferentiated hookworm species (17 of 45; 38%), *T. trichiura* (10 of 45; 22%), *A. caninum* (8 of 45; 18%), *A. duodenale* (6 of 45; 13%), *A. ceylanicum* (3 of 45; 7%), and *A. lumbricoides* (1 of 45; 2%). No studies identified in this review featured data on the presence of *N. americanus*. Figure 2.4 presents individual study TPs with confidence or credible intervals (where appropriate) for each of these parasites, with each line a distinct reported prevalence from a different host, diagnostic technique or Indigenous community location. Fifty-six out of 75 (75%) of TP measures were greater than their originally reported AP. Studies for each parasite are summarised into pooled TP estimates with 95% confidence intervals.

2.4.1 *Ancylostoma caninum*

For some species as well as for unidentified hookworm infections, prevalence estimates varied markedly. In the case of *A. caninum*, however, the pooled TP estimate of 77% and confidence interval presented in a tighter band of 64% to 91%, reflecting most of the contributing individual estimates. Lower estimates such as those from the study by Slapeta *et al.* (2015) all came from studies that used saturated salt flotation diagnostic

methods and were from more arid locations which may be less suited to environmental development of hookworm larvae (Appendix 1). Almost all other individual study estimates were from tropical or subtropical regions such as the Kimberley or Far North Queensland. Studies not suitable for meta-analysis included a study where 48% of dog faecal samples from a Kimberley community were found to contain *A. caninum*, though these were reported only when *Giardia duodenalis* - the focus of the study - was absent in samples (Hopkins et al. 1993). While TP data in humans is notably absent for *A. caninum* in Figure 2.4, two sets of case studies reported eosinophilic enteritis caused by adult worms in Northern Queensland (Prociv and Croese 1990; Walker et al. 1995).

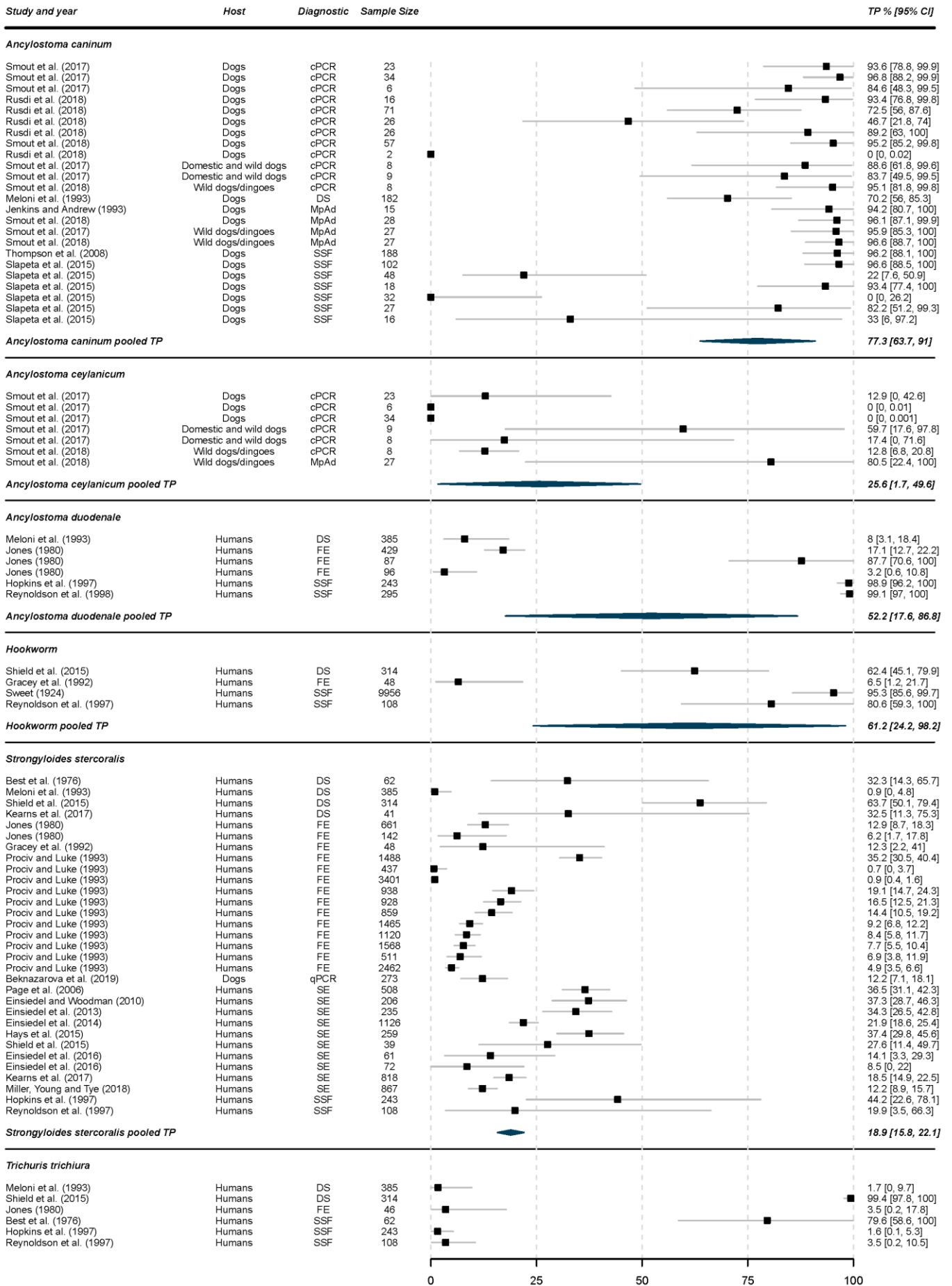


Figure 2.4 Forest plots for parasite species with pooled true prevalence. Individual true prevalence (TP) data with 95% confidence or credible intervals for each reported apparent prevalence within papers with pooled TP and 95% confidence intervals for each parasite species or designated as hookworm where species were not reported. For details on diagnostic tests and test accuracy see Table 2.2

2.4.2 *Ancylostoma ceylanicum*

Two studies of *A. ceylanicum* by Smout *et al.* (2017, 2018) reported widely differing prevalence estimates despite these samples being collected from the same region in Far North Queensland. It should be noted that sampling in one of these studies featured collection of soil from around communities rather than directly from animals. These limited number of studies represent some of the smallest sample sizes of those included in these analyses, which contributes to both their wide individual confidence intervals and the pooled TP estimate of 26% (95% CI 2% to 50%). One study unsuitable for meta-analysis detected *A. ceylanicum* in an unreported number of samples in Broome (Palmer *et al.* 2007).

2.4.3 *Ancylostoma duodenale*

A. duodenale produced a pooled TP estimate of 52% and a wide confidence interval of 18% to 87%, with no individual TP estimates falling within this range. Individual estimates fell at the extremes of the TP scale. Two of these studies, both of which make up prevalence estimates at the lower extreme of the scale, have a higher risk of bias due to poor representation of the population in the sample group. The study by Meloni *et al.* (1993) sampled children only and the study by Jones did not use random sampling or include details on the age of participants (Jones 1980; Meloni *et al.* 1993). One study not suitable for meta-analysis from East Arnhem Land in the Northern Territory found an AP of 15%, though risk of bias was also high due to poor sample selection (Watson and Tozer 1986). All other studies were from the Kimberley region in Western Australia. It should also be noted that these studies are significantly dated in comparison to other included studies, with the most recent being published in 1998.

2.4.4 Hookworm

Several of the aforementioned hookworm species may have contributed to the undifferentiated individual prevalence estimates seen from these studies, producing a

pooled TP estimate of 61% (95% CI 24% to 98%). These studies also showed varying degrees of risk of bias. The study by Gracey *et al.* (1992), which had a notably lower TP estimate than other studies at 6%, featured sampling from children up to two years of age while the other studies feature a more representative sample group. The report of the Australian Hookworm Campaign 1919-1924 by Sweet, while featuring a large sample size of 9,956 individuals, had a poor description of study methodology and no details on study participant demographic details or locations sampled in this nationwide survey. It is important to note also that the data contributing up the pooled TP estimate spans 91 years, during which time several efforts to combat hookworm infection have occurred and may have contributed to significant variation in prevalence.

Five other studies not suitable for meta-analysis featured data on the presence of undifferentiated hookworms, although only two of those could be disaggregated from non-Indigenous results and had denominators necessary to establish AP. A 1921 study by Lambert found an AP in humans of 77% across Queensland, although the description of sampling and methodology was poor and some data may overlap with that of the report by Sweet (Lambert 1921; Sweet 1924). Another study by Fryar and Hagan found an AP of only 4%, though a poor description of methodology and sampling group leads to a moderate risk of bias (Fryar and Hagan 1997).

2.4.5 *Strongyloides stercoralis*

The larger number of included studies, many of which feature large sample sizes has permitted a pooled TP estimate of 19% with a relatively narrow confidence interval of 16% to 22% for *S. stercoralis* infection in Australian Indigenous communities. A study by Kearns *et al.* (2017) which investigated the effects of an ivermectin mass drug administration had a baseline TP estimate of 32% (95% CI 11% to 75%) when examining direct faecal smears from 41 children, but had a TP of 18% (95% CI 15% to 22%) when examining 818 serological samples from the wider community including adults. Similar differences are seen between the TP estimates derived by different diagnostic methods in the study by Shield *et al.* (2015). Studies by Best *et al.* (1976), Meloni *et al.* (1993),

Gracey *et al.* (1992), and Prociv and Luke (1993) also have an increased risk of bias due to inclusion of children only (Best *et al.* 1976; Gracey *et al.* 1992; Meloni *et al.* 1993; Prociv and Luke 1995). Only one included study featured data on the presence of the parasite in dogs, although the aim of this study was focused on genetic characterisation of *Strongyloides* infections in dogs rather than prevalence (Beknazarova *et al.* 2019).

Ten studies unsuitable for meta-analysis featured data on the presence of *S. stercoralis*. Of those, only three could be disaggregated from non-Indigenous results or had denominators necessary to determine AP. While these studies had AP estimates of 4%, methodology and poor sampling strategy (Gunzburg *et al.* 1992; Gracey *et al.* 1996; Fryar and Hagan 1997).

2.4.6 *Trichuris trichiura*

Six studies were included for quantitative analysis of *T. trichiura* prevalence. These featured individual TP estimates at either extreme of the prevalence scale. The heterogeneity of individual study data from locations in which *T. trichiura* infection was either highly prevalent or absent, is therefore likely to contribute to a misleading pooled TP estimate. Thus, a pooled TP estimate was excluded from Figure 2.4. As mentioned above, the studies by Meloni *et al.* (1993) and Best *et al.* (1976) sampled only children and have a higher risk of bias. Four studies not suitable for meta-analysis included data on the presence of *T. trichiura*, though only two studies presented data where AP could be determined. Both these studies also featured data on *Strongyloides* and had moderate and high risk of bias for the reasons outlined above.

2.5 Discussion

This review brings together for the first time, true prevalence data on STH infections capable of maturing in the gastrointestinal tract of dogs and humans in Australian Indigenous communities. The TP estimation methods used in this review allow comparisons to be made among studies using different diagnostic methodologies and

permit the calculation of pooled TP estimates for each parasite species. While these pooled TP estimates and confidence intervals may be useful to extrapolate to other Indigenous communities across Australia as presented in , there are several important caveats.

Firstly, it should be noted that while TP estimates have been calculated based on imperfect diagnostic tests, the sensitivity and specificity data used to calculate these are also imperfect themselves in that they are based on what was considered 'gold standard' at the time of calculation. In truth, even gold standard tests are imperfect and therefore TP calculations based on these should be viewed in this light.

The diagnostic methodologies in Figure 2.4 as well as Appendix 1 are expressed here as they were presented in the included papers. In the case of hookworm species *A. caninum* in dogs and *A. duodenale* in humans, coproscopic methods examining eggs including SSF, FE and DS are unable to determine species due to the identical morphological appearance of *Ancylostoma* spp. eggs (Palmer et al. 2007; Traub et al. 2008). This calls into question whether hookworm species were accurately identified by these methods or if the species were assumed based on which host the samples came from and should therefore be reclassified as undifferentiated hookworm infections. This would reclassify all papers with data on *A. duodenale* and more than half of those featuring *A. caninum*. Included studies featuring *A. ceylanicum*, by comparison, used methods such as cPCR and MpAd which permit species identification. While reclassification of these individual *A. caninum* and *A. duodenale* TP estimates may be more accurate, it is unlikely to clarify the pooled TP estimate of hookworms in Indigenous communities any further.

The importance of seasonal variation in the TP data collated in this study is unclear. Larval hypobiosis of *A. caninum* in dogs and humans and *A. duodenale* in humans has been described, with reactivation of egg shedding infections under favourable climatic conditions (Croese 1995; Bradbury and Traub 2016). This phenomenon may have impacted prevalence in dry seasons, however timing of sample collection was rarely

specified beyond specific years in the included studies making seasonal patterns difficult to establish.

Similarly, *S. stercoralis* may have intermittent shedding of larvae and uneven distribution of larvae in faeces, making faecal detection methods less sensitive (Robertson et al. 2017; Ross et al. 2017). Other challenges also arise regarding the method of detection of *S. stercoralis* infection. Studies included in this review featured both direct methods, in which the presence of larvae was detected in the faeces indicating current infection, and indirect methods, in which serology was conducted to detect antibodies against *Strongyloides*. While serology is useful for detecting response to treatment, the acute phase of infection may not elicit detectable antibody responses for several months, and sensitivity and specificity of serology varies with test cut-off values (Page et al. 2006; Shield and Page 2008; Watts et al. 2016). Hyperinfective states may also have unreliable results on serology, although these cases would likely be detectable with faecal samples (Mejia and Nutman 2012; Page et al. 2016). While some of the included studies used agar plate culture as a follow-up to confirm *S. stercoralis* diagnosis, none used it as a primary means of diagnosis, nor did they use Baermann culture. While these are known to be more sensitive than most other faecal diagnostics, difficulties associated with sample storage and transport in order to keep larvae alive may have prevented the use of these methods (Watts et al. 2016).

A. ceylanicum and *T. trichiura* meta-analysis resulted in wide confidence intervals making meaningful conclusions on TP difficult. While *A. lumbricoides* was detected in one study, the single sample testing positive via undisclosed means and the high risk of bias in the study meant that this result was unlikely to be of significance (Gunzburg et al. 1992).

While some studies are numerous and large enough to contribute to TP estimates with narrower confidence intervals, such as those of *A. caninum* and *S. stercoralis*, it is important that these prevalence data are acted upon. Alarming, while the TP data in Figure 2.4 cover time periods of 1993-2018 and 1976-2019 for *A. caninum* and *S. stercoralis*, respectively, neither demonstrate significant reductions in prevalence

during those periods. The high TP of *A. caninum* in dogs presents a significant risk of environmental contamination in these remote communities where other factors such as tropical climate and barefoot walking also favour zoonotic infection or accidental ingestion. The vague and often mild symptoms of many of these STH infections has often led them to be omitted from differential diagnosis lists and undertreated in humans and animals alike (Fisher et al. 1993; Bisoffi et al. 2013; Miller et al. 2014; Beknazarova et al. 2018). Missed infections with hookworm or whipworm may lead to protracted anaemia, malaise and stunting in younger humans and animals, or in the case of *S. stercoralis*, hyperinfection can lead to severe disease and death. Routine testing and treatment programs which take a One Health approach considering all relevant hosts are required to establish more localised prevalence patterns both spatially and seasonally, and to catch infections early before serious sequelae occur.

Finally, few of the included studies included Indigenous community members as part of research teams. If meaningful policy development and community engagement is to be achieved, future studies must include Indigenous leadership and community involvement as part of surveillance and treatment efforts, as well as striving for the highest degrees of ethical standards.

2.6 Conclusion

From the data presented in this review, it is likely that the prevalence of STH infections in Australian Indigenous communities has been underestimated in most cases based off imperfect diagnostic methodology. The use of coproscopic methods which cannot differentiate hookworm species also calls into question the true presence and prevalence of some of these species, and whether confusion has occurred based on host species.

It is difficult to draw significant conclusions in relation to the TP of several of the included STH species in Indigenous communities, and more contemporary data across several host species is needed to achieve a clearer TP estimate. However, both *A. caninum* and *S. stercoralis* appear to remain endemic in similar proportions to those in the past 30-40

years. Knowledge gaps remain for *A. duodenale*, *N. americanus*, *A. lumbricoides*, the role of dogs as reservoirs of infection with *S. stercoralis*, as well as the importance of the increasing population of cats as reservoirs of zoonotic STH infection. By further understanding true prevalence across host species, a clearer picture of parasite status can be achieved which can guide culturally relevant, Indigenous-led policy and effective treatment and prevention strategies. Through these holistic, One Health approaches it may be possible to diminish and eliminate these important parasites.

Chapter 3: Impact of dog health programs and risk factors for canine soil-transmitted helminth infection in remote Aboriginal communities in West Arnhem Land

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Raw C, Tudor E, Traub RJ and Wiethoelter A. Impact of dog health programs and risk factors for canine soil-transmitted helminth infection in remote Aboriginal communities in West Arnhem Land

3.1 Abstract

Dogs hold important spiritual, cultural and practical roles in remote Aboriginal communities, often enjoying a free-roaming, independent lifestyle. Remote community access to veterinary services may be limited, and dog populations and zoonotic disease prevalence may increase. This study evaluates the impact of annual West Arnhem Land Dog Health Program visits on dog populations and health in three West Arnhem communities over an eighteen-year period using indicators including numbers of dogs per household and body and skin condition score. Faecal samples were collected from a subset of dogs and tested via qPCR for endoparasites. True prevalence was estimated alongside odds ratios for risk factors for infection using logistic regression modelling.

Program evaluation showed trends over time towards ideal scores in mean body and skin condition in each community. The median and range of dogs per household also showed declining trends over this period. Overall true prevalence was 86.9% (95% CI 80.4 to 91.8) for *Ancylostoma caninum* and 14.4% (95% CI 9.6 to 20.9) for *Strongyloides* spp. The odds of being *A. caninum* positive for dogs living in a household with three or more dogs was 7.01 times (95% CI 1.99 to 24.69) that of dogs from a household with less than three dogs when adjusting for community, age and desexed status.

This study demonstrates that culturally responsive, long-term animal health programs can have substantial impacts in remote Aboriginal communities, allowing community members to keep the number of dogs they wish, while reducing risk factors for zoonotic parasite infection and improving health measures in dogs.

3.2 Introduction

Dogs occupy a wide range of important roles in Australian Aboriginal and Torres Strait Islander communities; from traditional pet companions to nighttime guardians against intruders or unwanted spirits, cooperative hunting partners, spiritual figures in dog dreaming country and even as members of the intricate kinship system (Senior et al. 2006; Smith and Litchfield 2009; Constable et al. 2010). Due to the popularity of dogs, their free-roaming nature and the frequent inaccessibility of veterinary care and population control due to remoteness and lack of funding, the population size of dogs in these communities can be significantly larger than that of their urban counterparts (Burleigh et al. 2015; Molloy et al. 2017). A study of 20 Indigenous communities in the Northern Territory found an average of 24.4 dogs per 10 occupied households compared with an overall average of 3.9 dogs per 10 households in Australia in 2015 (Burleigh et al. 2015). With these increased numbers comes a greater risk of poorer health outcomes not only for dogs but also for the humans with which they share their environment. Risk of injury to dogs and humans from dog bites is greater, as is the risk of infectious diseases, including zoonoses (Smout et al. 2017a). For example, there can be increased exposure and infection with hookworms (*Ancylostoma* spp.) and threadworms (*Strongyloides stercoralis*) which can enter dogs and people percutaneously (Landmann and Prociw 2003). Behaviours which increase bare skin contact with soil, such as barefoot walking, can increase human infection risk and are commonly observed in remote Aboriginal communities along with issues with environmental hygiene (Ngu et al. 2012). Hookworms can produce mild to severe disease in dogs including haemorrhagic enteritis and anaemia (Traub et al. 2021). In humans a range of clinical manifestations from skin irritation to severe gastrointestinal distress can manifest (Bradbury and Traub 2016). *Ancylostoma caninum* has previously been thought of as a parasite only capable of producing patent, egg-shedding infections in dogs, with infestations being limited to adult worms in the human small intestine without reproducing (Prociw and Croese 1990; Croese et al. 1994; Walker et al. 1995). Recent evidence of egg-shedding infections with sequencing confirmation in humans, however, suggest that patent human infections are possible (Ngcamphalala et al. 2020). *Strongyloides stercoralis* infections in dogs and

people may lead to clinical manifestations ranging from asymptomatic to mortality rates of 87% in the case of hyperinfection or disseminated strongyloidosis. In these manifestations an immunocompromised or immunosuppressed host experiences a significant increase in fecundity of the parthenogenetic female worms in which large numbers of larvae emerge and undergo hepatopulmonary or disseminated migration simultaneously (Taylor et al. 2014; Page et al. 2016; Thamsborg et al. 2017). A systematic review and meta-analysis of human and zoonotic dog STH infections in Australian Aboriginal and Torres Strait Islander communities between 1918 and 2020 found pooled true prevalence estimates of 77.3% (95% CI 63.7 to 91.0) and 18.9% (95% CI 15.8 to 22.1) for *A. caninum* and *S. stercoralis*, respectively, indicating that these parasites remain endemic despite efforts to reduce their prevalence (Raw et al. 2022). Ivermectin is the treatment of choice for *Strongyloides* spp. infections and recent evidence also supports its off-label efficacy and effectiveness in remote community settings for the treatment of *A. caninum* infections in dogs (Paradies et al. 2019; Raw et al. 2024).

Animal health programs present a means to address many of the challenges to dog health and welfare faced in remote Aboriginal and Torres Strait Islander communities as well as the subsequent effects dogs may have on human wellbeing. The impact of animal health programs and success in addressing community and stakeholder concerns may vary dependent on program methodology and, crucially, the cultural competence of the personnel involved (Brown et al. 2014; Hiby et al. 2017; Ma et al. 2020b; Kennedy et al. 2021a; Kennedy et al. 2021b). Animal health programs delivered in Australian Aboriginal communities typically operate as either a contracted provider funded by local government or as a volunteer arrangement, with veterinarians providing services as individuals, or as part of clinics, educational institutions such as universities or charity organisations such as Animal Management in Rural and Remote Indigenous Communities (AMRRIC) or Vets Beyond Borders. Most reported animal health programs include the core activities of population management through surgical and/or chemical sterilisation, parasite control through treatment and preventative administration, and treatment of sick and injured animals (Wilks and Williamson 1998; Bradbury and Corlette 2006; Ma et al. 2020b). Vaccination and educational programs may also be

provided (Willis and Ross 2019). While currently no accreditation or standards for remote Aboriginal community veterinary programs exist, guidance documentation and policies for program planning, implementation and evaluation have been developed by the Australian Veterinary Association, AMRRIC and the International Companion Animal Management Coalition (ICAM) (Phelan et al. 2007; Australian Veterinary Association 2012; International Companion Animal Management Coalition 2019). Evaluation is also a key component of ongoing programs. Animal population density and demographics monitoring along with body and skin condition scoring are frequently cited in program evaluation recommendations, though broader measures determining impact beyond animal physical health may be used, such as evaluation of human-animal interactions, incidence of dog bites and other public health indicators (International Companion Animal Management Coalition 2015).

The aim of this study is to characterise a long-term animal health program in three remote Aboriginal communities, examine its impacts as well as to explore the prevalence, risk factors and genetic groupings of soil-transmitted helminth infections in dogs in these communities. This evidence will help to inform best practice in future animal health program planning, implementation and evaluation, as well as guiding zoonotic disease control within a One Health framework.

3.3 Methods

3.3.1 Study setting and population

Arnhem Land is a region of 97,000 km² in the north of the Northern Territory, Australia with a population of approximately 15,000 people of which 86% are Aboriginal and/or Torres Strait Islander (Australian Bureau of Statistics 2021). The region's climate is classified as tropical within the modified Köppen climate classification system, (Stern et al. 2000) with the primary weather station in the region recording a mean annual rainfall of 1461 mm and mean temperature range of 22.6 to 30.8°C between 1944 and 2023 (Bureau of Meteorology 2016).

The West Arnhem Land Dog Health Program (WALDHeP), part of Melbourne Veterinary School, University of Melbourne, provides annual animal health visits to the mainland community of Gunbalanya and the island communities of Warruwi and Minjilang (see Figure 3.1). Final year veterinary students, working under direct supervision of volunteer veterinarians, have been involved in WALDHeP since its inception. Students undergo a selection process to join the program and take part in two cultural competence preparation sessions before program visits as well as further training and debriefing sessions throughout visits.

Animal health visits in these communities include surgical desexing, parasite treatment, sick and injured animal consultations, humane euthanasia when requested by owners, educational programs and community animal health monitoring through record keeping during house-to-house visits. Commencing in 2005 and servicing Gunbalanya and its associated homelands annually, the program expanded to incorporate Warruwi and Minjilang in 2015 by request of local people. These three communities were chosen for program evaluation in the research presented herein due to the long-term program delivery by WALDHeP and associated longitudinal data.

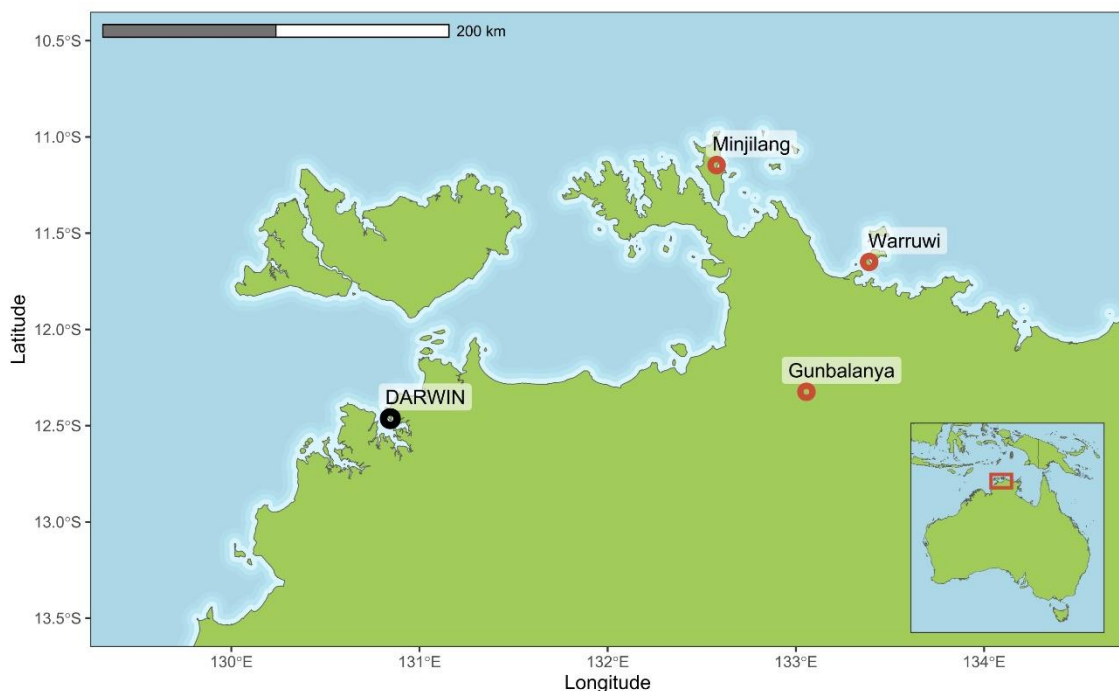


Figure 3.1 Locations of study communities of Gunbalanya, Warruwi and Minjilang

3.3.2 Survey data collection

During animal health visits, data were collected from house-to-house surveys in each community by the authors (CR and ET). Owners received veterinary advice, parasite treatment with 200 µg/kg off-label oral ivermectin on bread, or surgical desexing for their dogs. Data were collected either on customised paper forms, and later collated and transferred to an electronic spreadsheet or on mobile phones into a customised digital database (from 2018 onwards for Gunbalanya and Waruwi). While data collection from all houses within the communities was always attempted, the absence of owners or animals from some houses prevented a census each year. Treatments were conducted only with owner permission.

Animal data recorded included address, animal name and description, sex, desexed status, age group, body condition score, skin score, and treatments provided including surgeries. Dog age was advised by owners during consultation or was estimated by an experienced veterinarian on examination. Age group classifications included puppies which were less than six months old, young dogs which were six months to two years old, adults which were two to eight years old, and old dogs which were greater than eight years old. Body condition score was based on the Purina 9-point body condition scale (1 being emaciated and 9 being morbidly obese). Skin score was assessed on a 5-point scale, where a score of 1 is normal skin and fur and 5 is a completely hairless dog with thickened, inflamed skin (Laflamme 1997; Hiby et al. 2017). Further data were derived from numbers of treatments and surgeries provided as well as personnel records and number of days worked for each annual visit.

Human census data for the number of occupied households and human population in each community were collected from the Australian Bureau of Statistics (Australian Bureau of Statistics 2021). Estimated dog populations were calculated by multiplying the number of houses by 2.44 (Burleigh et al. 2015). Daily rainfall and average temperature records were collected from observations taken at airstrips in each community by the Bureau of Meteorology, with three months of rainfall totalled prior to the visit date (Bureau of Meteorology 2016).

3.3.3 Dog sampling

In 2019 a cross-sectional study of a subset of dogs was conducted in the three communities. The aim was to examine the prevalence and risk factors associated with *A. caninum* infections as well as the genetic haplotypes present in *S. stercoralis* infections to determine their zoonotic potential. Sample sizes were calculated using the `epi.sssimpleestb` function from the `epiR` package (v. 2.0.60, Stevenson *et al.*, 2023) in R (v. 4.2.2, R Core Team, 2022) using RStudio. Inputs included an expected prevalence of 77.3% based on published *A. caninum* data, (Raw *et al.* 2022) a diagnostic sensitivity of 97.3%, (Masseti *et al.* 2020) diagnostic specificity of 99.2%, (Mejia *et al.* 2013) using 95% confidence and 10% desired precision as well as assumed dog populations of 143, 66 and 72 for Gunbalanya, Warruwi and Minjilang, respectively. Required sample sizes obtained were 68 for Gunbalanya, 44 for Warruwi and 47 for Minjilang.

Samples were collected from dogs rectally while they were anaesthetised for surgical desexing or, if rectal collection could not be conducted, a fresh ground sample which could be identified to the dog was collected. Faecal samples were immediately stored in 5% potassium dichromate solution at a 1:2 ratio for transport at approximately 4°C to the University of Melbourne for laboratory analysis.

Owners were advised of research processes and aims and provided written consent for their dogs to be recruited into the study. If owners did not consent or were not present to provide consent, their dogs were not recruited. This study was approved by the University of Melbourne Animal Ethics Committee (ID 10298).

3.3.4 Coproscopic methods

One gram of faeces from each sample was subjected to a quantitative centrifugal faecal float using a combined solution of saturated sodium chloride and sucrose which was checked via hydrometer for a specific gravity of 1.27 (Gibbons *et al.* 2015; Takano *et al.* 2024). Parasite eggs were counted manually when identified under a light microscope, then counts were multiplied by the inverse of the faecal sediment measured in the

centrifuge tube to convert to a measure of eggs per gram (EPG) as per Zendejas-Heredia *et al.* (2021).

3.3.5 DNA extraction

Approximately 200mg of faeces from each sample underwent DNA extraction using a Maxwell® RSC PureFood GMO and Authentication Kit (Catalog no. AS1600, Promega Corporation, Madison, USA) in the Maxwell® RSC 48 Instrument (Catalog no. AS8500, Promega Corporation, Madison, USA) using a modified method as described by Massetti *et al.* (2022a).

3.3.6 Multiplex qPCR screening

Extracted DNA from each sample was subjected to multiplex qPCR assays to allow detection of four canine hookworm species including *A. caninum*, *Ancylostoma ceylanicum*, *Uncinaria stenocephala*, and *Ancylostoma braziliense* in addition to *Strongyloides* spp. according to protocols established by Massetti *et al.* (2020 and 2022a). Internal amplification controls were performed using previously published equine herpes virus (EHV4) primers (EHV-F, EHV-R), probe (EHV probe) and synthetic DNA fragments containing the target EHV4 sequence (gBlock® Gene Fragments, IDT® Technologies, Skokie, USA). DNA extraction controls were performed with previously published primers (MAM-F, MAM-R) and probes (MAM probe) targeting mammals (Massetti *et al.* 2020). Synthetic DNA fragments (gBlock® Gene Fragments, IDT® Technologies, Skokie, USA) including the target sequence of each parasite species were used as positive controls and no-template negative controls were included in all runs. Assay amplification and detection was conducted using a five channel AriaMx Real-time PCR System for all samples (Agilent, Santa Clara, USA), with data analysis performed using Agilent Aria software.

3.3.7 Conventional PCR amplification and sequencing

Samples positive for *Strongyloides* spp. on qPCR screening were subjected to conventional PCR for amplification of small subunit (SSU) ribosomal DNA highly variable regions (HVR) I and IV as well as the cytochrome c-oxidase subunit 1 (*cox1*) gene. Extracted DNA HVR I and IV regions were amplified according to protocols established by Barratt *et al.*, while *cox1* regions were amplified according to a nested PCR protocol established by Repetto *et al.* (Barratt *et al.* 2019; Repetto *et al.* 2022). Amplification of the target region was confirmed by assessing gel electrophoresis results against published molecular sizes for each amplicon. All PCR products confirmed to be of target length were sent at room temperature to Macrogen (Seoul, South Korea) for capillary electrophoresis sequencing.

3.3.8 Sequence and phylogenetic analysis

Consensus sequences were obtained through forward and reverse strand assembly using Geneious Prime (v. 2024.0.3 Dotmatrix, Boston, USA). Each strand was visually checked and cleaned of ambiguous chromatogram peaks, then trimmed to target sequence lengths. HVR-I and HVR-IV sequences were compared for sequence similarity using BLAST and aligned against published GenBank (GB) sequences (National Library of Medicine, Bethesda, USA). A Clustal Omega multiple *cox1* sequence alignment was conducted with published *cox1* sequences retrieved from canine and human hosts from GenBank. Geneious Tree Builder was used to generate a neighbour-joining method cluster dendrogram utilising 100,000 bootstrap replicates and a *cox1* sequence from *A. ceylanicum* as an outgroup (GB: NC035142).

3.3.9 Statistical analysis

Community animal data, animal health program and laboratory data were checked for consistency and completion and validated in an electronic spreadsheet (Microsoft Excel v. 1908, Microsoft Corporation, Redlands, USA). Data were analysed and plotted in R

using RStudio and contributed packages lme4 (v. 1.1-34, Bates *et al.*, 2023), emmeans (v. 1.8.7, Lenth *et al.*, 2023), ggplot2 (v. 3.4.2, Wickham *et al.*, 2023), epiR (v. 2.0.60, Stevenson *et al.*, 2023) and blme (v.1.0-5, Dorie *et al.*, 2020).

Dog demographic data including age group, sex, desexed status, body and skin condition as well as apparent endoparasite prevalence were described and stratified by community. The geometric mean EPG was calculated for each age group and community. True prevalence was estimated for each parasite species in each community based on the method described by Rogan and Gladen (1978) using the epi.prev function from the epiR package assuming diagnostic sensitivity of 97.3% based on Massetti *et al.* (2020) and diagnostic specificity of 99.2% based on a similar qPCR by Mejia *et al.* (2013).

Logistic regression models were used to determine odds ratios for the association between *A. caninum* qPCR result and the variables of community number of dogs at house, sex, desexed status and age group. Age group was collapsed to combine puppies with young dogs and adults with old dogs due to small numbers in each category. A directed acyclic graph (DAG) was developed based on available literature and putative associations based on veterinary field experience (Figure 3.2). It was used to determine relevant adjustment sets. Justifications for all links between variables are detailed in Appendix 2. Average temperature and rainfall in a community are determined by its geographic location. These variables remain consistent within a community although the identity of that community does not directly influence those variables. Furthermore, as sampling did not occur longitudinally there was no variation in either of these variables within a community leading to high collinearity. Therefore, only community was included in the regression models. Body condition score and skin score were not included in the DAG as they are complicated by unclear direction of causation. While poorer skin and body condition scores may be caused by hookworm or threadworm infection, they could also be a clinical sign of other comorbidities which in turn may predispose dogs to patent hookworm or threadworm infections. Measures of association between body or skin condition scores and qPCR parasite status were instead assessed using a Pearson's Chi-squared test. To overcome the limitations of small numbers per category, body condition score was collapsed into three categories:

underweight (1-3), ideal weight (4-6) and overweight (7-9). Similarly, skin score was collapsed into normal (1), minor hair loss (2-3) and major hair loss (4-5).

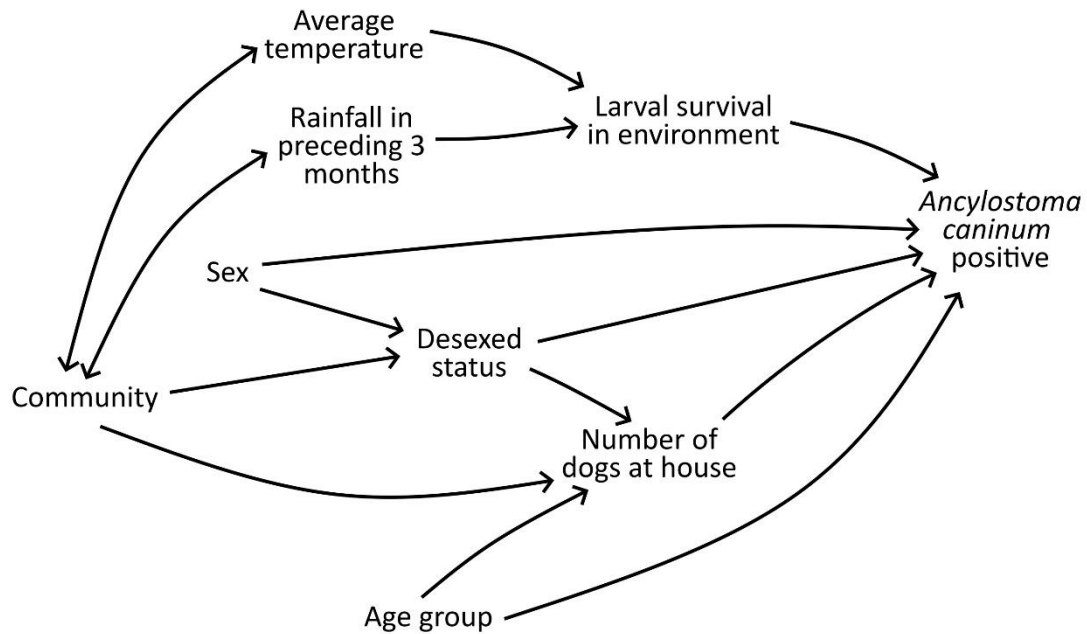


Figure 3.2 Directed acyclic graph showing putative causal paths linking explanatory variables to *Ancylostoma caninum* qPCR test result status

3.4 Results

3.4.1 Animal census and animal health program data

Animal health visits were conducted in Gunbalanya from 2005 to 2023 and in Waruwi and Minjilang from 2015 to 2023 (Table 3.1). Visits occurred annually each dry season in June or July except for 2020 and 2021 in which lockdowns due to COVID-19 prohibited travel to remote communities. While visits were conducted between 2005 and 2008 in Gunbalanya, paper records with community animal data and procedure data could not be recovered.

Program visits between 2005 and 2014 included 2 to 3 veterinarians spending approximately 4 days in Gunbalanya, equivalent to between 8 to 12 days of veterinary

service with one veterinarian, henceforth termed vet days. In 2015 a larger group provided 20 vet days to Gunbalanya followed by 10 vet days in Minjilang and 15 vet days in Warruwi. From 2016 to 2019 similar sized groups provided 20 vet days in Gunbalanya before separating to provide 8 and 12 vet days to Warruwi and Minjilang, respectively. In 2022 and 2023, Gunbalanya, Warruwi and Minjilang were provided 30, 8 and 6 vet days, respectively.

The number of houses with dogs visited varied by year with a median of 54 in Gunbalanya (range 38 to 101), 28 in Warruwi (range 17 to 41), and 22 in Minjilang (range 21 to 28). The numbers of dogs consulted on per workday also differed between visits, with a median of 42 in Gunbalanya (range 30 to 62), 23 in Warruwi (range 16 to 34), and 24 in Minjilang (range 18 to 57). Consistent house-to-house animal data collection methods allowed a high community coverage of dog-owning households each year. While visit coverage of the community remained consistent, data recording in Gunbalanya in 2016 was deficient due to a change of staff, and recorded figures for this year are not indicative of the true numbers of households visited or dogs consulted on. Demographic data of dogs in each year also varied, though balance between numbers of males and females was mostly observed with an average of 51% (range 41% to 61%) male dogs recorded during visits across all communities and years.

Table 3.1 Community animal data and animal health program data from Gunbalanya, Warruwi and Minjilang during years of animal health program delivery

Year	Vets : vet students	Work days	Occupied houses†	Est. dog pop.‡	Houses audited	Dogs consulted on	Male n (%)	Female n (%)	Spay	Castrate	Euth.	Ivermectin doses delivered n (% all dogs)	Median dogs per house (range)	Mean body cond. score	Mean skin score
Gunbalanya															
2005	2:1														
2006	3:4		145	353											
2007	3:5														
2008	3:3														
2009	3:3	4			38	177	108 (61)	69 (39)				61 (34)	5 (1-11)	4.55	2.13
2010	3:5	4			51	176	98 (56)	78 (44)	60	61	11	142 (81)	3 (1-10)	4.60	1.24
2011	3:5	4	151	368	74	248	117 (47)	131 (53)	82	70	3	222 (90)	3 (1-16)	4.33	1.51
2012	3:5	4			60	187	93 (50)	94 (50)	38	32	5	154 (82)	3 (1-15)	4.51	1.44
2013	3:3	4			51	163	84 (52)	79 (48)	16	24	5	142 (87)	3 (1-12)	3.50	2.60
2014	3:4	4			54	167	74 (46)	87 (54)	42	36	6	167 (100)	3.5 (1-12)	3.44	2.44
2015	5:7	4			70	140	78 (56)	62 (44)	13	18	2	120 (86)	2 (1-12)	4.69	1.64
2016	5:7	4	185	451	22 [§]	37 [§]	15 (41)	22 (59)	21	13	1		1 (1-5)	4.00	1.00
2017	5:6	4			71	155	83 (54)	72 (46)	16	11	1	129 (83)	1 (1-9)	4.71	1.65
2018	5:4	4			49	143	72 (50)	71 (50)	28	18	0	138 (97)	2 (1-9)	4.82	1.36
2019	5:7	4			62	170	91 (54)	79 (46)	25	22	0	121 (71)	2 (1-9)	5.13	1.27
2020¶															
2021¶			217	529											
2022	6:6	5			101	259	112 (43)	149 (57)	27	29	0	240 (93)	1 (1-12)	4.83	1.33
2023	6:6	5			44	151	56 (51)	54 (49)	16	15	0	151 (100)	2 (1-7)	4.91	1.20

† Occupied house number data retrieved from Australian Bureau of Statistics Census QuickStats

‡ Dog population estimated from a figure of 24.4 dogs per 10 houses from Burleigh *et al.* (2015)

§ Data recording in Gunbalanya in 2016 was incomplete and was not indicative of the number of dogs consulted on in the animal health program

¶ COVID lockdowns prohibited travel to remote communities in 2020 and 2021, and regular animal health programs were not conducted

Table 3.1 continued

Year	Vets : vet students	Work days	Occupied houses†	Est. dog pop.‡	Houses audited	Dogs consulted on	Male n (%)	Female n (%)	Spay	Castrate	Euth.	Ivermectin doses delivered n (% all dogs)	Median dogs per house (range)	Mean body cond. score	Mean skin score
Warruwi															
2015	5:7	3			28	104	62 (60)	42 (40)	24	33	4	57 (55)	3 (1-9)	4.07	2.08
2016	2:3	4	72	175	30	90	43 (48)	47 (52)	22	11	0	44 (49)	2 (1-7)	4.62	1.85
2017	2:2	4			41	113	52 (46)	61 (54)	12	9	0	95 (84)	3 (1-7)	4.54	1.33
2018	2:2	4			17	66	36 (55)	30 (45)	15	25	12	66 (100)	3 (1-13)	4.70	1.16
2019	2:3	4			26	92	41 (45)	51 (55)	29	21	0	90 (98)	3 (1-9)	4.53	1.30
2020¶															
2021¶			85	207											
2022	2:3	4			27	87	45 (52)	42 (48)	13	0	3	87 (100)	3 (1-8)	4.70	1.20
2023	2:3	4			29	78	41 (53)	37 (47)	10	5	0	77 (99)	2 (1-7)	4.84	1.05
Minjilang															
2015	5:7	2			28	114	56 (49)	58 (51)	30	22	0	37 (32)	3 (1-17)	3.79	1.61
2016	3:4	4	49	119	23	86	39 (45)	47 (55)	8	8	0	58 (67)	3 (1-10)	4.40	1.17
2017	3:4	4			22	94	49 (52)	45 (48)	10	15	3	65 (69)	4 (1-10)	3.82	1.00
2018	3:2	4			20	72	38 (53)	34 (47)	9	10	0	67 (93)	3 (1-8)	4.95	1.03
2019	3:4	4			21	86	46 (53)	40 (47)	7	6	0	62 (72)	3 (1-11)	4.90	1.04
2020¶															
2021¶			55	134											
2022	3:3	2			25	70	36 (51)	34 (49)	3	0	1	42 (60)	2 (1-8)	4.68	1.12
2023	3:3	2			21	56	29 (52)	27 (48)	2	2	1	46 (82)	2 (1-8)	4.87	1.07

† Occupied house number data retrieved from Australian Bureau of Statistics Census QuickStats

‡ Dog population estimated from a figure of 24.4 dogs per 10 houses from Burleigh *et al.* (2015)

§ Data recording in Gunbalanya in 2016 was incomplete and was not indicative of the number of dogs consulted on in the animal health program

¶ COVID lockdowns prohibited travel to remote communities in 2020 and 2021, and regular animal health programs were not conducted

Numbers of spay and castrate surgeries performed in each community tended to decrease over time. For example, in Gunbalanya in 2011 a total of 82 spays and 70 castrates were performed, while in 2023 only 16 spays and 15 castrates were performed, with fluctuations in surgery numbers in the intervening years. More surgeries were performed in Gunbalanya in 2022 compared to 2019 after a two-year interval between visits due to COVID-19. The median number as well as the range of dogs at each dog-owning house exhibited a downward trend with some fluctuation in each community. In Gunbalanya, the median number of dogs decreased from 5 in 2009 to 2 in 2023. In both Warruwi and Minjilang the median number of dogs decreased from 3 in 2015 to 2 in 2023.

Ivermectin doses were given to a median of 84% of dogs seen across all years and communities (range 32% to 100%). The mean body condition score showed a trend of improvement toward the ideal score of 5 in each community, with an average improvement of 0.36 between 2009 and 2023 in Gunbalanya and an average improvement of 0.77 and 1.08 points between 2015 and 2023 in Warruwi and Minjilang, respectively (Figure 3.3). Skin scores also showed improvement toward the ideal score of 1 across the periods of program delivery, with an average improvement of 0.93 points between 2009 and 2023 in Gunbalanya and an average improvement of 1.03 and 0.54 between 2015 and 2023 in Warruwi and Minjilang, respectively.

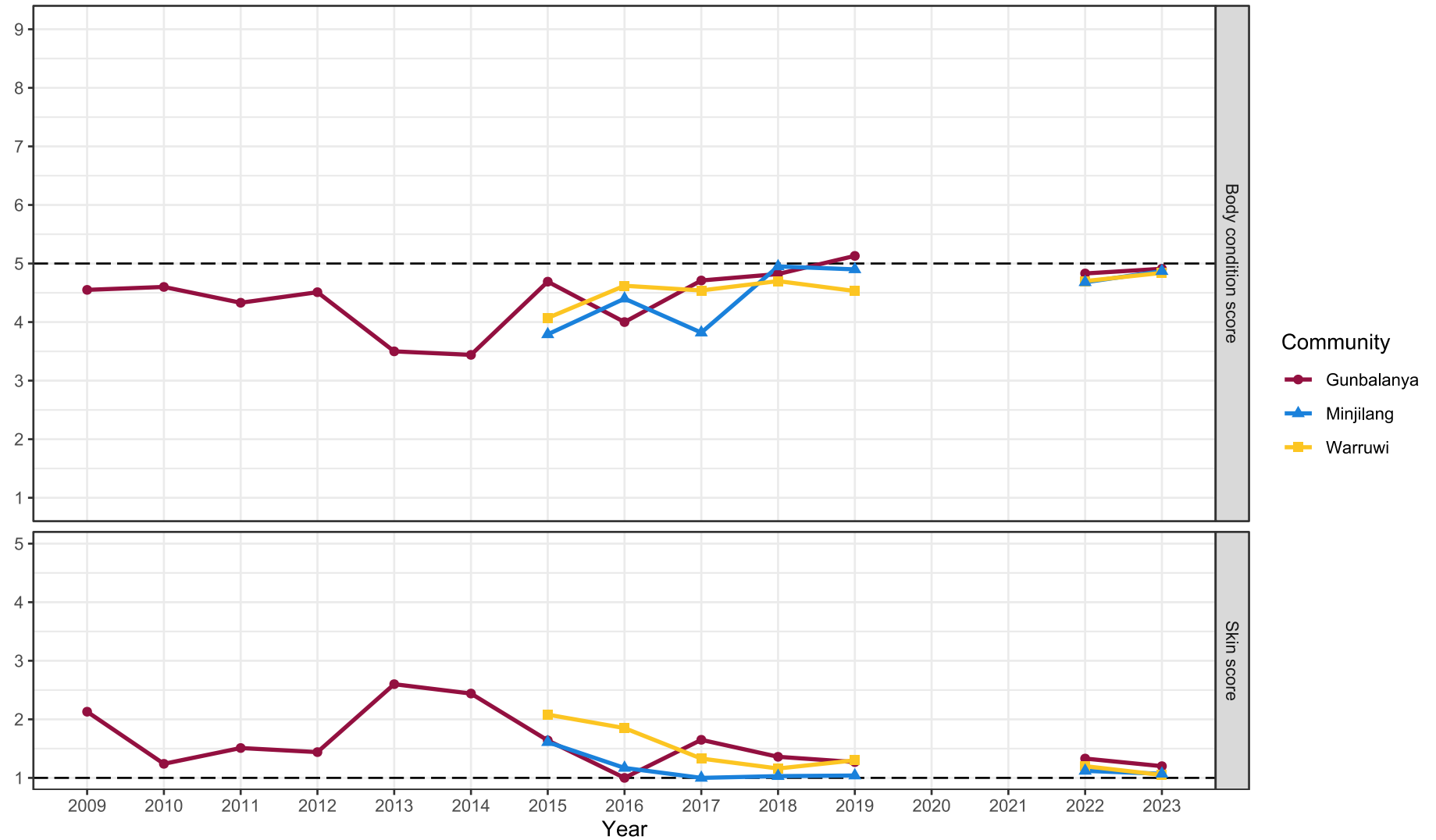


Figure 3.3 Mean body and skin condition scores of dogs in each community over years of program delivery, showing trends towards the ideal body condition score of 5 and the ideal skin score of 1 which are indicated by dashed lines

3.4.2 Soil-transmitted helminth prevalence and risk factors

In 2019, a subset of 163 dogs was sampled to estimate STH prevalence. Sufficient sample sizes were achieved in Gunbalanya (n = 91) and Warruwi (n = 46), but only 26 out of the target of 47 dogs were sampled in Minjilang. *Ancylostoma ceylanicum*, *U. stenocephala* and *A. braziliense* were not detected in any multiplex qPCR assays. The overall true prevalence for *A. caninum* across all communities was 86.9% (95% CI 80.4 to 91.8) (Table 3.2). Despite the varying demographics of dogs in each community, the community-level prevalence estimate was closely aligned with the overall estimate. True prevalence for *Strongyloides* spp. across all communities was 14.4% (95% CI 9.6 to 20.9). The highest true prevalence estimates were seen in Minjilang, with 90.8% (95% CI 72.8 to 98.6) and 19.1% (95% CI 8 to 38.4) for *A. caninum* and *Strongyloides* spp., respectively. Neither body condition score nor skin score was statistically significantly associated with *A. caninum* ($\chi^2=1.9$, $p=0.38$ and $\chi^2=3.9$, $p=0.14$, respectively) or *Strongyloides* spp. infection ($\chi^2=2.4$, $p=0.3$ and $\chi^2=0.1$, $p=0.95$, respectively).

Crude odds ratios from logistic regression quantified the individual associations of community, number of dogs at a house, sex, desexed status or age group with *A. caninum* infection (Table 3.3). After incorporating adjustment sets established from the DAG, logistic regression revealed that dogs from households with three or more dogs had 7.01 times higher odds of infection (95% CI 1.99 to 24.69) compared to dogs from households with fewer than three dogs. This result was adjusted for community, age and desexed status and was statistically significant with a p-value of 0.007. Entire dogs had 2.53 times higher odds of infection (95% CI 0.84 to 7.63, p-value 0.1) compared to desexed dogs after adjusting for community and sex.

Table 3.2 Demographic, intensity and prevalence data for a subset of 163 dogs sampled for soil-transmitted helminths in 2019 in each community based on qPCR results and assuming diagnostic sensitivity of 97.3% and specificity of 99.2%

	Gunbalanya	Waruwi	Minjilang	All communities
Sex n (%)				
Male	33 (38)	22 (49)	7 (35)	62 (41)
Female	53 (62)	23 (51)	13 (65)	89 (59)
Unknown	5	1	6	12
Desexed n (%)				
Yes	16 (19)	5 (11)	1 (5)	22 (15)
No	70 (81)	40 (89)	18 (95)	128 (85)
Unknown	5	1	7	13
Age group n (%)				
Puppy	18 (22)	4 (9)	4 (20)	26 (18)
Young	13 (16)	16 (36)	4 (20)	33 (23)
Adult	48 (59)	23 (52)	11 (55)	82 (56)
Old	3 (4)	1 (2)	1 (5)	5 (3)
Unknown	9	2	6	17
Body condition n (%)				
Underweight (score 1-3)	5 (6)	10 (23)	11 (55)	26 (18)
Ideal weight (score 4-6)	73 (88)	32 (74)	9 (45)	114 (78)
Overweight (score 7-9)	5 (6)	1 (2)	0 (0)	6 (4)
Unknown	8	3	6	17
Skin condition n (%)				
Normal (score 1)	52 (63)	27 (63)	16 (76)	95 (65)
Minor hair loss (score 2-3)	28 (34)	14 (33)	4 (19)	46 (31)
Major hair loss (score 4-5)	3 (4)	2 (5)	1 (5)	6 (4)
Unknown	8	3	5	16
Geometric mean hookworm eggs per gram of faeces (SD)				
Puppy	445 (54)	1951 (7)	98 (36)	443 (40)
Young	94 (63)	235 (40)	350 (52)	172 (45)
Adult	340 (20)	136 (22)	327 (56)	259 (24)
Old	43 (26)	0 (0)	372 (0)	31 (23)
All age groups	295 (29)	216 (28)	201 (36)	253 (29)
Apparent prevalence (95% CI)				
<i>Ancylostoma caninum</i>	83.5 (74.6, 89.7)	84.4 (71.8, 92.4)	88.5 (71, 96)	84.7 (78.3, 89.4)
<i>Strongyloides</i> spp.	14.3 (8.5, 22.9)	13 (6.1, 25.7)	19.2 (8.5, 37.9)	14.7 (10.1, 21)
True prevalence (95% CI)				
<i>A. caninum</i>	85.7 (76.4, 92.2)	87 (73.6, 95)	90.8 (72.8, 98.6)	86.9 (80.4, 91.8)
<i>Strongyloides</i> spp.	14 (8, 22.9)	12.7 (5.5, 25.8)	19.1 (8, 38.4)	14.4 (9.6, 20.9)

Table 3.3 Crude and adjusted odds ratios for infection with *Ancylostoma caninum* in dogs using logistic and mixed effects modelling with addition of random effect of individual dogs

Variable and category	Positives / n (%)	Crude OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value	Adjustment set for total effects
Community			0.83			NA
Gunbalanya	76/91 (84)	Reference				
Minjilang	23/26 (88)	1.51 (0.45, 6.94)				
Warruwi	39/46 (85)	1.1 (0.43, 3.08)				
Dogs at house			0.002		0.007	Community, age, desexed
<3	12/19 (63)	Reference		Reference		
≥3	69/73 (95)	4.79 (1.86, 13.45)		7.01 (1.99, 24.69) [†]		
Sex			0.34			NA
Female	74/89 (83)	Reference				
Male	55/62 (89)	1.59 (0.63, 4.41)				
Desexed			0.06		0.1	Community, sex
Yes	16/22 (73)	Reference		Reference		
No	113/128 (88)	2.82 (0.9, 8.12)		2.53 (0.84, 7.63)		
Age			0.96			NA
Puppy and young	50/59 (85)	0.98 (0.39, 2.53)				
Adult and old	74/87 (85)	Reference				

[†] Interpretation: After adjusting for the effect of community, age and desexing, the odds of being *A. caninum* positive for a dog living in a household with three or more dogs was 7.01 times higher (95% CI 1.99 to 24.69) compared to dogs from a household with less than three dogs.

3.4.3 *Strongyloides* spp. sequencing

Out of the 163 dogs sampled in 2019, 24 dogs were positive on qPCR screening for *Strongyloides* spp. All 24 samples were subjected to conventional PCR with gel electrophoresis to confirm that DNA of the target length was successfully amplified. The HVRIV region in one dog (WD177), HVRI and *cox1* in another dog (GD110) and all three regions in a third dog (GD095) amplified successfully. All amplicons were successfully sequenced except for the GD110 *cox1* amplicon and all sequences were submitted to GenBank.

Sequences from sample GD095 (GB accession number: PQ301468-PQ301470) showed 100% identity with published GenBank sequences identified as HVRI haplotype V (GB accession number: MN076361) and HVRIV haplotype B (GB accession number: MK468672) with a *cox1* sequence with 83.69% bootstrap consensus support most closely aligned with known dog genotypes in clade 2 (Figure 3.4). Sample GD110 (GB accession number: PQ301471) showed 100% identity with HVRI haplotype II (GB accession number: MK778085), while the WD177 HVRIV sequence (GB accession number: PQ301472) showed 98.3% identity with *Strongyloides ratti* (GB accession number: LN609412).

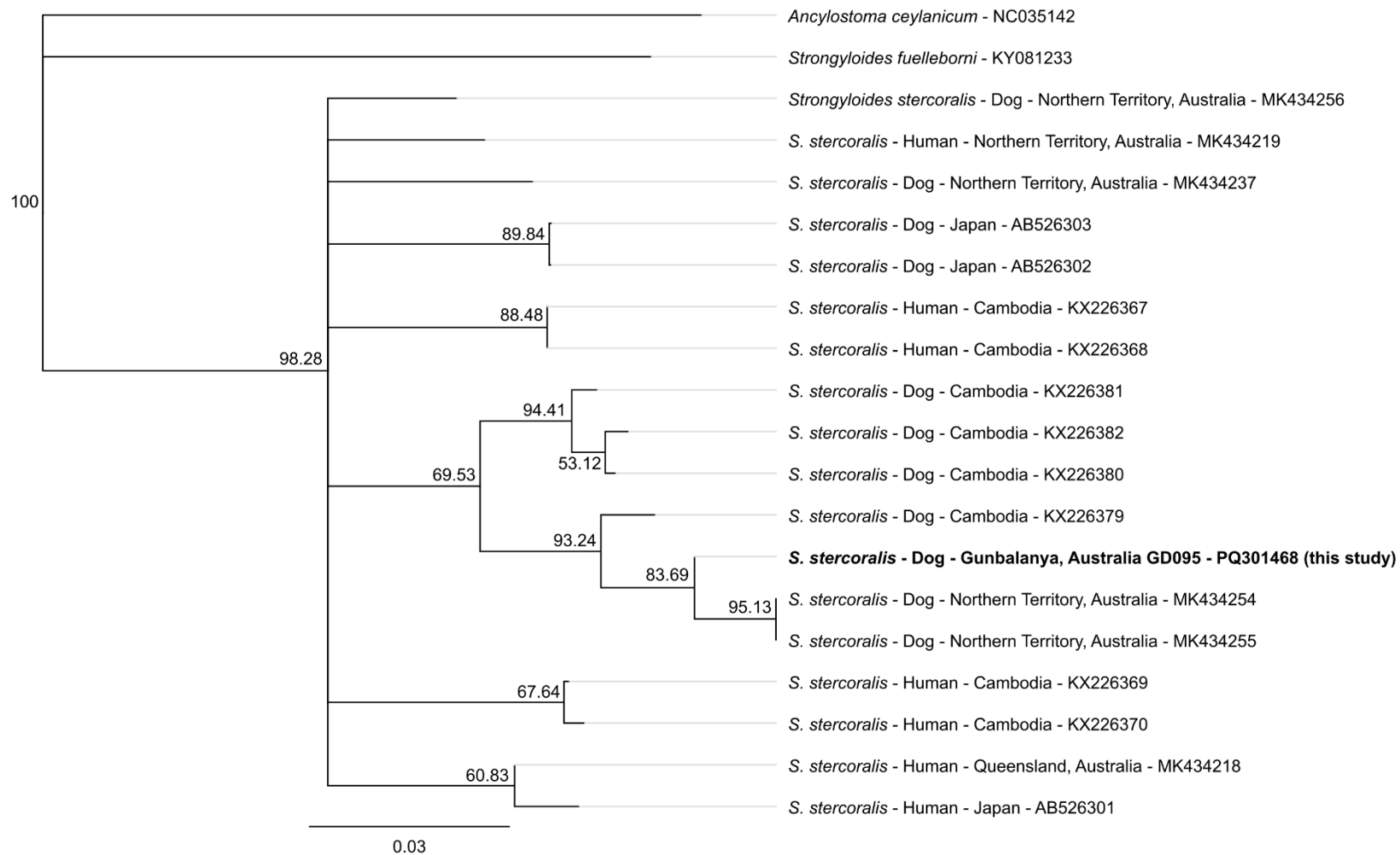


Figure 3.4 Neighbour-joining cluster dendrogram of *Strongyloides stercoralis cox1* sequences including GD095 from this study together with published sequences from human and canine hosts with their geographic location and GenBank accession number

3.5 Discussion

The impacts of programs such as WALDHeP are evident in the number of dogs treated and desexed and have most likely been the main factor in the changes in the numbers of dogs per house and the improvement in average body and skin condition over time. Continual program delivery over a period of almost twenty years and successful expansion to include two more communities are also notable in this setting. This ongoing program's delivery and expansion have been driven by factors that are more important and enduring than merely availability of funding or the need for veterinary services. Relationship building, reliability and sustainability are key aspects of this program.

Relationships have been established and strengthened with local Aboriginal people in Gunbalanya and its associated homelands for twenty years based on a foundation of cultural humility, and with that a level of trust in the care afforded to community dogs by the veterinarians involved. Body and skin condition scores of dogs in the community showed clear improvements following desexing surgeries and parasitic treatments delivered by WALDHeP. While not a formal part of the research presented herein, local people have indicated during informal conversations that they have also noticed these positive changes and in turn feel more confident in having their own dogs desexed or treated. This may be despite possible unease felt towards animal management staff based on past experiences with personnel sent to manage dog populations through culling programs (Brookes et al. 2020; Bennett and Archer-Lean 2023). Kennedy *et al.* (2021) emphasise the importance of considering all stakeholders in addressing animal health program challenges in Aboriginal communities, as well as the knowledge and power frames influencing the behaviour and impact of those stakeholders (Kennedy et al. 2021a; Kennedy et al. 2021b). While dog overpopulation was identified as the most pressing issue by Indigenous and non-Indigenous community members as well as animal management staff, the proposed means by which to enact change and the value placed on community dogs varied markedly. Non-Indigenous locals favoured introduction of by-laws including dog registration and movement restrictions equivalent to those found in urban settings and were identified as having greater power to enact these changes as

many were local government employees. In contrast, Indigenous locals favoured culturally appropriate management of dog populations acknowledging the significance of dogs in daily life including the importance of independence and free movement, while limiting the enforcement of by-laws. This highlights the wealth of knowledge Indigenous people have regarding the lives and importance of their animals, though their power to enact systemic change was reported to be less than that of other stakeholders (Kennedy et al. 2021a). Cultural competence, decolonisation of veterinary programs and consultation with local people have also been highlighted as key to program planning and evaluation in studies of veterinary services in underserved communities in North and South America (LaVallee et al. 2017; Daigle et al. 2023; Rojas-Sanchez et al. 2024).

Important relationships have also been established with local government representatives who facilitate programs through providing accommodation, vehicle use, and some staff support in communities. Program reliability further contributes to trusting relationships as dog owners know that WALDHeP will return at regular intervals as promised. Sustainability is ensured by maintaining and nurturing a team of available veterinarians experienced in remote community work, which is where veterinary student involvement is key. Programs such as WALDHeP afford veterinary students opportunities to develop cultural competence in Aboriginal communities as well as their professional and clinical skills. As a result of their positive experiences and growth, many students choose to continue this work as graduate veterinarians, creating a larger available pool of culturally competent veterinarians with ongoing relationships with communities. Cultural understanding and relationship building underpinned by reliability and sustainability take time to establish – often several years. Due to local government budgeting constraints, veterinary service contracts for remote communities are often limited to twelve months. Assessments of program impact on a short timescale of one to two years does not account for these important factors and may not reveal the longer-term impacts on dog health and wellbeing as demonstrated in this study. Funding on these timescales may also hinder sustainable relationship building necessary for long-term impact.

The numbers of dogs consulted on and treated in this study appear considerably smaller than the dog population estimate based on the figure of 24.4 dogs per 10 occupied households proposed by Burleigh *et al.*, though it is likely to be closer to the true dog population (Burleigh *et al.* 2015). Based on the data of 20 communities in the Burleigh *et al.* study, it is unclear what access these communities had to population management and how stable their dog population was at the time. Other studies surveying dog populations in remote Aboriginal communities report figures of 2.7 dogs per dog-owning house and 26.2 dogs per 100 people, making comparisons challenging both in their reporting methods and the limited information on availability of population management in those studies (Hudson *et al.* 2018; Riley *et al.* 2020). The majority of households in each community were visited each year in the present study, though many houses were unoccupied and therefore had no dogs. The median and range of number of dogs per house, however, decreased over time, demonstrating the effectiveness of long-term annual visits for population management. This is important in terms of program impacts but more importantly it illustrates that animal health programs enable owners to keep the number of dogs they choose rather than the number they find themselves with because of indiscriminate breeding. This acknowledges owner goals and agency as well as the significance of animals in people's lives (Kennedy *et al.* 2021a; Kennedy *et al.* 2021b).

The changes in body and skin condition seen in this study are similar to those observed in a study by Riley *et al.* (2020) evaluating the impacts of an animal health program operated by AMRRIC in the West Daly region of the Northern Territory. The study reported a 77% increase in prevalence of dogs with a body condition score of 4-5 over a 12-month period as well as a 9% increase in the prevalence of dogs with no hair loss, equivalent to a skin score of 1. The present study reveals that improvements in individual animal and average community body and skin condition can continue over several years but are not necessarily linear. Factors likely to contribute to fluctuations in these measures include abundance of food, owner care, as well as morbidity from diseases including parvovirus, transmissible venereal tumours, scabies, ticks, ehrlichiosis

and soil-transmitted helminths, each of which may have seasonal or climatic variations (Wilks and Williamson 1998; Bradbury and Corlette 2006).

One challenge in evaluating animal health programs is that the full scope of work conducted by WALDHeP is not captured in these reported data. Educational interactions with local community members, sick or injured dog consultations or treatment of species other than dogs can be very impactful on both animal and human health and often happen incidentally. These interactions are woven naturally into the flow of WALDHeP visits, making it difficult to capture and therefore to quantify.

Another challenge, particularly in early years of the program, was record keeping. In a volunteer program with limited periods of time spent in resource limited settings, focus was mostly placed on delivery of services with comprehensive record keeping when time permitted. Animal health monitoring on a range of measures in these settings provides an important means of accurately measuring impact, refining practices and providing evidence to supporting stakeholders and warrants greater attention (International Companion Animal Management Coalition 2015). Interruptions in program delivery due to COVID-19 make analysis of impact more challenging, as do alterations to the size of the team or numbers of workdays spent in each community.

Ancylostoma caninum infections were present in more than eight out of ten dogs in these communities, while *Strongyloides* spp. infections were found in more than one in ten dogs. These prevalence estimates are in line with pooled true prevalence estimates published in a systematic review of STHs in Australian Indigenous communities of 77.3% (95% CI 63.7 to 91.0) and 18.9% (95% CI 15.8 to 22.1) for *A. caninum* and *Strongyloides* spp., respectively (Raw et al. 2022). The continued endemism of both *A. caninum* and *Strongyloides* spp. reported here despite access to annual animal health programs for up to twenty years suggests that more frequent anthelmintic administration is necessary to reduce burden or achieve community elimination. Recent research indicates that the dosage of ivermectin used in this program is efficacious in the short term and effective in reducing prevalence of *A. caninum* in community dogs with three-monthly treatment frequency (Raw et al. 2024). While no published data exists examining the field efficacy

of ivermectin used to treat *Strongyloides* spp. in dogs in these settings, it has been shown to reduce prevalence in humans and concomitant dog and human treatment is recommended for optimal effect (Kearns et al. 2017; Beknazarova et al. 2017).

Appropriate options for both on- and off-label treatments are required in this setting to ensure consistent parasite control (Raw et al. 2024). Depending on the treatments used, these may be administered by rangers or environmental health worker staff and thus may not require veterinary visits in short intervals. However, the frequency of animal health program visits is relevant not only for zoonotic parasite control, but also for population management and animal welfare. Infrequent visits can lead to accelerated population growth and may mean that animals suffer with disease for long periods before veterinary attention is accessible. Further research is warranted to develop recommendations on the optimal frequency of animal health program visits in remote Aboriginal communities to ensure dog populations are managed in culturally sensitive, effective and cost-effective ways. Community education and communication is also key to maintaining engagement with preventive medicine. Effective programs should empower communities in the management of the health of their animals rather than encouraging a reliance on annual treatments which are unlikely to lead to elimination or meaningful control of parasites in the community.

In terms of risk factors for *A. caninum* infection, dogs from households with three or more dogs had seven times higher odds to test positive for *A. caninum* on qPCR compared to dogs from households with fewer than three dogs after adjusting for the effects of community, age and desexing. This is important because the number of dogs in a house is something that owners can control and animal health programs can assist with. This is exemplified by the changes in the median number of dogs per household described in this study, as well as in other studies conducted in remote community settings (Bradbury and Corlette 2006). While entire dogs had greater odds of infection with *A. caninum*, this finding did not reach the significance threshold p-value of 0.05. However, biologically, this association may be relevant as entire dogs roam further and may have a greater chance of encountering contaminated environments (Molloy et al. 2017). Further, a study in India found entire males to have higher prevalence of

hookworm infection, which was attributed the suppressive effect of testosterone on innate resistance to parasites (Traub et al. 2014).

The sampling methodology applied in this study presents a potential source of bias as the majority of dogs sampled were dogs anaesthetised for desexing. As such, many more entire dogs were sampled than desexed dogs and fewer puppies and old dogs were sampled as they were not appropriate surgical candidates. In addition, the age group classification used also represents a potential source of bias as it is subjective and may not be reflective of true age groupings or risk factors associated with each age group. This bias in sampling may have influenced prevalence or odds ratio estimates in this study, while an overall insufficient sample size may limit the precision of these estimates. Insufficient dog sample numbers in Minjilang were due to low numbers of dog owners present in the community to give consent for inclusion in the study, as well as limited numbers of surgical procedures performed in 2019. Reduced sample size also meant that statistical power was insufficient for modelling accounting for the hierarchical structure of the data of dogs within households within communities. However, mixed-effects modelling conducted in a study in the Torres Strait Islands indicated that greater than 99% of random effects variation was attributable to individual dogs rather than communities, and a similar attribution would be expected in this study (Raw et al. 2024) Despite these limitations, the collected data still provide valuable insights, particularly given the scarcity of information available from hard-to-reach populations, providing a better understanding of the unique challenges and needs within these communities. Nevertheless, the findings should be interpreted with caution, and further research with larger sample sizes is recommended to validate and expand upon these results.

There was no evidence of human-infecting *Strongyloides* spp. haplotypes being present in dogs in these communities, though recent evidence of a human infection with what was thought to be a dog-only haplotype may challenge these host-based haplotype classifications (de Ree et al. 2024). Haplotype analyses in these communities was limited, however, as the success of sequencing *Strongyloides*-positive samples was low. Intermittent and low-level shedding of larvae in *Strongyloides* spp. infections are known

to make detection and amplification challenging. Further studies implementing larval concentration techniques which can boost sequencing success are warranted to better understand the zoonotic risk of this parasite in these settings (Sultana et al. 2013; Robertson et al. 2017). With a better understanding of zoonotic risk, One Health intervention strategies can be implemented to control these parasites across all relevant hosts.

This study demonstrates that consistent, long-term animal health programs can have substantial impacts in remote Aboriginal communities, though they must have foundations of trusting relationships, reliability and sustainability. These impacts may be seen in improvements to dog body and skin condition or in supporting owners to keep their desired number of dogs, which can also have significant effects on their risk of STH infection. Through holistic, culturally responsive practices and long-term commitments, substantial improvements to dog health are possible with additional benefits to the health of the people who care for them.

Chapter 4: A comparative field efficacy trial of three treatment programs against endo- and ectoparasites in naturally infected dogs

Presented as published:

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4.1 Abstract

Tropical climates in remote Aboriginal and Torres Strait Islander communities in northern Australia are conducive to the transmission of canine helminths such as hookworms, as well as ectoparasites such as fleas and ticks. In addition to their veterinary importance, these parasites may present a zoonotic risk either directly, or as potential vectors for bacterial pathogens. These factors necessitate efficacious and effective antiparasitic treatment programs for community dogs. A cluster-randomised trial was performed on three islands in the Torres Strait to examine the short-term efficacy and medium-term effectiveness of three treatment programs. Treatments administered included oral oxibendazole/praziquantel (ParaGard®) and oral afoxolaner (Nexgard®); topical moxidectin/imidacloprid (Advocate®) and imidacloprid/flumethrin collars (Seresto®); and off-label oral ivermectin (Bomectin®). Canine faecal samples were collected and examined for endoparasites by faecal flotation and real-time PCR at baseline, 7-11 days after treatment and 6 months later. The proportion of dogs positive for *Ancylostoma caninum* at baseline and negative at day 7-11 was 9% (95% CI 4.4-17.4) for dogs treated with oxibendazole, 56.4% (95% CI 41-70.7) for moxidectin, and 89.7% (95% CI 73.6-96.4) for ivermectin. Faecal flotation results showed a greater than 90% egg reduction in 29.2% (95% CI 19.9-40.5) of dogs treated with oxibendazole, 79.4% (95% CI 63.2-89.7) for moxidectin, and 95% (95% CI 76.4-99.1) for off-label ivermectin. Elimination of ectoparasite infestation was observed at day 7-11 in 69.9% (95% CI 56.7-80.1) of dogs treated with afoxolaner, 80% (95% CI 60.9-91.1) with imidacloprid/flumethrin collars, and 0% (95% CI 0-11.7) for off-label ivermectin. Mixed effects modelling revealed only treatment group to be significantly associated with outcome measures. Based on these study results, the poor efficacy of oxibendazole against *A. caninum* renders it inept for treatment, while ivermectin and moxidectin were suitable. Ivermectin was unsuitable for ectoparasite treatment due to its poor efficacy, while afoxolaner and imidacloprid/flumethrin collars appear suitable.

4.2 Introduction

In tropical climates, and particularly in remote community settings, canine endoparasites and ectoparasites and the diseases they vector cause significant morbidity and mortality in dogs and are also responsible for some of the most important and well recognised zoonoses affecting humans (Chen et al. 2012; Traub et al. 2015; Baneth et al. 2016; Raw et al. 2022). Endoparasites such as hookworms of the genus *Ancylostoma* spp., threadworms (*Strongyloides* spp.) and roundworms (*Toxocara canis*) constitute some of the most prevalent canine zoonotic helminths of stray, semi-domesticated and pet dogs throughout tropical regions of the world (Bradbury and Traub 2016; Page et al. 2016). Infections with these parasites can result in asymptomatic to serious clinical manifestations in dogs and people. For example, *Ancylostoma* spp. infections can cause profound haemorrhagic enteritis and anaemia in dogs, depending on parasite species and worm burden. *Ancylostoma* spp. infection in humans may cause cutaneous larva migrans, or in the case of *Ancylostoma caninum*, eosinophilic enterocolitis (Bradbury and Traub 2016; Traub et al. 2021). While most human intestinal infections with *A. caninum* were found to be caused by a single adult worm, more recent evidence suggests that patent infections are potentially possible (Ngcamphalala et al. 2020). Infection with *Toxocara canis* may manifest as ocular toxocariasis with vision loss or retinal damage or as visceral toxocariasis with wheezing, asthma, fever, or abdominal pain (Chen et al. 2018).

High burdens of fleas (*Ctenocephalides felis*) and brown dog ticks (*Rhipicephalus linnaei*) in community dogs contribute to the spread of tick-borne diseases ehrlichiosis, hepatozoonosis, babesiosis and anaplasmosis, while fleas may pose a zoonotic risk for the transmission of bartonellosis and flea-borne spotted fever (Dantas-Torres 2010; Barrs et al. 2010; Teoh et al. 2016; Teoh et al. 2018). In addition to the risk of vector-borne diseases, pruritis caused by even transient flea or tick infestations or bites may predispose humans to chronic secondary skin infections with potential sequelae of impetigo, rheumatic fever, or rheumatic heart disease (O'Donel Alexander 1984; Elliot et al. 2006).

As in Aboriginal communities across other parts of Australia, dogs in Torres Strait Islander communities may have many different roles including companion, hunting partner, source of protection, or cultural or spiritual roles (Senior et al. 2006; Smith and Litchfield 2009; Constable et al. 2010). These important roles, as well as the often free-roaming nature and large populations of dogs in these communities, may place community members at risk of acquiring parasite and flea-borne zoonotic pathogens either directly through close contact, or indirectly through contact with, or ingestion of parasitic stages in contaminated soil and bedding (Smout et al. 2017a).

Efficacious endo- and ectoparasitic treatments are essential to mitigate the morbidity related to canine parasites. The remoteness of many Australian Aboriginal and Torres Strait Islander communities means that veterinary visits may be limited, sporadic or ultimately unattainable due to logistical or financial barriers. As such, identifying effective antiparasitic treatment programs which can be administered regularly without the need for veterinary oversight is of value to these communities. Off-label treatments require veterinary oversight to be administered as they are being used outside of the registered and labelled use (Department of Agriculture Forestry and Fisheries 2014). Such treatments have formed the mainstay of remote community veterinary antiparasitic treatment despite scarcity of evidence of their effectiveness in these settings. Evaluating the efficacy of off-label treatment is therefore of value, particularly to the veterinarians, local government departments or non-government organisations (NGOs) owing to their potential cost effectiveness (Wilks and Williamson 1998; Bradbury and Corlette 2006). With these factors in mind, the aim of this study is to examine the short-term efficacy and medium-term effectiveness of two labelled antiparasitic treatment programs in comparison to the off-label usage of ivermectin in a remote Torres Strait Islander community setting. The resulting evidence will inform antiparasitic programs which can be administered by community members either with or without veterinary oversight.

4.3 Materials and methods

4.3.1 Study setting and population

The Torres Strait Islands comprise over 270 small islands in the Torres Strait between the northernmost tip of mainland Australia in the state of Queensland and Papua New Guinea spanning an area of over 48,000 km². Sitting at the border of equatorial savanna and monsoonal climate regions based on a modified Köppen climate classification system (Stern et al. 2000), the primary weather station for the islands recorded a mean annual rainfall of 1736 mm and mean temperature range of 24.7 to 30.5°C between 1995 and 2023 (Bureau of Meteorology 2023).

Dogs on three remote islands were enrolled in this cluster-randomised trial. Islands were selected based on recommendations from the Torres Strait Islands Regional Council regarding adequate dog numbers present as well as community consultation and acceptance of the proposed study. Locations of the selected islands are shown in Figure 4.1. Torres Strait Islander community engagement and leadership was crucial to this study. In-person consultation was conducted with local Environmental Health Worker staff to ascertain what was important to the community and to develop a feasible study methodology. This was followed by consultation with elders and elected council representatives of all island groups regardless of their inclusion in the study, and approval of a formal research proposal. This study was also approved by the University of Melbourne Animal Ethics Committee (ID:10298).

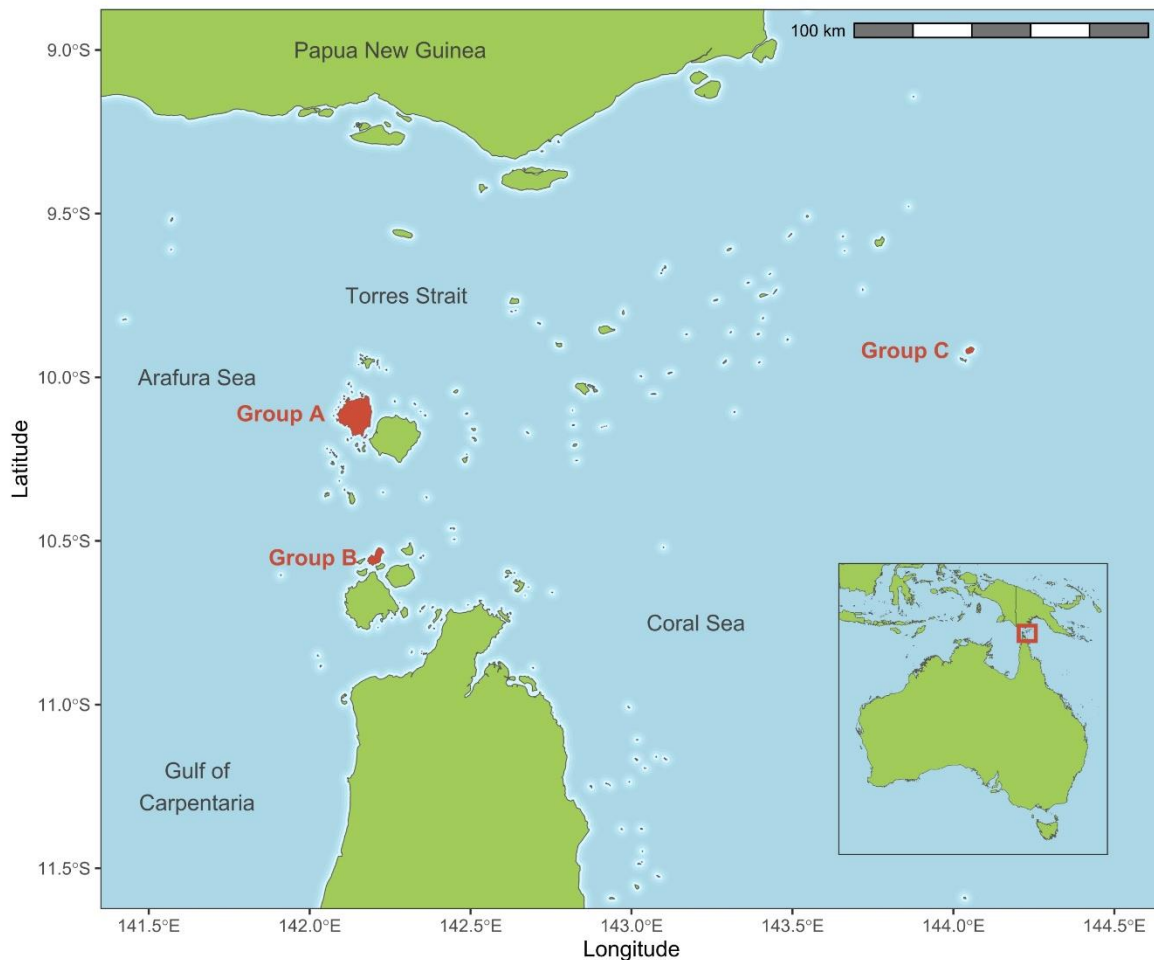


Figure 4.1 Torres Strait region map with shaded and labelled study islands

All dog owners on each selected island were approached to provide verbal and written consent to have their dogs recruited into the study. Dogs were not recruited if owners did not consent or were not present to provide consent. Dogs on each island were assigned to the same treatment arm to ensure consistent administration of ongoing treatments and to reduce the risk of environmental contamination influencing other treatment group outcome measures. Treatment arms consisted of; Group A - oral tablets administered at 22.5 mg oxibendazole/5 mg praziquantel per kilogram bodyweight (ParaGard®, Boehringer Ingelheim) and oral chews administered at 2.5 mg afoxolaner per kilogram bodyweight (Nexgard®, Boehringer Ingelheim); Group B - topical 1% moxidectin/10% imidacloprid applied at 0.1 ml per kilogram bodyweight (Advocate®, Elanco) and a 10% imidacloprid/4.5% flumethrin polymer matrix collar

(Seresto[®], Elanco) administered according to the labelled instructions and; Group C - off-label oral ivermectin (Bomectin[®], Elanco) administered at 200 µg/kg in bread with flavoured paste. As ivermectin administration in this context is off-label usage, it required oversight from a registered veterinary practitioner.

4.3.2 Data collection

At baseline, dog and owner names and address details were collected for the purpose of follow-up reidentification. Other dog details recorded at the time of enrolment included sex, sterilisation status, estimated weight, and age group. Age group information was provided by dog owners at the time of enrolment or was estimated by a veterinarian on examination of the dog. Age group classifications consisted of puppies which were less than six months old, young dogs which were six months to two years old, adults which were two to eight years old, and old dogs which were greater than eight years old. Any overt skin lesions were noted, and a targeted patch examination technique of predilection sites was used to establish a semi-quantitative measure of tick burden on each dog as described by Brianti *et al.* (2013). Briefly, a tick score of zero indicates no ticks detected, a score of 1 indicates between 1-5 ticks detected, a score of 2 indicates 6-20 ticks detected, a score of 3 indicates 21-50 ticks detected, a score of 4 indicates 51-100 ticks detected and a score of 5 indicates over 100 ticks detected. The same system was employed to determine flea burden. Single faecal samples were collected from each dog rectally, or from the ground if rectal collection was not possible and a fresh ground sample identifiable to the dog was available. All faecal samples were immediately stored in DNA/RNA Shield (Zymo Research, Irvine, USA) at a 1:2 ratio for transport at room temperature to the University of Melbourne for laboratory analysis. At this point, treatments were administered per specified treatment arm and dogs remained under their owners' care thereafter.

Follow-up sampling was conducted by the same method 7-11 days post-treatment. This timeframe allows detection of reduction or cure of initial infection whilst avoiding new or re-infections as it is shorter than the prepatent period of *Ancylostoma* spp. Dogs were

reidentified from recorded data to allow comparison of baseline and post-treatment data. Repeat measures of flea and tick count were also recorded.

Dogs again remained in their owners' care and were treated according to their treatment arm three months post-baseline. Treatments were administered by trained local Environmental Health Workers. Six months post-baseline, dogs were reidentified and underwent repeat faecal sampling and flea and tick counting.

4.3.3 Coproscopic and molecular methods

One gram of faeces was subjected to a quantitative faecal float using a centrifugal faecal flotation (CFF) method with saturated sodium chloride and sucrose (specific gravity 1.27). Parasite eggs were manually counted and converted to eggs per gram (EPG) by multiplying counts by the inverse of the faecal sediment measured in the centrifuge tube to allow sample comparison.

DNA was extracted from 200mg of faeces of each sample using the Maxwell® RSC PureFood GMO and Authentication Kit (Catalog no. AS1600, Promega Corporation, Madison, USA) with the Maxwell® RSC 48 Instrument (Catalog no. AS8500, Promega Corporation, Madison, USA) using a modified method as described by Massetti *et al.* (2022).

Extracted DNA was subjected to multiplex qPCR assays for the detection of four species of canine hookworm including *Ancylostoma caninum*, *Ancylostoma ceylanicum*, *Uncinaria stenocephala*, and *Ancylostoma braziliense* as well as *Strongyloides* spp. according to published protocols (Massetti et al. 2020; Massetti et al. 2022). Internal amplification controls were performed using equine herpes virus (EHV4) primers (EHV-F, EHV-R), probe (EHV probe) and EHV4 synthetic DNA fragments containing the target sequence (gBlock® Gene Fragments, IDT® Technologies, Skokie, USA). DNA extraction controls were performed with mammalian primers (MAM-F, MAM-R) and probe (MAM probe) (Bialasiewicz et al. 2009; Hii et al. 2018; Massetti et al. 2020). Synthetic DNA fragments containing the target sequence of each parasite species (gBlock® Gene

Fragments, IDT® Technologies, Skokie, USA) were used as positive controls and no-template negative controls were included in all runs. A five channel AriaMx Real-time PCR System (Agilent, Santa Clara, USA) was used for the amplification, detection, and data analysis of all samples (Agilent Aria software).

4.3.4 Statistical analysis

Demographic and physical examination and laboratory data were recorded on paper then transferred, cleaned, and validated in an electronic spreadsheet (Microsoft Excel v. 1908, Microsoft Corporation, Redlands, USA). Recoding of variables was conducted where necessary and data was analysed and plotted in R (v. 4.2.2, R Core Team, 2022) using RStudio and contributed packages lme4 (v. 1.1-34, Bates *et al.*, 2023), emmeans (v. 1.8.7, Lenth *et al.*, 2023), ggplot2 (v. 3.4.2, Wickham *et al.*, 2023), epiR (v. 2.0.60, Stevenson *et al.*, 2023) and terra (v.1.7-55, Hijmans *et al.*, 2023). Flea and tick scores were combined to an ectoparasite score and subsequently used as a binary variable (present/absent) to account for low frequencies. Similarly, age group categories were collapsed to dogs under one year of age and dogs over one year of age to account for low frequencies.

Dog demographic data including age group, sex and desexed status as well as qPCR-based endoparasite prevalence, hookworm EPG distributions and ectoparasite prevalence were described for each treatment arm. Short-term data between baseline and day 7-11 post-treatment permitted the calculation of efficacy measures for each treatment; those being the performance of each treatment under close to ideal conditions which do not include new re-infections. Cure rates (CR) were calculated as a percentage in which the number of dogs qPCR-positive for a parasite species pre-treatment and negative 7-11 days post-treatment was divided by the total number of dogs positive for the parasite species pre-treatment. 95% confidence intervals were calculated for prevalence and CR estimates using the epi.conf function in the epiR package. Cure rates for ectoparasites were also conducted in the same manner for each treatment arm and demographic group.

For dogs testing positive for hookworm eggs at baseline, egg reduction rates (ERR) were calculated as a percentage, where the 7-11 days post-treatment count was subtracted from the baseline count and divided by the baseline count. Per World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines and International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) guidelines as adopted by the Australian Pesticides and Veterinary Medicines Authority (APVMA), a 90% ERR threshold was used to indicate an anthelmintic as efficacious for label claim requirements (Vercruyse et al. 2002; Geurden et al. 2022; Beugnet et al. 2022). In the present study, the proportion of dogs achieving a 90% or greater ERR in each treatment and demographic group was calculated along with 95% confidence intervals.

Considering all timepoint data up to six months permits the calculation of effectiveness measures which, in contrast to efficacy measures, are inclusive of real-world influences such as re-infection. Generalised linear mixed models were used to assess associations between treatment group and EPG and treatment group and ectoparasite infestation based on Poisson and binomial family models, respectively. Individual dog and island were included as random effects and age group, sex and desexed status were included as fixed effects. Backward stepwise variable selection was used to arrive at the final model considering a p-value of <0.05 significant. A data dispersion ratio was calculated from the sum of residual squares divided by the number of observations. R code for this analysis is included in Appendix 3.

4.4 Results

Treatment arms consisted of 80 dogs in Group A, 51 in Group B and 44 in Group C at baseline. Populations varied in each treatment group with respect to the representation of age group, sex and desexed status. Demographic data for dogs in each treatment group at baseline are shown in Table 4.1. Adult dogs were the largest age group in each treatment group followed by young dogs. No puppies were included in Group C. Proportions of male dogs were higher in Groups A and C, while more females were found

in Group B. More desexed dogs were present in Group C while more entire dogs were present in Groups A and B. One dog in Group B was not present for resampling at post-treatment follow up and was therefore excluded from efficacy analysis. 18 dogs from Group A, 15 dogs from Group B and 20 dogs from Group C had either died or were not present for sampling at the six-month timepoint and were therefore excluded from medium-term effectiveness analysis. All other dogs were present for sampling at all time points.

Table 4.1 Dog demographic data from each treatment group

Variable and category	Total n (%)	Group A n (%)	Group B n (%)	Group C n (%)
Age group				
Puppy	11 (6.3)	4 (5)	7 (13.7)	0 (0)
Young	45 (25.7)	18 (22.5)	10 (19.6)	17 (38.6)
Adult	103 (58.9)	53 (66.2)	32 (62.7)	18 (40.9)
Old	16 (9.1)	5 (6.2)	2 (3.9)	9 (20.5)
Sex				
Female	74 (42.3)	33 (41.2)	26 (51)	15 (34.1)
Male	101 (57.7)	47 (58.8)	25 (49)	29 (65.9)
Desexed				
Yes	69 (39.4)	27 (33.8)	19 (37.3)	23 (52.3)
No	106 (60.6)	53 (66.2)	32 (62.7)	21 (47.7)

Mammalian DNA extraction controls were positive for all samples. Only *A. caninum* and *Strongyloides* spp. were detected by the multiplex qPCR and only *A. caninum* was detected at levels allowing for before-and-after comparison in individual dogs. Overall baseline qPCR-based prevalence of *A. caninum* was 83.9% (95% CI 77.7-88.6) with 97.5% (95% CI 91.3-99.3) in Group A, 78.4% (95% CI 65.4-87.5) in Group B and 65.9% (95% CI 51.1-78.1) in Group C. Baseline microscopy-based EPG varied widely, with a geometric mean of 219 (range 0-14,430) and high degrees of skewness (4.85) and kurtosis (26.04). Baseline EPG was highest in puppies, with three puppies (and a single adult) shedding more than 10,000 EPG. Individual dog *A. caninum* EPG counts, flea score and tick score at each time point are presented in Figure 4.2.

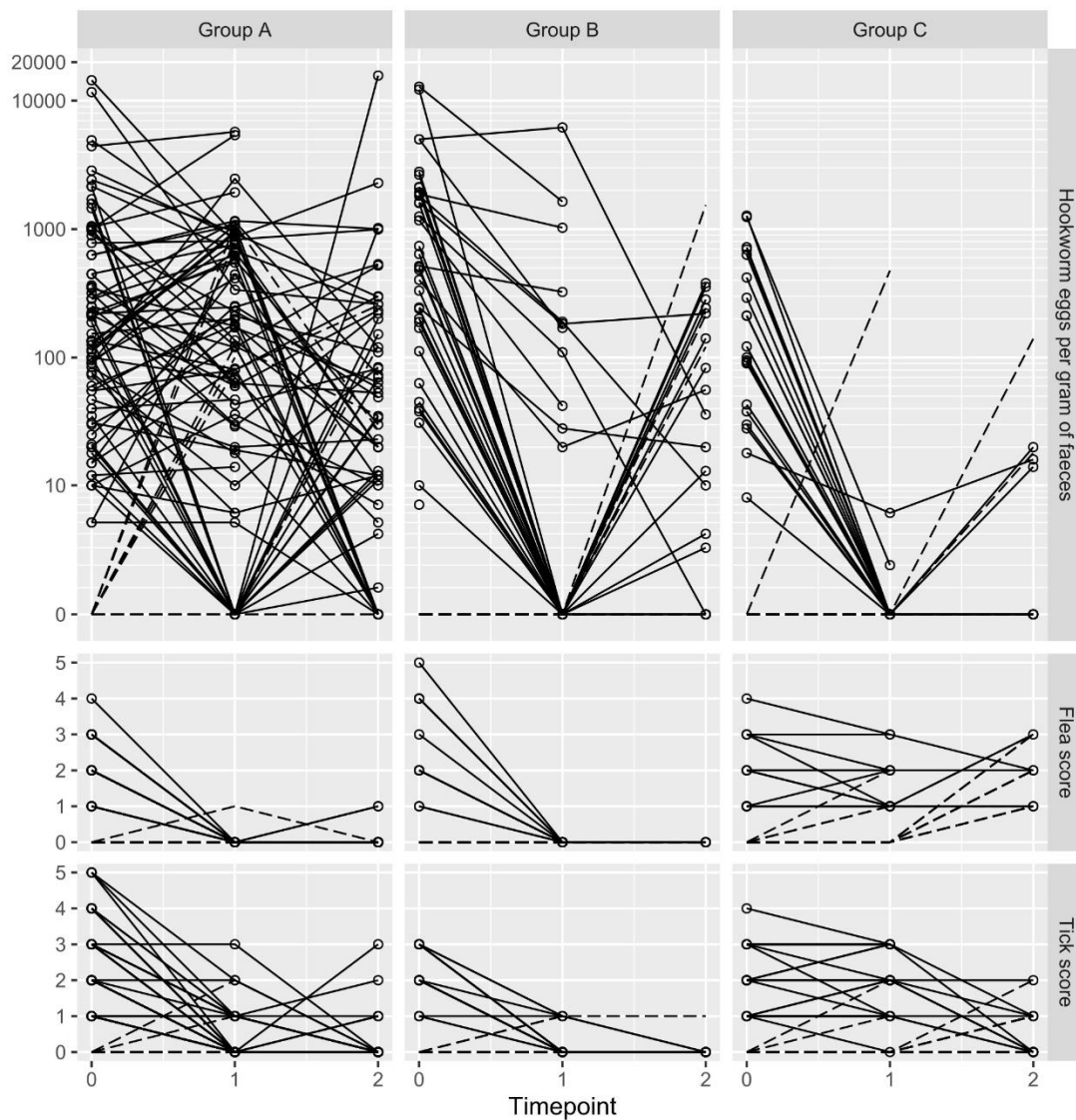


Figure 4.2 Trellis plot of hookworm eggs per gram, flea score and tick score at baseline (timepoint 0), 7-11 days following treatment (timepoint 1) and 6 months later (timepoint 2). Each line shows results of an individual dog. Dotted lines represent dogs with a baseline count or score of zero.

Cure rates and ERR results for dogs which tested positive to *A. caninum* via qPCR CFF and positive for ectoparasites via patch examination are shown in Table 4.2. Baseline prevalence for fleas was 37.5% (95% CI 27.7-48.5) for dogs in Group A, 23.5% (95% CI 14-36.8) for Group B and 36.4% (95% CI 23.8-51.1) for Group C. Baseline prevalence for ticks was 65% (95% CI 54.1-74.5) for dogs in Group A, 39.2% (95% CI 27-52.9) for Group B and 52.3% (95% CI 37.9-66.2) for Group C. Positive or negative ectoparasite status derived from this led to the calculation of cure rates presented in Table 4.2.

Table 4.2 Endoparasite and ectoparasite outcome measures at 7-11 days post-treatment by treatment and demographic group for dogs which were positive at baseline

Variable and category	<i>A. caninum</i> qPCR cure rate		Dogs achieving <i>A. caninum</i> 90% egg reduction rate		Ectoparasite cure rate	
	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
Total	146	37.7 (30.2-45.8)	126	53.2 (44.5-61.7)	110	53.6 (44.4-62.7)
Treatment						
Group A	78	9 (4.4-17.4)	72	29.2 (19.9-40.5)	56	69.6 (56.7-80.1)
Group B	39	56.4 (41-70.7)	34	79.4 (63.2-89.7)	25	80 (60.9-91.1)
Group C	29	89.7 (73.6-96.4)	20	95 (76.4-99.1)	29	0 (0-11.7)
Age group						
Puppy	9	22.2 (6.3-54.7)	9	55.6 (26.7-81.1)	8	75 (40.9-92.9)
Young	38	47.4 (32.5-62.7)	32	46.9 (30.9-63.6)	27	40.7 (24.5-59.3)
Adult	89	32.6 (23.7-42.9)	78	55.1 (44.1-65.7)	64	60.9 (48.7-71.9)
Old	10	60 (31.3-83.2)	7	57.1 (25-84.2)	11	27.3 (9.7-56.6)
Sex						
Female	63	39.7 (28.5-52)	54	53.7 (40.6-66.3)	48	56.2 (42.3-69.3)
Male	83	36.1 (26.6-46.9)	72	52.8 (41.4-63.9)	62	51.6 (39.4-63.6)
Desexed						
Yes	54	44.4 (32-57.6)	44	48.8 (38.3-59.4)	38	44.7 (30.1-60.3)
No	92	33.7 (24.9-43.8)	82	61.4 (46.6-74.3)	72	58.3 (46.8-69)

Coefficient estimates for the final EPG Poisson and ectoparasite infestation binomial models are presented in Table 4.3. Neither age group, sex nor desexed status were significantly associated with EPG or ectoparasite infestation between baseline and day 7-11 or between this time point and 6-months and were thus removed from the final model. Intraclass correlations were calculated, with greater than 99.9% of the variation in EPG and ectoparasite infestation attributable to differences between dogs, rather than island clusters. Overdispersion was present in the EPG model with a data dispersion ratio of 406.

Table 4.3 Mixed effects model outputs for associations with changes in eggs per gram of faeces and ectoparasite infestation

Variable	Category	Association with <i>A. caninum</i> EPG of faeces			Association with ectoparasite infestation		
		Coefficient estimate	Standard error	<i>p</i> -value	Coefficient estimate	Standard error	<i>p</i> -value
Fixed effects							
Treatment group				<0.001*			<0.001*
	Group A	<i>Reference</i>			<i>Reference</i>		
	Group B	-0.90	0.54	0.097	-1.62	0.71	0.023
	Group C	-3.81	0.58	<0.001	-0.15	0.73	0.84
Timepoint				<0.001*			<0.001*
	Baseline	<i>Reference</i>			<i>Reference</i>		
	Day 7-11	-0.53	0.01	<0.001	-3.62	0.70	<0.001
	6 months	-0.54	0.01	<0.001	-5.23	0.94	<0.001
Treatment x timepoint				<0.001*			0.002*
	Group A x baseline	<i>Reference</i>			<i>Reference</i>		
	Group B x Day 7-11	-1.29	0.01	<0.001	0.21	0.86	0.803
	Group C x Day 7-11	-2.05	0.05	<0.001	3.81	0.96	<0.001
	Group B x 6 months	-1.22	0.02	<0.001	-0.08	1.54	0.957
	Group C x 6 months	-2.20	0.07	<0.001	24.2	209.02	0.908
Random effects							
	Individual dog variance	8.94			5.28		
	Island cluster variance	<0.001			<0.001		

* Variable level *p*-values were calculated using the `joint_tests` function from the `emmeans` package

4.5 Discussion

This study found that the treatment administered to each animal group was the most significant factor associated with reductions in *A. caninum* egg shedding (EPG) as well as presence or absence of ectoparasite infestation. Results indicate that demographic variables of age group, sex and desexing status are not associated with anti-parasiticide efficacy and effectiveness in this setting.

For treatment of *A. caninum*, off-label ivermectin performed best in terms of both qPCR CR and 90% ERR. This supports the findings of a treatment trial by Bhanjadeo *et al.* (2022), for which ivermectin administered at 200 µg/kg body weight to twelve dogs infected with *A. caninum* with a mean EPG of 1,725 at baseline, produced a CR and EPG reduction of 100% at day 15 post-treatment. Studies have also demonstrated high efficacy of ivermectin against *A. caninum* at doses as low as 10 µg/kg (Wang *et al.* 1989; Daurio *et al.* 1993). In Australia, administration of ivermectin in dogs at doses above 6 µg/kg body weight represents off-label use. The lack of registered treatments may be partly due to the presence of the ABCB1 gene mutation often present in collie breeds and their crosses, which makes them more sensitive to toxic effects of ivermectin at doses used to target gastrointestinal helminths (Marsh and Lakritz 2023). In the author's experience, the dogs living in remote Aboriginal and Torres Strait Islander communities tend to be medium-sized crossbreeds often known as 'Australian camp dogs', and very rarely include collie dog genetics. The risk for these dogs is low and testing for gene mutations is not necessary (Beckers *et al.* 2022). Nonetheless, care must be taken in populations which may have the ABCB1 gene mutation. Off-label drugs cannot be purchased by dog owners and require veterinary oversight, which comes at greater cost either at an individual dog level or in community-level animal health programs. Off-label usage also means that there may be less standardisation in the method and dose administered compared to commercially produced animal treatments, especially oral treatments. In this study, ivermectin was soaked into bread and covered with peanut butter for palatability, which was well accepted by the dogs, though palatability is often a challenge in these settings. However, acceptance cannot always be relied upon for any oral treatment in any dog whether it be commercially available or off-label.

While a study by Hellman *et al.* (2003) of the efficacy of topical moxidectin/imidacloprid in 131 naturally hookworm infected dogs found a geometric mean ERR of 99.92% 8 to 13 days following treatment, the proportion (29.2%) of dogs achieving a 90% ERR in the present study and low CR of 56.4% did not support this treatment's efficacy to the same degree. One possible explanation for the reduced efficacy may be the inability to control for the application of the product to a dry coat and the avoidance of wetting the coat within 24 hours of application (Australian Pesticides and Veterinary Medicines Authority 2003). Since dogs could not be supervised after treatment, it is possible that the rapid skin absorption of the moxidectin component of the product may have been disrupted. Moreover, individual clearance of moxidectin from the system may vary between dogs of different body condition score owing to their differing levels of adipose tissue, though this would be more relevant to moxidectin's sustained larvicidal effect than its immediate adulticidal efficacy (Bousquet-Mélou *et al.* 2021). Furthermore, differences in the distribution of body condition score did not differ significantly between treatment groups and would not sufficiently explain any differences in observed treatment effects.

Efficacy of oxibendazole based on this study was demonstrated to be poor against *A. caninum*. While tableting of dogs is the most difficult of the three endoparasitic treatments to administer in this study and is generally prone to failure due to dogs not accepting tablets, these treatments were all administered by a trained, registered veterinarian and all treatments were confirmed to have been swallowed. Individual dog data in Figure 4.2 shows multiple cases in which dogs were not only without cure or egg reduction but appear to have increases in egg counts following treatment. Several confounding factors are known to influence successive faecal egg counts in the same individual such as time of sampling, faecal consistency, and host diet (Morgan *et al.* 2022). These effects may have been masked in the other treatment groups by treatment effects but were more evident in Group A due to a lack of efficacy.

Only a single study is known to have examined the efficacy of oxibendazole against hookworms in dogs. In this study, oral oxibendazole at a dose rate of 15mg/kg administered to naturally infected dogs found a 94.6% reduction of *A. caninum* based on the reduction in the arithmetic mean EPG from baseline to 8-10 days post-treatment

(Overgaauw and Boersema 1998). The finding of such a high arithmetic mean ERR is surprising compared to the findings of the present study which used a higher dose rate of 22.5mg/kg. The fact that only 11 dogs were initially infected with *A. caninum* in the Overgaauw and Boersema study, along with a lack of reported confidence intervals and accurate demographic data calls the validity of the presented results into question. Poor efficacy of oxibendazole, as with other benzimidazoles, may be related to its low aqueous solubility further compounded by the relatively rapid gut transit times of dogs (Sánchez et al. 2000). For that reason, efficacy of benzimidazoles is predominately time- rather than dose-dependent, with optimal efficacy typically only seen after repeated doses over three to five days (Page 2008). By contrast, in a recent study involving the development of an *in vitro* egg hatching assay to determine the ovicidal effects of anthelmintics it was revealed that oxibendazole, despite its poor adulticidal and larvicidal properties, demonstrated high potency against hookworm eggs, while eggs exposed to moxidectin or ivermectin showed relatively unchanged levels of maturation and hatching (Easland et al. 2023). This may point to a potential use for benzimidazoles in combination with an efficacious adulticidal and larvicidal treatment to immediately reduce environmental shedding of viable eggs, though further *in vivo* studies are necessary. Further studies are also required to investigate the potential for resistance to benzimidazole anthelmintics, and indeed all anthelmintics used for mass drug administration to treat *A. caninum*, especially with mounting evidence of β -tubulin gene fenbendazole resistance in this species (Marsh and Lakritz 2023).

Analysis of ectoparasite cure rates found oral afoxolaner given to Group A and imidacloprid/flumethrin collars given to Group B to be highly efficacious, which supports the findings of previous studies by Brianti *et al.* (2013) and Fankhauser *et al.* (2016). While the product label of Advocate® and Seresto® state that the products are still efficacious against ectoparasites after swimming, free-roaming dogs in these island settings frequently swim in salt water. Nevertheless, regular wetting of the coat did not appear to reduce efficacy of the imidacloprid and flumethrin concentrations within the coat in the hours to days after application in this study. Group C demonstrated very poor ectoparasitic efficacy, and although macrocyclic lactones are known to have lethal

paralytic effects on arthropods at the time of exposure, this could not be observed at the time of follow-up and either the same or new flea and tick burdens were observed (Page 2008).

Random effects variance for island clusters in mixed effects modelling in this study was very low. While a lack of treatment randomisation would ordinarily be a limitation in many treatment trials, here it was a necessary study design feature. The impact of mass treatments was being assessed on a community, rather than individual animal level, including the ability of mass treatment to reduce environmental shedding and in turn re-infection rates. Had dogs been randomly allocated on each island, treatments with poor efficacy could have led to greater environmental contamination with parasites and greater chances of reinfection for all dogs over time, which may have reduced apparent medium-term effectiveness for what were otherwise more effective treatments. Realistically, differences in location in terms of veterinary and owner care would have been negligible and given that the time between pre-treatment and post-treatment sampling was insufficient to allow new patent reinfections, any differences based on location would have been minimal.

Random effects variance for individual dogs was, in comparison to island clusters, much higher. To allow maximal inclusion of dogs from areas with limited populations for the sake of statistical power, all dogs from all demographics were enrolled. Ideally at least eighty dogs would have been included in each treatment arm with a more equal distribution of age groups. While attempts were made to choose islands with the largest dog populations, a wave of parvovirus in the study islands leading to the deaths of several dogs immediately prior to initial sampling precluded reaching the planned sample size. Low numbers of dogs in the puppy and old age categories meant that collapsing these categories was necessary and that more detailed examination of age group associations with changes in outcome were not possible in mixed effects modelling. While puppies had the highest baseline EPG, it is biologically doubtful that age group alone would affect the clearance of infection holding all other variables constant. Other factors and comorbidities affecting young or old animals may affect their susceptibility to infection, however.

The Poisson model showed a high degree of overdispersion, which may be expected from field based faecal egg count data in which a large proportion of counts were zero along with some counts above 14,000 EPG. This overdispersion made for challenging model selection and meant that model fit parameters remained imperfect, even when other distributional assumptions were used. The presented final model selection and structure, however, is sufficient to demonstrate that associations between treatment and EPG or ectoparasite infestation were significant and that associations with demographic factors and cluster groups were not.

Access to efficacious and effective antiparasitic treatments is important in any setting, but particularly in remote Aboriginal and Torres Strait Islander communities where access to veterinary care and animal health products can have additional barriers and where the potential risks of zoonotic disease are especially relevant. The results of this study demonstrate that single-dose oxibendazole/praziquantel (ParaGard®) has poor efficacy against the zoonotic dog hookworm *A. caninum*, while moxidectin/imidacloprid (Advocate®) and off-label ivermectin at 200 µg/kg appear efficacious. Furthermore, afoxolaner chews (NexGard®) and imidacloprid/flumethrin collars (Seresto®) are efficacious against flea and tick infestation and may aid in preventing the spread of vector-borne diseases.

With the benefit of up-to-date efficacy data relevant to remote community field sites, local organisations can make informed decisions to help develop effective One Health programs and manage the risks of parasitic disease for all human and animal community members.

Chapter 5: Cost-effectiveness of treatment for canine parasites in remote Indigenous communities

Presented as submitted for peer review to EcoHealth:

Raw C, Traub RJ, Wiethoelter A, Wiseman V, Watts C. Cost-effectiveness of treatment
for canine parasites in remote Indigenous communities

5.1 Abstract

Zoonotic canine parasites and the vector-borne diseases they may carry can cause high morbidity and mortality in dogs and people. Many remote Indigenous communities in the north of Australia have numerous free-roaming dogs, tropical climates favouring parasite development, and limited access to veterinary care, which can promote high prevalence of zoonotic parasites. To successfully combat parasites, effective, cost-effective, and feasible treatment programs are needed.

We compared canine parasite treatments in a Torres Strait Islander community setting, including oxibendazole/praziquantel tablets (OXI), moxidectin/imidacloprid spot-on (MOX), off-label oral ivermectin (IVM), afoxolaner chews (AFO), and flumethrin/imidacloprid collars (FLU). Cost surveys estimated the total annual and per-dog cost of each program. Markov modelling determined the cost per dog free of infection for each program using a government payer perspective over six-month and four-year time horizons.

The annual cost per dog treated was \$54.53 for OXI, \$95.44 for MOX, \$22.85 for IVM, \$219.79 for AFO, and \$133.95 for FLU. IVM was less costly and more effective than other treatments against hookworm. FLU dominated in ectoparasite treatment and prevention. Sensitivity analyses supported these results.

This study contributes cost-effectiveness data to inform parasite treatment program policy with aims of significant reductions in zoonotic canine endo- and ecto-parasite and vector-borne disease prevalence, and subsequent reductions in environmental contamination with infectious parasite stages.

5.2 Introduction

The warm and humid environments of northern Australia favour the development and spread of several species of internal and external parasites that impact the health of dogs (O'Donel Alexander 1984; Gordon et al. 2017). Many of these parasites are also responsible for well-recognised zoonotic diseases transmitted to humans (Smout et al. 2017a; Raw et al. 2022). Dogs are commonly free-roaming in remote Aboriginal and Torres Strait Islander communities in Australia and can live near humans or as fringe-dwelling wild dogs (Constable et al. 2010; Ma et al. 2020a; Bennett and Archer-Lean 2023). Their mobility, proximity to and interaction with other animal species including humans provides ample opportunity for disease exchange either directly or indirectly through contact with, or ingestion of contaminated soil, food, water or via bites from arthropod vectors such as ticks and fleas (Smout et al. 2017a).

Important examples of canine zoonoses in the Australian tropics are soil-transmitted helminths (STH) including hookworms (*Ancylostoma* spp.) and threadworms (*Strongyloides* spp.) (Bradbury and Traub 2016; Traub et al. 2021; Raw et al. 2022). While ectoparasites such as fleas, ticks and mites are not known to complete their lifecycles on humans, even transient infestations caused by sharing bedding with an infested dog can lead to transmission of vector-borne diseases such as bartonellosis, flea-borne spotted fever or symptoms of hypersensitivity in the form of pruritis (Barrs et al. 2010; Teoh et al. 2016; Teoh et al. 2018). This pruritis, while seemingly innocuous, can be a precursor to secondary chronic skin disease, rheumatic fever or rheumatic heart disease (O'Donel Alexander 1984; Elliot et al. 2006).

Recent research indicates that known zoonotic STHs such as *Ancylostoma caninum* and *Strongyloides* spp. are endemic in dogs in remote communities, with high levels of flea and tick infestations also detected (Raw et al. 2022; Raw et al. 2024). Due to barriers of remoteness and limited funding, veterinary care may be sporadic, infrequent or non-existent in these communities, and the potential for continued parasite endemicity and zoonotic risk to humans is high (Bennett and Archer-Lean 2023). As such, cost-effective

approaches towards the control and elimination of these parasites in dogs is critical as part of broader, culturally responsive animal health programs.

The control of parasites in remote Indigenous communities relies upon efficacious, effective and feasible treatments. The Australian Pesticides and Veterinary Medicines Authority (APVMA) registers antiparasitic treatments following evidence that the product meets safety, efficacy, trade and labelling criteria (Department of Agriculture Forestry and Fisheries 2014). Labelling criteria of registered treatments designate a specific use such as treating hookworm in dogs. Many antiparasitic treatments can be administered by owners provided that the labelled use is adhered to. ParaGard® (Boehringer Ingelheim), Advocate® (Elanco), NexGard® (Boehringer Ingelheim) and Seresto® (Elanco) are labelled for the control and treatment of parasites in dogs and can be purchased and administered by owners. Products such as these may be more recently developed and covered by patents, increasing unit costs (Christie et al. 2013). Other treatments may be used in an off-label manner, though these decisions must be made and administered or overseen by a veterinarian, thereby incurring additional costs. Ivermectin is an off-label treatment in dogs and has been the mainstay of many veterinary or NGO parasite treatment programs in remote Aboriginal and Torres Strait Islander communities for decades (Wilks and Williamson 1998; Bradbury and Corlette 2006; Burleigh et al. 2015). Evidence of ivermectin's efficacy in this setting has recently been established (Raw et al. 2024) and as it is no longer covered by a patent it is inexpensive to procure.

Failure to control parasite species may have drastically different consequences with varying zoonotic potential, life cycles and pathogenic effects. For instance, high prevalence of *A. caninum* in dogs may lead to greater environmental contamination with hookworm eggs and infective larvae and a higher risk of zoonotic infection. A realistic and worthwhile goal is therefore to reduce environmental contamination and animal burdens, thereby minimising zoonotic transmission as well as animal morbidity. While goals of reducing environmental and animal burden are similar for external parasites such as ticks and fleas, they have vastly different life cycles, modes of infestation and pathogenic effects (O'Donell Alexander 1984; Dantas-Torres 2010). Treatments may

instead be targeted at killing immediately following bites or ideally repelling before bites occur to avoid vector-borne disease transmission. While complete community elimination of fleas, ticks or hookworms would be desirable, it is unlikely due to wild host species such as wild dogs, dingoes or feral cats harbouring these parasites and contributing to environmental burdens (Dantas-Torres 2010; Smout et al. 2017b; Clark et al. 2018).

Cost-effectiveness studies of human antiparasitic treatments have been performed in many countries throughout the world (Turner et al. 2015), however cost-effective parasite control strategies in dogs are scarce. A scoping review on the economic evaluation of control strategies aimed at reducing cystic echinococcosis in humans highlighted the incompleteness of cost data and lack of detailed analysis or sensitivity analysis to inform this topic (Widdicombe et al. 2022). Cost-effectiveness measures of animal health programs can guide policy and funding decisions by local government or non-governmental organisations (NGOs) with potential enhancements in cost-effectiveness when considered within a One Health framework acknowledging the interconnectedness of human, animal and environmental health. One Health policy also needs to be culturally relevant to be most effective, recognising how culture informs interactions between people, animals, and the environment. In remote Aboriginal and Torres Strait Islander communities, local culture may be vastly different from other countries or other regions in Australia. An example of this is the spiritual and cultural importance of dogs to many Aboriginal communities for whom dogs are part of their Dreaming (Smith and Litchfield 2009; Constable et al. 2010). As such, cultural interactions with and management of dogs needs to be considered in analyses and resulting policy decisions.

Current practice varies between communities and may involve labelled or off-label treatments, with choices based on provider access to treatments, veterinarian preferences or cost. Despite high prevalence of parasites, many remote communities do not have any current dog parasite treatment programs in place. Thus, this study aims to inform future treatment programs by determining the cost-effectiveness of three

treatments for the zoonotic canine hookworm *A. caninum* and three ectoparasite treatments for fleas and ticks under field conditions in the Torres Strait Islands.

5.3 Methods

5.3.1 Overview

In this study, we compared the cost-effectiveness of three endoparasite treatments and three ectoparasite treatments administered according to labelled instructions or in an off-label manner. The perspective for this cost-effectiveness analysis was the local government payer, as animal health programs are funded by the local council in these Torres Strait Islander communities and are funded by government bodies in some other Aboriginal communities (Ma et al. 2020b). Treatments were to be delivered by council staff to ensure appropriate storage and administration of treatments. Two separate time horizons were examined: the first at six months to reflect the study period; and a longer time horizon of four years to examine the effect of real-world application of treatments on community parasite prevalence.

5.3.2 Study setting and population

The location and study population has been described in detail elsewhere (Raw et al. 2024). Briefly, dogs on three remote tropical islands in the Torres Strait in Queensland, Australia were included in this study. The Torres Strait spans an area of over 48,000 km² with Papua New Guinea and the northernmost extent of Queensland as its northern and southern borders, respectively. Islands were selected based on recommendations from the Torres Strait Islands Regional Council regarding dog numbers. Community leadership and consultation were crucial to the design and completion of this research. Consultations were conducted with local Environmental Health Worker staff, followed by consultation with elders and elected council representatives of all island groups and approval of a formal research proposal. Environmental Health Workers were involved in

all stages of field work, communicating with community members and providing treatments.

As many dogs as possible were enrolled on each island with owner consent. Dog populations varied on each island in the region regarding numbers, the balance of sex, age group and whether dogs were desexed or entire, though these factors were not found to be significant regarding treatment efficacy (Raw et al. 2024).

5.3.3 Interventions

All enrolled dogs on each island were assigned to the same treatment arm and given treatments for both internal and external parasites as described below. Treatments were selected to allow comparison of registered treatments administered according to labelled instructions versus off-label use. Registered treatments were selected based on availability of sufficient quantities, and off-label use of ivermectin was selected based on its current widespread use in remote community animal health programs (Wilks and Williamson 1998; Burleigh et al. 2015). All treatments, both labelled and off-label, were dose-banded, meaning that the following minimum target doses were administered. For the treatment of *A. caninum*, dogs received three-monthly treatments of either: i) oral tablets administered at 22.5 mg oxibendazole/5 mg praziquantel per kilogram bodyweight (ParaGard[®], Boehringer Ingelheim) given according to labelled instructions (treatment group OXI); ii) topical combination 1% moxidectin/10% imidacloprid (Advocate[®], Elanco) applied according to labelled instructions at 0.1ml per kilogram bodyweight (treatment group MOX); or iii) off-label oral ivermectin (Bomectin[®], Elanco) administered at 200 µg/kg in bread with flavoured paste (treatment group IVM). For the treatment of fleas and ticks, dogs received either i) oral chews administered at 2.5 mg afoxolaner per kilogram bodyweight (NexGard[®], Boehringer Ingelheim) administered monthly according to labelled instructions (treatment group AFO); ii) a 10% imidacloprid/4.5% flumethrin polymer matrix collar (Seresto[®], Elanco) applied according to labelled instructions (treatment group FLU); or iii) off-label oral ivermectin treatment administered at 200 µg/kg (IVM). Treatment coverage was 95% based on efficacy study

field data (Raw et al. 2024). This study was approved by the University of Melbourne Animal Ethics Committee (ID:10298).

5.3.4 Cost data collection

Costs were estimated to conduct an ongoing treatment program assuming no associated research activities. Costs included capital costs of equipment for preparation of treatments and operational costs such as vehicle rental and fuel usage, purchase of parasite treatment, consumable transport and personnel costs including council and veterinary staff. Based on field data, approximately 14% of dogs in the FLU treatment group lost their collars each six-month period, and a replacement cost was included to account for additional collars used. All other treatments were administered topically or orally based on the weight of the dog and did not require replacement. Council environmental health worker staff costs were derived from publicly available council award rates and calculated based on an hourly rate inclusive of council administration costs (McLennan 2021).

The time taken to administer treatments and the proportion of dogs in each weight group were based on observed dogs and treatment timing in this field trial. Vehicle usage costs per hour were derived from council fleet information inclusive of administration costs and were based on the time in use for the program.

The costs of transporting parasite treatments from the supplier to the study islands were obtained from supplier shipping quotes and communication with council staff (E Gunn, personal communication, May 5, 2021). Parasite treatment costs were based on wholesale veterinary supplier prices (Provet 2021).

Approximate tender costs for private veterinary clinics to provide services to the council region were obtained from council staff (E Gunn, personal communication, August 7, 2023). The overall tender cost of \$135,000 is inclusive of veterinary staff wages, travel, and accommodation for veterinary staff to visit all communities across the Torres Strait Islands Regional Council area. As these visits are also covering desexing and general

animal health purposes, only 15 minutes per 100-dog cohort per cycle was included in this cost-effectiveness analysis, which accounts for the estimated veterinary time required to provide advice and oversight for ivermectin treatment. Total programs costs per island were divided by the number of dogs treated to calculate a cost per dog treated. Prices are in June 2023 Australian dollars adjusted from 2021 figures from the time of the field trial using the Reserve Bank of Australia’s Inflation Calculator (Reserve Bank of Australia 2023). Cost inputs are presented in Table 5.1.

Table 5.1 Base case costs for antiparasitic treatment interventions in dogs

	ParaGard® tablets (OXI) treating worms	Advocate® spot-on (MOX) treating worms	Off-label ivermectin (IVM) treating worms, fleas, ticks	NexGard® chews (AFO) treating fleas, ticks	Seresto® collars (FLU) ^a treating fleas, ticks
5kg dog treatment cost	\$2.58	\$17.92	\$0.08	\$12.83	\$54.23
10kg dog treatment cost	\$4.37	\$19.71	\$0.16	\$13.53	\$54.23
20kg dog treatment cost	\$6.54	\$19.71	\$0.32	\$13.53	\$54.23
40kg dog treatment cost	\$13.08	\$21.75	\$0.63	\$14.38	\$54.23
Average 27.5kg dog treatment cost	\$9.20	\$20.55	\$0.43	\$13.88	\$54.23
Time to give treatment to each dog (hours)	0.05	0.033	0.017	0.05	0.055
Staff cost per dog treated^b	\$2.22	\$1.46	\$0.75	\$2.22	\$2.44
Time to prepare each treatment (hours)	-	-	0.004	-	-
Staff cost per dog treatment prepared^b	-	-	\$0.18	-	-
Cost of equipment to prepare treatment^c	-	-	\$0.49	-	-
Veterinary oversight time per dog treated (hours)	-	-	0.0025	-	-
Veterinary oversight cost^d	-	-	\$3.18	-	-
Vehicle hire cost per dog treated^e	\$1.09	\$0.72	\$0.37	\$1.09	\$1.20
Treatment transport per dog treated^f	\$1.13	\$1.13	\$1.13	\$1.13	\$1.13
Average cost per dog treated each treatment cycle	\$13.63	\$23.86	\$5.71	\$18.32	\$66.98
Treatment cycle length (months)	3	3	3	1	6
Annual cost per dog	\$54.53	\$95.44	\$22.85	\$219.79	\$133.95
Annual cost per 100 dog cohort	\$5,453.03	\$9,544.49	\$2,285.86	\$21,979.20	\$13,395.76

a Same cost for collars for all sizes of dog

b Staff salary cost based on hourly rate of \$44.37

c Equipment cost to prepare IVM treatments (needle, syringe, knife, cutting board) divided by 100 dog cohort

d Cost to provide veterinary advice and oversight for administration of off-label treatments including travel, accommodation and support staff based on an hourly rate of \$1271.20 and divided by the 100 dog cohort

e Vehicle hire cost from fleet department based on hourly rate of \$21.75 multiplied by the time to treat each dog

f Transport for treatment for 100 dogs from warehouse to islands via local government office

5.3.5 Intervention outcomes

Medium- to long-term effectiveness was modelled based on study results from a published field efficacy trial conducted in 2021, from which the following efficacy and prevalence data were drawn (Raw et al. 2024). Dogs were considered cured of hookworm infection if they were tested positive via quantitative PCR at baseline and were negative 7-11 days post treatment. For ectoparasites, if dogs had fleas or ticks at baseline and were free of any observable fleas or ticks 7-11 days post-treatment, they were deemed cured. Efficacy against canine hookworms was 9% (95% CI 4.4 to 17.4) for OXI, 56.4% (95% CI 41 to 70.7) for MOX, and 89.7% (95% CI 73.6 to 96.4) for IVM. Efficacy against fleas was 100% (95% CI 88.6 to 100) for AFO, 100% (95% CI 75.8 to 100) for FLU and 0% (95% CI 0 to 19.4) for IVM. Efficacy against ticks was 67.3% (95% CI 53.8 to 78.5) for AFO, 80% (95% CI 58.4 to 91.9) for FLU and 4.3% (95% CI 0.8 to 21) for IVM.

Baseline prevalence for modelling effectiveness was the average of the baseline prevalence across all trial locations, which was 72.4% (95% CI 65.1 to 78.9) for *A. caninum*, 33% (95% CI 26.1 to 40.4) for fleas and 54.3% (95% CI 46.6 to 61.8) for ticks. To establish a dynamic model, a hookworm reinfection constant was calculated based on field trial data to account for the proportion of dogs reinfected during each three-month period. This constant was multiplied by the proportion of infected dogs in each community which would determine the degree of environmental contamination with infective hookworm larvae. A reinfection constant was also applied to flea and tick models.

Effectiveness was measured as the proportion of dogs free of hookworms, fleas, or ticks at each time horizon. A survival analysis for the prevalence of *A. caninum* infections in dogs for each treatment cycle was conducted with 95% confidence intervals of efficacy for each treatment.

Modelling was based on a population of 100 dogs per treatment group. Weights of dogs were estimated at the time of the field efficacy trial, of which 10% of enrolled dogs were 5kg, 10% were 10kg, 30% were 20kg and 50% were 40kg. The arithmetic mean dog weight was 27.5kg, which was used to establish treatment costs for the dog population.

5.3.6 Cost effectiveness analysis

Calculated incremental cost-effectiveness ratios were defined as the cost per dog free of infection at each time horizon for each parasite of interest. A Markov model was created with cycle lengths of three months, considering different treatment intervals for each intervention. All modelling and analyses were conducted using TreeAge Pro Healthcare, v23 (TreeAge Software, Inc, Williamstown, Massachusetts, USA), with model outputs plotted in Microsoft Excel v 1908 (Microsoft Corporation, Redlands, California, USA).

5.3.7 Sensitivity analysis

Multiple one-way deterministic sensitivity analyses were conducted based on the four-year time horizon to examine the effect of differences in treatment cost, numbers of dogs, mean weights of dogs, vehicle and staffing costs, time taken to administer or prepare treatments and treatment efficacy. Upper and lower values of treatment efficacy were based on the 95% confidence intervals of each treatment in the previously described field trial (Raw et al. 2024). Likely upper and lower values of treatment costs were derived from variation in online retailer and wholesaler estimates, excluding bulk purchase discounts for the upper value. Ranges for treatment transport were derived from the least and most expensive shipping supplier quotes. The range tested for staff costs was based on variation in council staff award rates and for vehicle costs was based on daily rate estimates in hiring costs for shortest and longest time used from council fleet services. The likely values for numbers of dogs treated was based on field experience of the numbers of dogs seen in remote communities while values for weights of dogs and the time needed to treat each dog was based on the ranges observed in this field study.

5.4 Results

The mean annual cost to treat each 100-dog cohort was \$5,453.03 for OXI, \$9,544.49 for MOX, \$2,285.86 for IVM, \$21,979.20 for AFO, and \$13,395.76 for FLU. The total costs of treatment per dog as well as treatment of the 100-dog cohort are presented in Table 5.1. The contribution of treatment costs to total costs per cycle varied between treatment groups. Treatment costs accounted for 68% of total costs per cycle for OXI, 86% for MOX, 8% for IVM, 76% for AFO and 81% for FLU. Veterinary oversight costs accounted for 56% of IVM cost per cycle.

For the treatment of hookworm, IVM dominated MOX and OXI at both the six-month and four-year time horizons in terms of cost per dog free of infection. For the treatment of fleas and ticks, while IVM had a low cost per dog free of infection due to its very low overall cost, its effectiveness was poor compared to FLU and AFO. FLU dominated AFO at each time horizon for both flea and tick treatment. Markov cohort analyses are presented in Table 5.2. This table shows the cumulative costs and cost per dog free of infection for each parasite of concern at each time horizon.

The reinfection constant for hookworm was calculated to be 0.25, while for ectoparasites it was 0.02. While rapid declines in hookworm prevalence were seen for MOX and IVM, the efficacy of OXI was insufficient to overcome reinfection rates in each treatment cycle. A survival curve of *A. caninum* prevalence in each treatment cycle is presented in Figure 5.1 and survival curves for flea and ticks are presented in Figure 5.2.

Table 5.2 Cumulative treatment costs and cost per dog free of infection at each time horizon

Parasite	Dogs free of infection at baseline (/100)	Treatment	Six month time horizon			Four year time horizon		
			Dogs free of infection (/100)	Cumulative cost	Cost per dog free of infection	Dogs free of infection (/100)	Cumulative cost	Cost per dog free of infection
Hookworm	28	ParaGard® tablets (OXI)	27	\$2,726.51	\$100.98	28	\$21,812.12	\$779.00
		Advocate® spot-on (MOX)	69	\$4,772.24	\$69.16	99	\$38,177.96	\$385.64
		Off-label ivermectin (IVM)	89	\$1,185.80	\$13.32	100	\$9,143.43	\$91.43
Fleas	67	NexGard® chews (AFO)	100	\$10,989.60	\$109.90	100	\$87,916.80	\$879.17
		Seresto® collars (FLU)	100	\$6,697.88	\$66.97	100	\$53,583.05	\$535.83
		Off-label ivermectin (IVM)	66	\$1,185.80	\$17.97	59	\$9,143.43	\$154.97
Ticks	46	NexGard® chews (AFO)	99	\$10,989.60	\$111.01	100	\$87,916.80	\$879.17
		Seresto® collars (FLU)	97	\$6,697.88	\$69.05	100	\$53,583.05	\$535.83
		Off-label ivermectin (IVM)	49	\$1,185.80	\$24.2	67	\$9,143.43	\$136.47

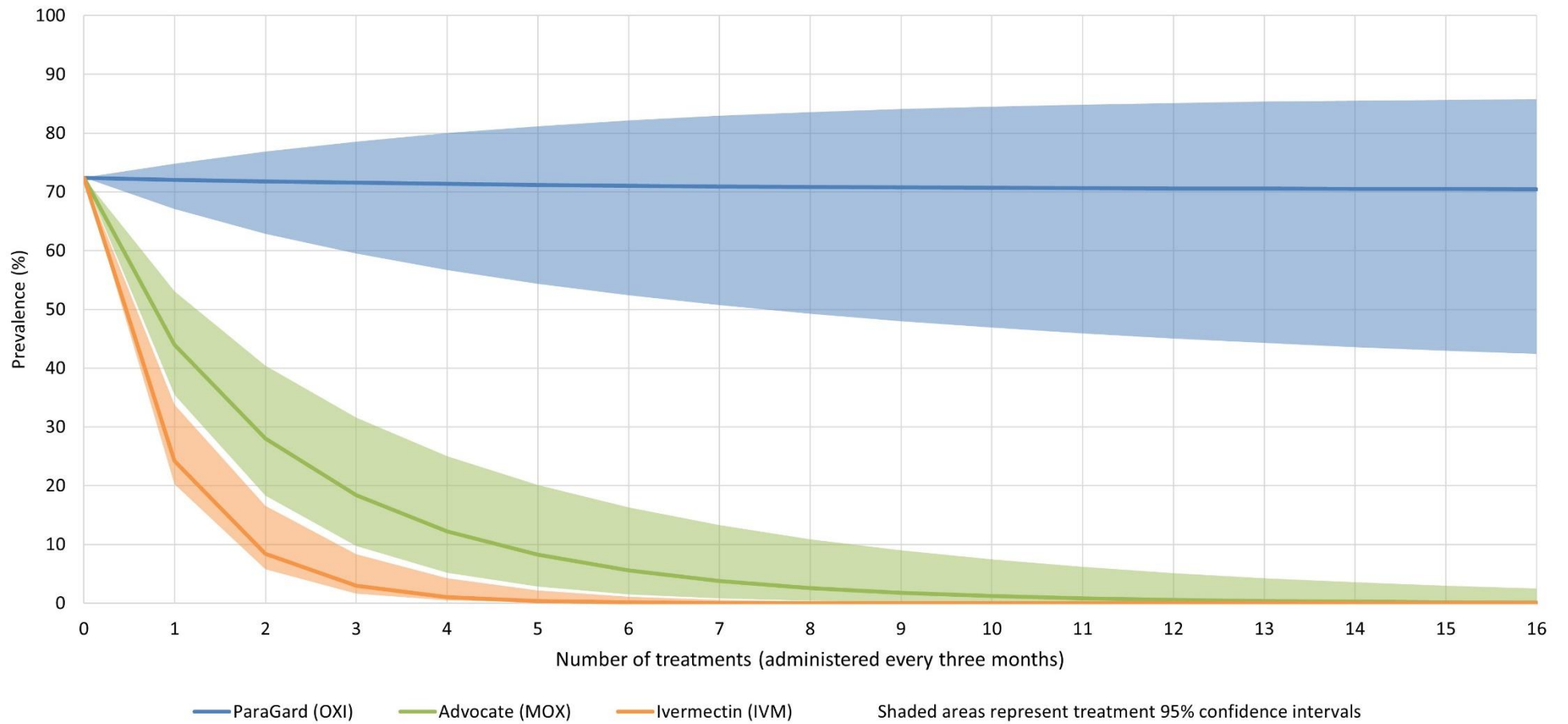


Figure 5.1 *Ancylostoma caninum* prevalence in dogs over time with treatment

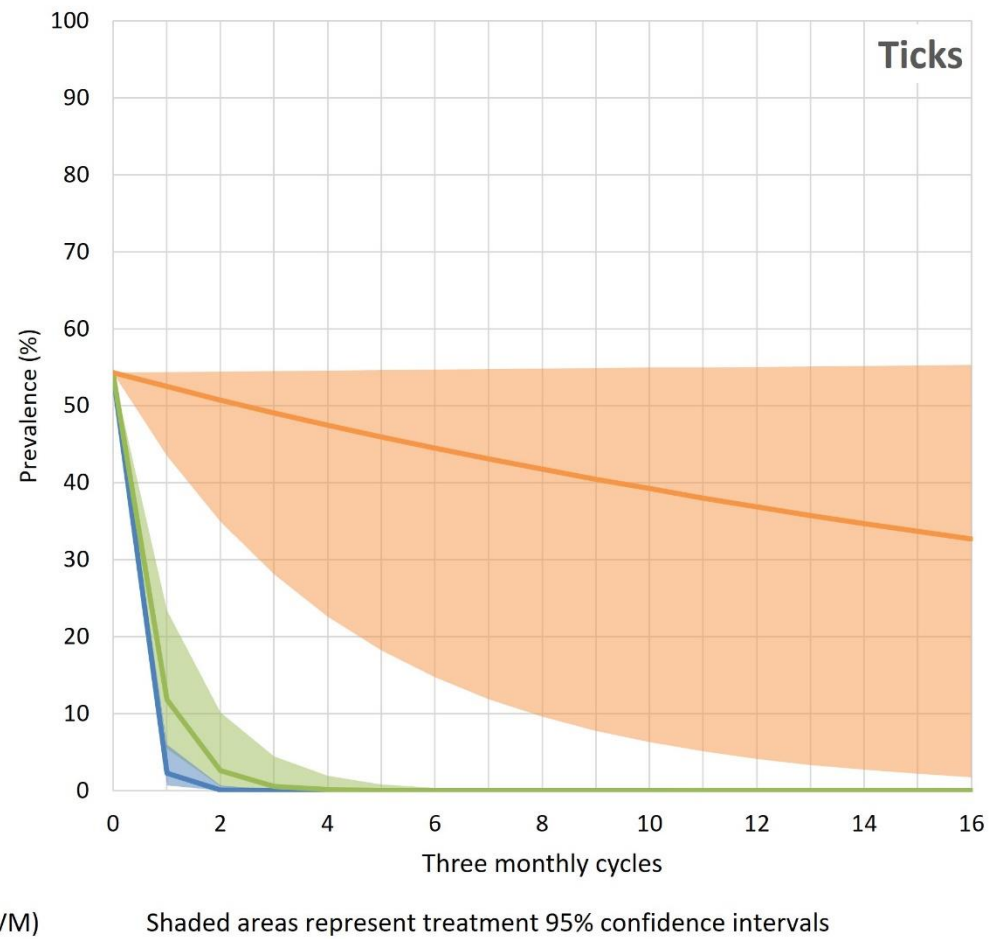
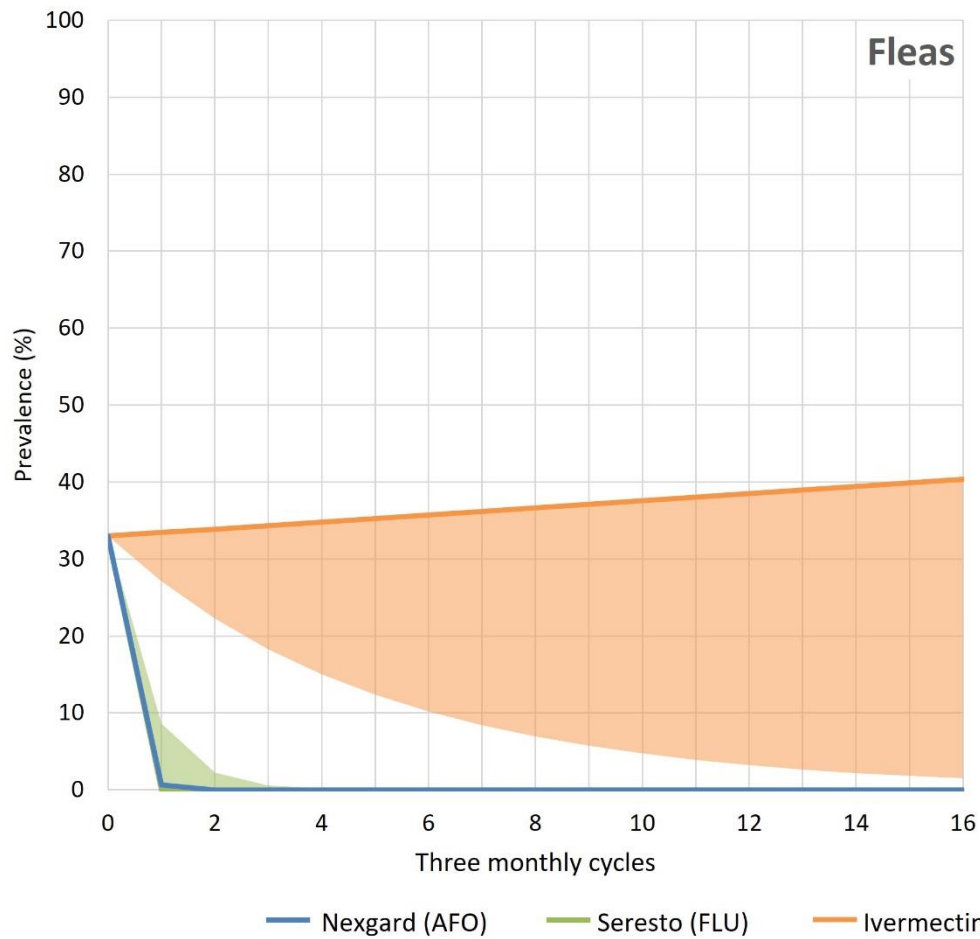


Figure 5.2 Flea and tick prevalence in dogs over time with treatment

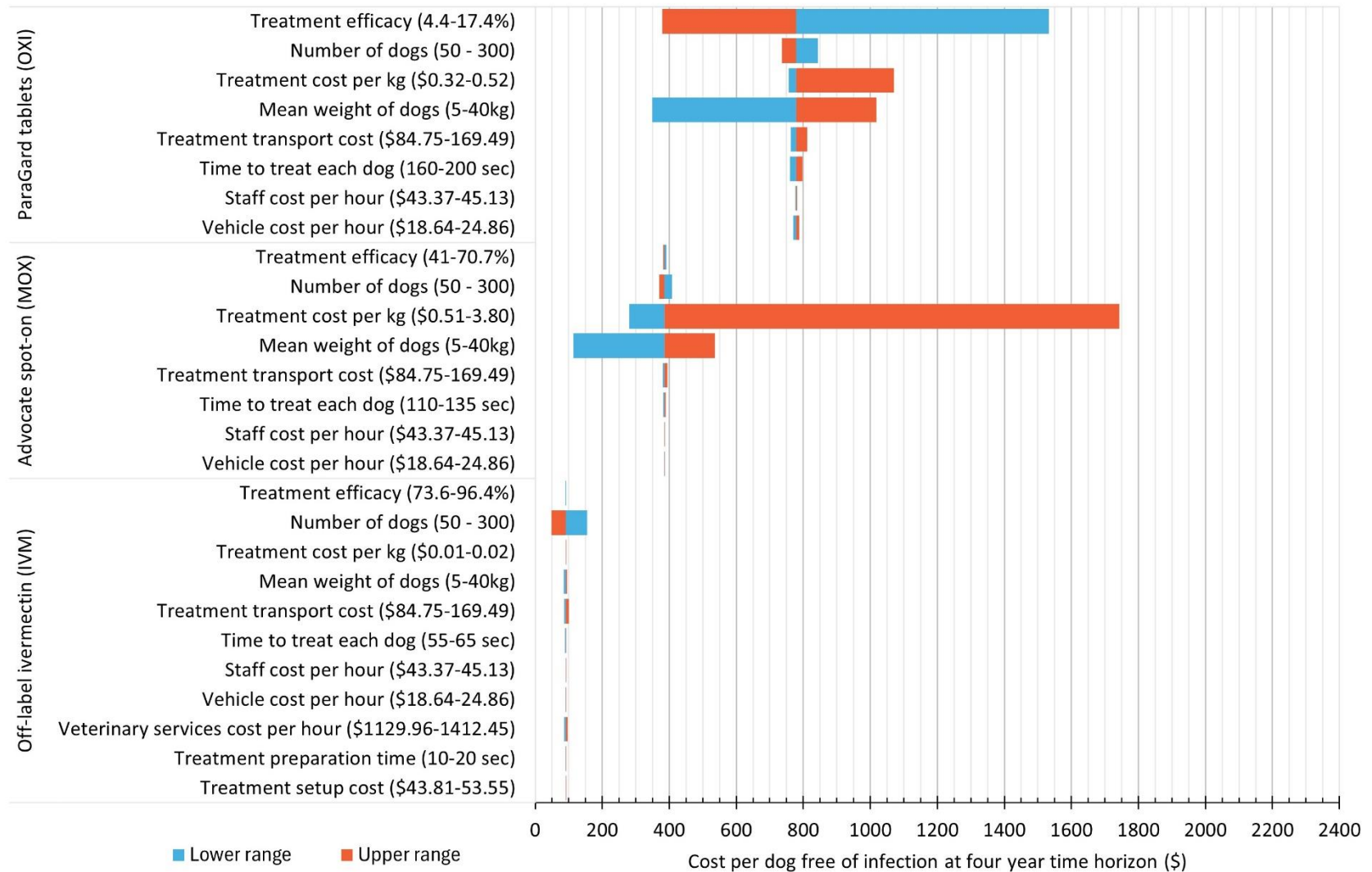


Figure 5.3 Tornado diagram of multiple one-way sensitivity analyses to examine the effect of variations in efficacy, cost, time and dog variables on the cost per dog free of *Ancylostoma caninum* infection at the four-year time horizon

5.4.1 Sensitivity analysis

Multiple one-way deterministic sensitivity analyses revealed the same treatment dominance results as presented in Table 5.2, in which IVM consistently dominated both MOX and OXI in terms of cost per dog free of hookworm infection at the four-year time horizon regardless of variations in the cost or efficacy parameters of all three treatments. Similarly, MOX had lower cost per dog free of infection for all analyses in comparison to OXI except in the case of efficacy for which OXI dominated at its upper range. Increasing the number of dogs resulted in increased efficiency as reflected by a lower cost per dog free of infection for each treatment. Sensitivity analyses are presented in a tornado diagram in Figure 5.3.

5.5 Discussion

Owing to its low cost and high effectiveness, IVM was shown to be cost-effective compared to OXI and MOX, dominating in all modelled cost-effectiveness analyses for canine hookworm including sensitivity analyses. For the four-year time horizon as shown in Table 5.2, IVM resulted in 257% and 1% more dogs being free of infection at 42% and 24% of the cost of OXI and MOX, respectively. While IVM was less costly than AFO and FLU, it was ineffective against ticks and especially fleas. AFO and FLU were both highly effective against fleas and ticks, with FLU dominating AFO in primary and sensitivity analyses. Diminishing returns of the cost per dog free of infection were seen in all treatment arms over the two time horizons due to the movement of modelled cohorts towards elimination of infection. High treatment coverage was a strength in this study, though this cannot be relied upon in all community settings. It is worth noting that MOX in this study was administered at an off-label frequency of once every three months as opposed to the labelled monthly recommendation, though this frequency is for heartworm prevention. On consultation, it was determined that monthly administration was not a feasible addition alongside the many duties of Environmental Health Worker staff. If monthly treatment was strictly adhered to, costs for MOX would triple across the modelled time horizons. Current literature for the effectiveness of moxidectin used

at longer intervals is lacking, with evidence of its use against mites, heartworm and hookworm being at either monthly or shorter intervals (Castro et al. 2019; Schraven et al. 2021).

Care should be taken in comparing the overall costs of on- and off-label treatments, as the costs of veterinary oversight for off-label treatments can be considerable. In this model 56% of the overall cost of ivermectin was due to veterinary costs. In the communities in this study, veterinary services were already being contracted for population control and general health care programs. A small amount of veterinary time was accounted for in this model to allow for the legally required veterinary oversight of off-label treatment as well as any training or discussions with animal health staff. Due to remoteness, communities often share veterinary services where a range of duties are completed including surgical or chemical neutering, parasite treatments and general health care (Wilks and Williamson 1998; Bradbury and Corlette 2006; Hiby et al. 2017). Efficiencies and economies of scale and scope may be achieved as numbers of dogs treated, numbers of communities visited, and range of veterinary activities performed increase (Dias et al. 2015). Under these arrangements it is feasible to include veterinary oversight for use of off-label treatments, but this may not be cost-effective if costs were not shared across communities due to very high travel and transport costs (Wilks 2000; Rural and Regional Affairs and Transport References Committee 2019).

Care should also be taken when considering the cost-effectiveness of treatments in isolation of other diseases. While ivermectin proved cost-effective for *A. caninum* infections, it was not cost-effective for ectoparasite infestations due to its lack of effectiveness. While ivermectin's effectiveness against other important zoonotic helminths such as *Strongyloides stercoralis* is untested in dogs in this setting, it is reported to be effective in shelter dogs and humans in remote Indigenous communities (Kearns et al. 2017; Hays et al. 2017; Paradies et al. 2019). To optimise the use of staff time in parasite treatment programs all potential internal and external parasite infections should be considered, especially those of zoonotic importance.

Treatment choice should be guided by priorities of the community and their desired outcomes. For fleas and ticks, prevention and repelling infestations is of primary importance due to the potential for vector-borne disease transmission from single bites (Otranto et al. 2008; Barrs et al. 2010; Teoh et al. 2018). For *A. caninum*, individual cure and reduction and elimination of community prevalence are meaningful aims. This is particularly relevant in a One Health framework given *A. caninum*'s zoonotic importance, the contribution to environmental contamination dependent on prevalence and host species range (Smout et al. 2017b; Raw et al. 2022). Modelling of human hookworm prevalence has revealed that driving overall prevalence below 2% via mass drug administration is likely to break transmission cycles resulting in community elimination and no further need for mass treatments (Truscott et al. 2017). Questions arise about the diagnostic sensitivity and specificity of tests used to determine when these thresholds have been reached and the cost of performing large numbers of tests required to detect infection in low prevalence settings (Lim et al. 2018). The alternative of ongoing mass treatment may involve lower costs compared to testing but carries a risk of anthelmintic resistance emergence (Bethony et al. 2006). Nonetheless, this study indicates that elimination may be feasible in this setting with the use of ivermectin, as 2% prevalence was reached after only four treatment cycles. Ongoing treatment may be administered at longer intervals of six months or potentially ceased following confirmation of elimination.

Without similar studies available on the topic of cost-effectiveness of parasite treatment programs in dogs it is challenging to draw comparisons to other settings. Most published analyses have examined cost-effectiveness of STH treatment strategies based on age group or target parasites, which has relevance in a broader One Health context but less relevance to the current study (Montresor et al. 2001; Turner et al. 2015). The results of this study are likely to be applicable to other Aboriginal and Torres Strait Islander communities in tropical and equatorial climatic zones in Australia with large populations of dogs. Such communities are present across the north of Queensland, Western Australia and the Northern Territory in which continued endemicity of *A. caninum* and *Strongyloides* spp. is evident (Raw et al. 2022). Communities with climates favourable to

STH and ectoparasite development and limited access to veterinary care or parasite treatments can also be found throughout Oceania (Bradbury and Traub 2016; Page et al. 2016; Beknazarova et al. 2016a; Traub et al. 2021). Similar regimens could also be cost-effective elsewhere for large populations of free-roaming dogs. Treatment, transport and staff costs may vary widely in non-Australian locations, and cultural and social factors determining human-animal interactions should be considered based on thorough community consultation.

The funder may also vary between locations and may include dog owners, government departments, or NGOs. While cost-effectiveness data may have meaning for the choice of parasite treatments by owners, it is the widespread, often multi-community council or NGO-operated programs with budgetary constraints that will benefit most from this information (Burleigh et al. 2015; Baker et al. 2018). Costs borne by owners may be limited to treatments, though costs borne by government may also extend to broad animal health or One Health programs. Efficiencies may be achieved even when different diseases are being targeted in different species within a One Health framework. In a study in Tanzania, an integrated health delivery platform conducted mass drug administration for STHs in humans and vaccination for rabies in dogs resulting in significant time savings and similar coverage when compared to each program delivered individually (Lankester et al. 2019). Additionally, it resulted in a 33% and 16% lower cost per deworming dose and rabies vaccination, respectively. Such research highlights an important area for further study in remote community settings, given that remoteness necessitates the efficient use of resources and time.

A limitation of this study is the uncertainty of model inputs such as the applied reinfection constant. While this was calculated from field trial data over a six-month period, it may not reflect the true rate of reinfection from environmental sources, particularly considering environmental variation between seasons. This is true for hookworms as well as fleas and ticks, which spend a greater proportion of their life cycle in the environment and may have a greater range of reservoir host species around communities, further adding to the complexity of real-world interactions (Dantas-Torres 2010). Modelling of flea and tick infection is also complicated by the mechanisms of

action of the included treatments. Flumethrin/imidacloprid collars act as repellents as well as parasiticides, whereas afoxolaner chews act on the parasite immediately after biting the host which makes measuring effectiveness challenging (Brianti et al. 2013; Shoop et al. 2014). Another limitation of this study is the lack of inclusion of dog population changes as a result of movement, births and deaths. In the study population dogs lived on remote islands and numbers have remained stable for some time, and a stable population was deemed appropriate. If this modelling was applied to less remote or less stable populations, this would need to be included in modelling. A further limitation was the lack of assessment of combinations of treatments which may reveal further economies if administered together. Further studies in remote community settings are warranted to examine other registered treatments which may be administered by owners, as well as considering combinations of treatments.

5.6 Conclusion

This study presents practical cost-effectiveness data for three endoparasite treatments and three ectoparasite treatments for dogs in remote northern Australian Aboriginal and Torres Strait Islander community settings. In these settings, reduction of environmental contamination with hookworm larvae may be possible by using ivermectin to treat infected dogs, and reduction of flea and tick bites in dogs may be feasible using combination flumethrin/imidacloprid collars. Practical and meaningful One Health outcomes are also possible in conjunction with regular anthelmintic treatments in people. Effective One Health programs involve community partnership and agency in health outcomes for animals, people, and the environment.

Chapter 6: General discussion

6.1 Thesis aims revisited

Several knowledge gaps in the available literature on zoonotic STHs and associated intervention strategies in Aboriginal and Torres Strait Islander communities were identified as the foundation of this thesis. Key among these were the lack of information on the true prevalence and the hosts affected by zoonotic STHs, risk factors for infection in dogs, evaluations of animal health programs, and effective and cost-effective treatments. Underpinning all aspects and aims of this thesis, however, is the importance of meaningful and culturally responsive engagement with Aboriginal and Torres Strait Islander communities.

6.1.1 Working with Aboriginal and Torres Strait Islander communities

Community engagement and leadership was essential in addressing the aims in this thesis, including the research presented in Chapters 3, 4 and 5. As Chapter 2 showed, in 100 years of published research, only 45 studies covered the prevalence of STHs in remote Aboriginal and Torres Strait Islander communities, underlining the challenges of working in these remote settings and also potentially the lack of sustainable community engagements.

A key aspect of the successful engagement for this thesis was the involvement of local environmental health workers employed by the local council. Before any research proposals regarding Chapter 4 and 5 were made, a visit to Moa Island in the Torres Strait was undertaken including meetings with local elders and environmental health workers from several communities who understood local issues. At these meetings information was presented on potential zoonotic concerns in community dogs with feedback sought from locals on what was important to them in this regard. Information was presented live as well as via posters (see Figure 6.1 as an example), which people could take back to their communities to discuss with others. After a period of several weeks to allow for community discussion and consultation, further meetings were held online with Torres Strait Island Regional Council environmental health workers, Queensland public health staff and AMRRIC staff where a collaborative research proposal was developed to meet

community needs and empower evidence-based decisions in communities. This proposal was presented to elders and elected officials across all Torres Strait Island communities regardless of their involvement in the research, with a result of unanimous support. Following sample analysis, results were first disseminated to all Torres Strait Island Regional Council environmental health workers during another in-person meeting. More posters were developed which allowed further dissemination of results to all communities using approachable language content and employing the use of pictograms to simplify reporting. Examples of initial and results reporting posters are shown in Figure 6.1. The experiences described in these engagement activities align with other research and recommendations of research practices, which highlight the need for culturally responsive, and community-led research (Kwaymullina 2016).

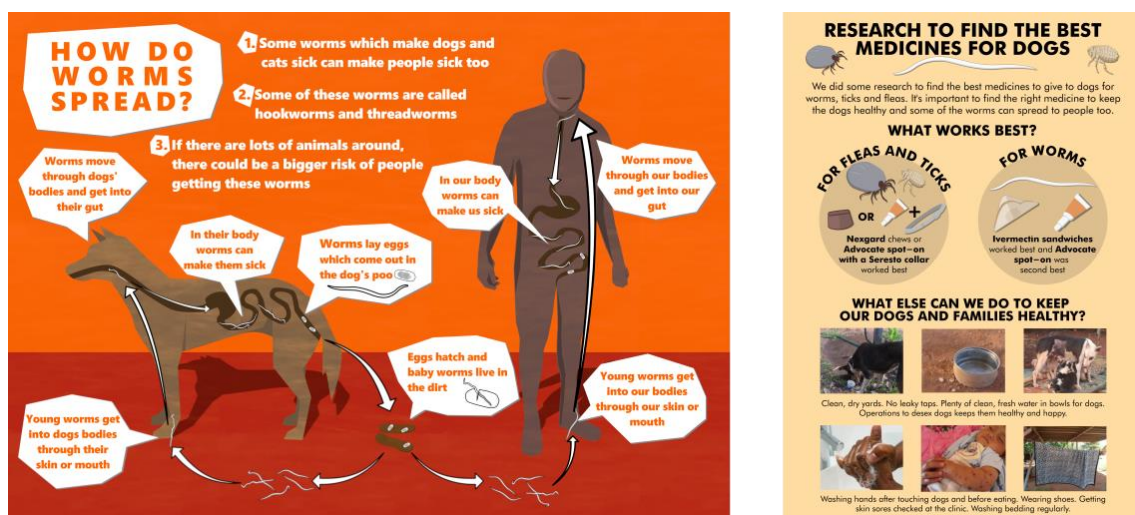


Figure 6.1: Posters designed for dissemination of information through Torres Strait Islander communities before (left) and after (right) research conducted in Chapter 4

Several specific challenges were present in access to remote communities or to specific areas of those communities within this thesis. In Chapter 3, while samples were successfully collected in 2019, further faecal sampling in West Arnhem in the subsequent years of 2020 and 2021 was not possible due to COVID-19 travel restrictions. In Chapter 4, the target number of 80 dogs per island at baseline could not be achieved in each treatment group due to a wave of parvovirus dog deaths affecting one island

and cultural practices for a funeral meant that parts of the community could not be entered meaning that sampling of dogs in that area was prohibited. While cultural practices have precluded research or animal health program activities in some areas of communities, respect towards cultural practices is paramount in fostering ongoing relationships (Kwaymullina 2016).

Funeral practices in Aboriginal and Torres Strait Islander communities are diverse and, in contrast to many Western practices, may continue for days or weeks depending on the identity of the deceased (Tonkinson and Burbank 2017). These practices often occur at the home of the deceased, where local people will prevent interruptions by placing barricades on nearby roads which restrict vehicle and non-local entry to the area. The timing of funerals may be dependent on several factors including transport of bodies from morgues or the presence of family in community and as such may not be predictable or avoidable from the perspective of external researchers (Tonkinson and Burbank 2017). As such, empathy, cultural literacy and flexibility are key aspects of cultural training for animal health program and research personnel.

6.1.2 Prevalence of STH infections in Aboriginal and Torres Strait Islander communities

The first aim of this thesis was to systematically review available literature, understand the locations, parasite and host species involved, and provide an estimate of the individual study and pooled true prevalence of STH infections in Australian Aboriginal and Torres Strait Islander communities. This aim was addressed in the systematic review and meta-analysis in Chapter 2 of this thesis. The key finding was that hookworm and *S. stercoralis* have pooled true prevalence estimates indicating continued endemicity in Aboriginal and Torres Strait Islander communities, and that domestic dogs as well as wild dogs, dingos and humans act as hosts in these settings. This indicates that prior efforts to reduce prevalence have not been effective, and that concerted, evidence-based approaches to control and elimination of STHs are needed. Prevalence of STHs in the Arnhem Land communities in Chapter 3 were consistent with the pooled true

prevalence estimates revealed in Chapter 2 with estimates of 86.9% (95% CI 80.4 to 91.8) and 14.4% (95% CI 9.6 to 20.9) for *A. caninum* and *Strongyloides* spp., respectively. Similar prevalence for *A. caninum* was also reported in Chapter 4 with a baseline estimate of 83.9% (95% CI 77.7 to 88.6).

Further, the systematic review highlighted the range of diagnostic methods utilised in prevalence studies over the last hundred years. Some of these methods had poor diagnostic accuracy, while others were inappropriate for identification of a parasite at species level or certain life cycle stages in a sample. Instead of diagnostic confirmation, several studies assumed hookworm species identified by coproscopy based on the common species typically found in the host. As such, important zoonotic species may have been misclassified. This emphasises the need for accurate, species-specific diagnostic methods in order to understand the significance of zoonotic STHs in Aboriginal and Torres Strait Islander communities.

The dearth of prevalence information for *A. ceylanicum* available for inclusion in Chapter 2 is concerning given its known zoonotic potential, recorded presence in Australia and status as the second most prevalent hookworm in humans the Asia-Pacific region (Traub 2013; Bradbury et al. 2017). Only 63 dogs and 35 dingoes in Aboriginal and Torres Strait Islander community settings had test results reported up to the date that the literature search was conducted, while only one autochthonous case in a human had been reported in the Pilbara region of Western Australia and was believed to be derived from dogs or cats (Koehler et al. 2013; Smout et al. 2017b; Smout et al. 2018). Interestingly, none of the 338 dogs from three Arnhem Land and three Torres Strait Island communities included in the results of all chapters in this thesis were positive for *A. ceylanicum* on qPCR. While dogs have been of primary zoonosis research interest, cats may also present a significant zoonotic risk (Smout et al. 2017a). In West Arnhem communities visited as part of WALDHeP cat populations have grown dramatically. In 2013 approximately five cats were seen in a community of 1500 people, however in 2023 in that same community we recorded over fifty cats living in one house. Given their increasing numbers and largely unknown STH infection burden, surveys are needed to shed light on the risks they may pose (Kennedy et al. 2020).

6.1.3 Impacts of long-term animal health programs

With a clearer understanding of prevalence of these important parasites, an obligation arises to determine optimal approaches to their control as well as to improve the overall wellbeing of dogs in these settings. The second thesis aim of examining the impacts of a long-term animal health program in three remote Aboriginal communities in Arnhem Land was addressed in Chapter 3. While short-term variation in measures was apparent between years, long-term trends were evident in improved body and skin condition scores as well as in reductions to the median number of dogs per household.

Animal health programs in remote Aboriginal and Torres Strait Islander communities have operated for decades in many locations, though community-based research to inform clinical and program decisions has, to date, been limited. As such, decisions by veterinarians may have been extrapolated from experience in urban settings, potentially leading to ineffective or culturally insensitive actions (Bennett and Archer-Lean 2023). Examples of this have been seen in reports of culling practices or strict enforcement of local by-laws on dog numbers (Brookes et al. 2020). Published data on the recommended frequency of visits is scarce. Reports of animal health programs recommend frequencies of every three months to three years, though these recommendations have not been based on field trial analyses (Wilks 2000; Burleigh et al. 2015). In guides for veterinarians undertaking animal health programs, AMRRIC does not advise a specific frequency but instead advises community consultation and balancing of community needs with availability of resources to develop sustainable programs (Phelan et al. 2007).

A key finding in enabling reporting on the impacts of remote community animal health programs was the importance of record keeping. Rigorous record keeping is key to establishing impact in remote community animal health programs, though keeping complete, detailed and secure records can present challenges. While record keeping for WALDHeP began with paper records, a customised digital database has been implemented, allowing exporting for more rapid analysis as well as incorporating GPS

and photographic details to aid in animal identification. Digital databases are also promoted in ICAM recommendations, with the importance of regular and secure backups emphasised (International Companion Animal Management Coalition 2015). This is a crucial aspect to record keeping in remote Aboriginal and Torres Strait Islander community programs, where internet access can be unreliable and experience prolonged outages.

6.1.4 Risk factors and genetic groupings of zoonotic STHs

The third thesis aim of exploring the prevalence, risk factors and genetic grouping of STHs in three remote Aboriginal communities in Arnhem Land was also addressed in Chapter 3. A key risk factor for canine infection with *A. caninum* was identified as coming from a household with three or more dogs compared to dogs from households with fewer than three dogs. This is particularly important as a risk factor that can be directly addressed by owners assisted by animal health programs, as seen in the reductions in median numbers of dogs per house in Chapter 3. This also emphasises the significance of owners having agency in the number of animals they choose to keep and the importance of community empowerment in animal health. While entire dogs had a greater odds of infection with *A. caninum* compared to desexed dogs, this finding was not statistically significant though it is biologically plausible, particularly in the case of entire males due to the immunosuppressive effects of testosterone (Traub et al. 2014). Studies from other settings have identified males as having a higher odds of infection and entire males as having a higher prevalence of hookworm infection than other dog demographics (Palmer et al. 2007; Traub et al. 2014). Given the lack of reported risk factors for canine parasite infection in this setting, further research is warranted to validate desexed status as a risk factor as well as to investigate other relevant risk factors.

Careful interpretation of these findings is required due to biased and small sample sizes as detailed in Chapter 3. However, the collected data still provide useful information in terms of risk factors for infection. Further investigation should be pursued to validate

these risk factors for *A. caninum* infection in these settings as well as risk factors for other parasites including *Strongyloides* spp.

A key finding in terms of genetic grouping of *Strongyloides* was the lack of evidence of zoonotic haplotypes of *Strongyloides* spp. found in these dogs. While only based on a small number of successfully sequenced samples, this result was expected as most dogs are reported to be infected with dog-only haplotypes (Barratt et al. 2019). However, in relation to other reported literature it is clear that vigilance is necessary due to reports of zoonotic strains in dogs from nearby remote Aboriginal communities, as well as recent reports of humans becoming infected with strains thought to only infect dogs (Beknazarova et al. 2019; de Ree et al. 2024).

Another challenge in addressing this aim was in sample collection for *Strongyloides* spp. genotyping. Sampling for STH detection in this thesis involved preservation with potassium dichromate or Zymo DNA/RNA Shield which are suitable for hookworm egg preservation, yet unsuitable for *Strongyloides* live larval isolation (Stracke et al. 2021). Concentration techniques including larval culture and the Baermann technique are useful for *Strongyloides* spp. isolation given the low levels of shedding in most infections (Bisoffi et al. 2013). This can provide more concentrated genetic material for successful sequencing but requires live larvae and therefore was not feasible with preserved samples (Zhou et al. 2019).

6.1.5 Efficacy and effectiveness of antiparasitic treatment programs

While program impacts and risk factors may assist in preventing infection, the high *A. caninum* prevalence reported in these three Arnhem Land communities as well as those found in Chapter 2 require treatment with efficacious and effective treatments. The fourth aim of this thesis was addressed in Chapter 4, in which the short-term efficacy and medium-term effectiveness of three treatments for endoparasites and three treatments for ectoparasites was assessed in a community field trial over a period of six months. Out of the three treatments tested – oxibendazole, moxidectin, and off-label ivermectin - this research identified that off-label ivermectin was the most efficacious

and effective for *A. caninum*, curing infection in nine out of ten cases. On the other hand, a single dose of oxibendazole resulted in cure in less than one in ten cases and was thus inept. Other published reports also support the use of ivermectin in treatment of *A. caninum* as well as for *S. stercoralis* (Paradies et al. 2019; Bhanjadeo et al. 2022; Chapman et al. 2024). While highly suitable for endoparasites, off-label ivermectin was inappropriate for the treatment of ectoparasites. Imidacloprid/flumethrin collars resulted in cure of ectoparasite infection in eight out of ten cases thus being the most effective choice, particularly considering their repellent effect, which is consistent with other studies assessing the efficacy of these collars (Otranto et al. 2008; Brianti et al. 2013).

While a three-monthly treatment frequency with ivermectin was shown to be effective in treating *A. caninum* in this thesis, ongoing blanket treatments may promote anthelmintic resistance (Fissiha and Kinde 2021). Resistance to benzimidazoles and pyrantel have recently been identified in *A. caninum*, though resistance genes and mechanisms of resistance development to macrocyclic lactones have not yet been identified in this parasite (Castro et al. 2019; Leutenegger et al. 2023; Marsh and Lakritz 2023). Longer-term field trials with variation in frequency of administration or treatment rotation may aid in reducing parasite prevalence while minimising the risks of development of resistance.

6.1.6 Cost-effectiveness of antiparasitic treatment programs

Finally, while some treatments may be effective, their cost-effectiveness is a key determinant in whether they are implemented within the often-limited budgets of local governments or NGOs. Cost-effectiveness data is particularly useful in guiding policy and targeting budgetary requirements for the control of canine parasites and protecting human populations from zoonoses. The fifth aim for this thesis was addressed in Chapter 5, by determining the cost-effectiveness of three treatments for *A. caninum* and three treatments for fleas and ticks under field conditions in a Torres Strait Islander community setting.

The key finding of this chapter was that despite the additional costs of veterinary oversight, off-label ivermectin was the most cost-effective endoparasite treatment with a cost of only AUD \$13.32 per dog free of infection at the six-month time horizon compared to \$69.16 for moxidectin. Imidacloprid/flumethrin collars were the most cost-effective for treating and preventing ectoparasite infections long-term despite their high unit cost, with a cost of \$66.97 per dog free of flea infestation or \$69.05 per dog free of tick infestation at the six-month time horizon. Afoxolaner chews had a higher cost per dog free of infestation, with \$109.90 for fleas and \$111.01 for ticks. While off-label ivermectin was less costly than either of these treatments for ectoparasites, it had very poor effectiveness.

Furthermore, this chapter showed that ivermectin also represented the lowest cost to achieve possible community elimination of *A. caninum* in the shortest period. From a baseline prevalence of 72.4%, *A. caninum* prevalence can be brought below 2%, the threshold of community elimination as defined in modelling of human STH studies (Truscott et al. 2017), within 4-6 three-monthly treatment cycles with off-label ivermectin. This represents a cost of \$2,285.86 per 100 dogs over four treatment cycles, with greater cost-efficiencies expected for larger dog populations as detailed in the sensitivity analysis. Comparison to other studies is challenging, as no published economic evaluations exist for canine antiparasitic treatments, let alone in remote Aboriginal and Torres Strait Islander community settings.

A challenge in addressing this aim existed in a lack of prior reports of effectiveness measures for use in economic evaluations of animal or One Health interventions. While quality-adjusted life years or disability-adjusted life years are commonly used as outcome measures in human cost-effectiveness analyses, these do not directly translate to studies involving animals. The measure used in Chapter 5 of dogs free of infection is valuable in terms of animal outcomes but also in their role as environmental contaminants leading to human infections. Further investigation of effectiveness measures applicable within animal and One Health interventions is necessary. Furthermore, while these analyses examined 3-monthly treatment frequency with ivermectin, which would be suitable and likely feasible within environmental health

worker or ranger work schedules, other treatment frequencies may be more cost-effective. As no published data exists, further economic evaluations of these variations are warranted.

6.2 Research recommendations

The findings of this thesis provide a foundation for several important research avenues which would further develop understandings of STH prevalence, risk factors for infection, effective treatment programs, treatment cost-effectiveness, and animal health program evaluations. Building on this work, future research should consider prevalence studies, sample collection strategies, diagnostic methodologies used, efficacy and effectiveness analyses and cost-effectiveness analyses.

6.2.1 Prevalence studies

The limitations of the included studies in the systematic review in Chapter 2 highlight the need for further prevalence studies on STHs in remote Aboriginal and Torres Strait Islander communities. Specifically, there is a need for concomitant animal and human prevalence studies to help identify potential zoonotic exchange. Given the existing knowledge gap and utilising synergies around sampling logistics, cats should also be included in future studies. Specific emphasis is warranted for *Strongyloides* spp. given their serious clinical outcomes, as well as *A. ceylanicum* given its zoonotic capacity. Prevalence studies of *A. ceylanicum* should focus on northern Queensland where it has most recently been identified.

Furthermore, as highlighted in Chapter 3, remote community animal health programs represent an important means of conducting prevalence reporting as part of program evaluation, treatment efficacy analyses and community elimination efforts. Regular prevalence surveys as part of program planning and evaluation are warranted to provide further evidence of impact and target interventions as well as to identify whether community elimination has been achieved.

6.2.2 Sample collection

The practicalities of sample collection, storage and transport to laboratories must be carefully considered in remote settings in which seasonal extremes or the vagaries of air or road transportation may cause significant complications or delays. Similarly, high temperatures found in tropical climates risk damage to collected faecal samples or may promote larval hatching from eggs. Challenges with transport delays and lack of refrigeration were encountered in this thesis and managed through appropriate sample preservation. Preservation media such as Zymo DNA/RNA shield should be utilised for faecal samples as it does not require refrigeration and samples remained suitable for both coproscopic and molecular analysis, even after several weeks of field work. Refrigeration wherever possible is nonetheless recommended as a precaution. However, faecal samples intended for *Strongyloides* spp. genotyping should be collected separately without the immediate use of DNA preservatives to allow for live larval concentration techniques such as larval culture or the Baermann technique. These techniques may need to be performed in the field, therefore suitable facilities and equipment are required such as agar plates for larval culture, or funnels, clamps and tubing and a microscope for the Baermann technique. Once larvae are isolated DNA preservatives should be used for sample transport for DNA extraction. This would support further research on genotyping of *Strongyloides* spp. in dogs in remote Aboriginal and Torres Strait Islander communities and thereby provide clarity on their zoonotic potential.

Sampling strategies for risk factor or efficacy analyses should pursue calculated sample targets ensuring sufficient numbers for statistical analyses. Careful consideration of sampling strategies taking hierarchical data structure into account will avoid biased samples. However, logistical constraints, the availability of resources including funding, personnel, and time as well as participant availability and cultural considerations will always have an unavoidable influence and need to be balanced.

6.2.3 Diagnostic methods

Diagnostic methods available to detect STH species have expanded dramatically over the last twenty years, becoming more accessible and less costly to perform. The research presented in Chapter 2 highlights these developments as well as the importance of accurate and species-specific diagnostic methods. Real-time PCR is recommended as a high-throughput method suitable for prevalence and efficacy studies in which multiple species may exist, as exemplified in Chapters 3 and 4 of this thesis. Further research establishing cycle threshold value to eggs per gram conversions for different parasite species and hosts would enable simultaneous prevalence and burden analyses. Analyses incorporating next-generation sequencing should also be conducted to aid in identifying specific molecular diagnostic targets.

For diagnostics in the field, faecal floatation is appropriate to detect hookworms and requires minimal equipment while providing a means of assessing worm burden. However, it does not permit species identification. Thus, positive samples should be stored in Zymo DNA/RNA Shield until molecular diagnostics can be used to confirm parasite species, thereby improving the understanding of zoonotic parasite distributions in remote Aboriginal and Torres Strait Islander communities.

6.2.4 Efficacy, effectiveness and cost-effectiveness analyses

While practical efficacy, effectiveness and cost-effectiveness analyses for three endo- and ectoparasiticides are presented in this thesis, further analyses are warranted given the broad range of treatment and repellent options available as well as the range of practical considerations in remote community animal health. Given the promising field efficacy and effectiveness results of ivermectin and moxidectin presented in this thesis, further investigation of other macrocyclic lactones such as topical selamectin is warranted using commercially available formulations. Other orally administered compounds should also be investigated further in remote community settings as dosing does not require handling of potentially difficult dogs, though palatability must also be ensured. Investigation of effectiveness in remote community settings must account for

the impact of environmental contamination on reinfection, and therefore treating all dogs in a community at the same point in time with the same treatment as conducted in Chapter 4 is recommended.

The potential emergence of anthelmintic resistance in remote community settings requires further investigation, both in terms of genotypic analysis for resistance genes as well as field trials assessing possible resistance and methods to combat its emergence. Long-term field trials of varying treatment frequencies should be conducted as longer intervals present a potential means to avoid emergence of resistance. Another area for research to avoid emergence of resistance is treatment rotation. Once several effective treatment classes have been established, these may form part of research rotating between these classes at regular intervals with ongoing assessment of effectiveness.

Further research of efficacy and effectiveness of parasite treatment programs in remote Aboriginal and Torres Strait Islander communities should also incorporate cost-effectiveness analyses to ensure that resulting recommendations are economically feasible. Any cost-effectiveness analyses must be careful to incorporate all associated costs for treatment as demonstrated in Chapter 5, including treatment and personnel transport, staff costs, vehicle costs and costs of any necessary veterinary oversight for off-label treatments. Further research is also necessary in establishing outcome measures which are applicable within animal and One Health intervention studies in this setting and account for outcomes in all affected species. Impacts recorded as part of animal health programs such as body or skin condition scores, clinical signs or dog mortality rates may have use as effectiveness measures in cost-effectiveness analyses and investigation into how they may be utilised is warranted.

6.3 Dog health program recommendations

The findings of this thesis are also valuable in developing policy for conducting impactful animal health programs incorporating effective and cost-effective parasite treatment and control programs. When planning, implementing and evaluating dog health

programs in Aboriginal and Torres Strait Islander communities, consideration should be given to parasite treatments, program visit frequency, record keeping, community engagement and program sustainability.

6.3.1 Parasite treatments

Nearly eight out of ten hosts in remote Aboriginal and Torres Strait Islander communities are infected with *A. caninum*, which may lead to chronic weight loss and severe anaemia in canines or cutaneous larva migrans and eosinophilic enteritis in humans. Furthermore, nearly two out of ten hosts are infected with *Strongyloides* spp. which can lead to serious manifestations of disseminated strongyloidosis with a high case fatality rate. As such, treatment and control programs on community level are essential. This thesis has shown that off-label ivermectin is efficacious and effective, but also highly cost-effective in the treatment of these parasites in this setting. Given its acceptability to dogs it can be readily administered by veterinary staff or, with veterinary oversight and training, environmental health workers and ranger groups. Administered to dogs on a three-monthly basis, it can be used for targeting community elimination of *A. caninum* at a cost of around AUD \$2,300 per 100 dogs per year. Treatment intervals of more than three months may lead to continued endemicity from ongoing environmental contamination and reinfection. Based on modelling in Chapter 5, communities which have had six cycles of regular three-monthly treatment may be able to reduce frequency of treatment to reduce risks of anthelmintic resistance development as well as costs. This decision should be based on ongoing prevalence surveys to confirm a break in transmission, which should be conducted at least every two years.

With the potential for environmental contamination to occur from dogs recently brought into a community, any new arrival dogs should be presented to veterinary or animal health worker staff and treated with at least two doses of ivermectin one month apart to ensure efficacy against any arrested larvae. New arrivals should also be monitored for any indication of them having genetics of at-risk breeds for ivermectin sensitivity to ensure that ABCB1 gene deletions do not enter the population. If a dog

with a perceived risk of sensitivity were to enter the population, treatment options other than macrocyclic lactones would need to be recommended for those dogs and their offspring.

For the control of ectoparasites, dogs should have imidacloprid/flumethrin collars applied due to their effectiveness, cost-effectiveness, and repellent effect. Collars should be checked regularly to ensure that they are in place, and stores of collars should be maintained to ensure that replacements can be fitted if any are lost. New collars should be fitted every six months to ensure coverage against ectoparasites. For 100 dogs without collar replacement this would constitute a cost of approximately AUD \$13,400.

6.3.2 Program visit frequency

Evidence presented in Chapter 3 highlights the changes possible in the numbers of dogs per house as well as body and skin condition with sustained annual visits over a long period. Program visits are thus recommended to occur at least annually with a view to long-term program maintenance and commitments. However, research presented in Chapters 3 and 5 indicate that annual parasite treatments do not reduce STH prevalence. More frequent visits are necessary to ensure that regular veterinary oversight of parasite treatment programs is maintained and that injuries and disease which would lead to poor welfare if left untreated are attended to as soon as possible. The inclusion of veterinary oversight of parasite treatment programs represents significant value-adding for regular veterinary services as it permits the use of more cost-effective treatments such as off-label ivermectin. This is advantageous compared to the labelled treatments communities may otherwise be restricted to using, as detailed in Chapters 4 and 5. Compared to ivermectin, oxibendazole and moxidectin would represent an additional annual cost of \$3,167.17 and \$7,258.63 per 100 dog cohort, respectively, and oxibendazole would not have an effect in reducing prevalence. Overall, however, considerations of animal health program visit frequency must balance community needs with availability of resources, and thorough community consultation should always occur.

6.3.3 Record keeping and reporting

Animal health programs must keep thorough records both from a veterinary regulatory perspective, but also to aid in measuring impacts which are relevant to community settings. Digital databases with offline capability should be used in preference to paper records, with the option for online backups when internet connections are available. Purpose-built software is available, such as the AMRRIC app, though more simple custom databases are also suitable.

Relevant measures to be recorded may vary widely between communities depending on dog population demographics, zoonotic disease prevalence, human health reporting or wildlife populations. Based on research presented in Chapter 3 of this thesis, a community census with records of body condition score, skin condition score, numbers of dogs per house and parasite treatments administered are recommended as a minimum. While traditionally animal health programs have focused on dogs, cat populations have dramatically increased in many remote communities, and the needs of each community should be determined with thorough consultation. The numbers, types and frequency of parasite treatments administered either by veterinary staff or local workers should be recorded and shared amongst all personnel involved in parasite treatment programs to ensure continuity of treatment and avoid superfluous dosing. Measuring the proportion of desexed dogs in a population is also recommended to identify specific areas of focus for future program visits. Statistical analysis workflows should be developed to aid in program planning as well as reporting to communities and local governments where necessary. Reporting to communities should be conducted in a format and language appropriate to the community and should include in-person discussions with community leaders and elders where possible.

6.3.4 Community engagement

Owner and community engagement must be a focus of animal health programs in Aboriginal and Torres Strait Islander communities in order to meet community needs and support self-determination. Measuring community engagement with animal health

program as part of impact assessments may be challenging but would be useful to guide practices in communities as well as to report impacts to supporters. Community engagement may take many forms depending on specific communities, but should involve preliminary conversations with elders, traditional owners and more formal Aboriginal corporations or local governments to ensure that community needs are considered in planning and met in implementation. Engagement should begin before any veterinary program activities occur as it is crucial to gain an understanding of local physical and social landscapes. This sets in motion the trusting relationships upon which a sustainable animal health program should be built.

6.3.5 Program sustainability

Finally, the success and sustainability of animal health programs in Aboriginal and Torres Strait Islander communities is dependent on the cultural competence of the personnel involved and maintenance of an available workforce to fulfill ongoing program commitments. Cultural competence is important in any veterinary practice setting, but particularly important on the part of veterinary staff who may not necessarily identify with the cultural, spiritual or linguistic background of the people they serve. In some communities, particularly those with histories of enforced dog culls or restrictions, trusting relationships may take years to develop. The progress of these relationships relies upon the attitudes and training of those engaged in animal health programs. Cultural competence training is essential for any personnel involved in remote Aboriginal and Torres Strait Islander community animal health programs and should take place formally before any program visits occur, with continued cultural competence debriefs occurring during and after visits. A base of culturally competent veterinary graduates has been vital to the continuation of WALDHeP as a sustainable animal health program capable of serving remote Aboriginal communities for twenty years.

6.4 Conclusion

The continued endemicity of STHs in remote Aboriginal and Torres Strait Islander communities represents an ongoing threat to canine health, a fundamental element of enduring health inequities faced by human populations, and a problem requiring One Health interventions. Remote community dog health programs occupy important roles in managing this threat through the provision of culturally responsive services and parasite treatment programs. Veterinary services can improve health impact measures and empower communities in managing the health of their animals as well as reducing risks of zoonotic parasite infection. Evidence-based parasite treatment programs utilising effective treatments given at regular intervals can enable community elimination of these parasites. Cost-effectiveness analyses provide funders with practical data to make informed decisions on which treatments will achieve community goals, which will be feasible within animal health program budgets, and which will deliver a greater return on investment. Culturally responsive, evidence-based and community-led animal health programs represent a way forward to dramatic reductions in the prevalence of zoonotic STHs in dogs in remote Aboriginal and Torres Strait Islander communities. Working within a One Health framework, these programs greatly improve not only the wellbeing of dogs, but also the communities with which they share their lives.

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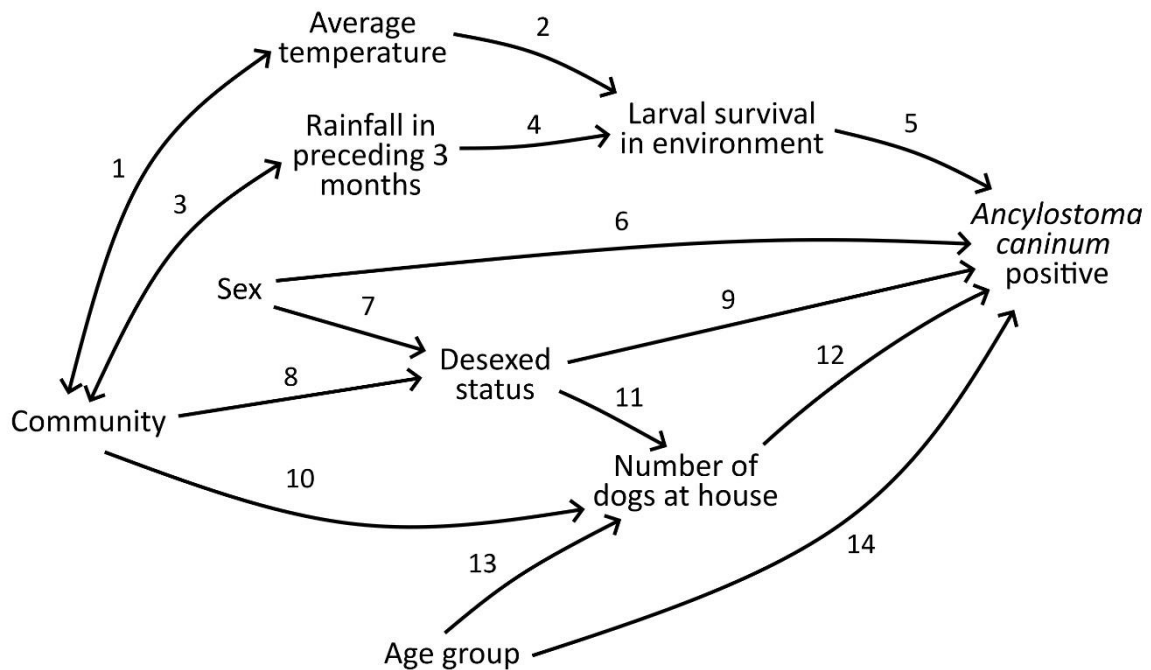
Appendices

Appendix 1: Extracted data and assessment of bias of all papers included in the systematic review in Chapter 2

This table is too large to fit into this document, but can be downloaded from this link:

<https://go.unimelb.edu.au/3898>

Appendix 2: Directed acyclic graph (DAG) and justification



Detailed justification of the links in the directed acyclic graph (DAG) in a cross-sectional study of risk factors for infection with *Ancylostoma caninum* in dogs in remote Aboriginal communities

1. 'community' -> 'average temperature'

Southern hemisphere locations with more northern latitudes may have higher average temperatures given their tropical or equatorial climates. Average temperature coincided with community in this study as samples were collected at only one point in time.

2. 'average temperature' -> 'larval survival in environment'

Warm environments are more favourable to larval development and survival (McCoy 1930; Brooker et al. 2006)

3. 'community' -> 'rainfall in preceding 3 months'

Southern hemisphere locations with more northern latitudes may have higher rainfall given their tropical or equatorial climates. Similar to average temperature,

however, rainfall coincided with community as samples were collected only once and over a very short period of time in each community.

4. 'rainfall in preceding 3 months' -> 'larval survival in environment'

Moist environments created by recent rainfall may be more favourable to larval development and survival (Brooker et al. 2006). A three-month window was selected to capture the seasonality of northern Australian climates, in which the wet season may lead to prolonged wetting of soil, while the dry season may mean that little to no rain falls for prolonged periods.

5. 'larval survival in environment' -> '*Ancylostoma caninum* positive'

Greater numbers of larvae surviving in and contaminating the environment may lead to greater rates of infection and animals testing positive.

6. 'sex' -> '*Ancylostoma caninum* positive'

Male and female dogs may have different behavioural tendencies, such as different tendencies to roam as found by Molloy *et al.* (2017) which may lead to greater exposure to contaminated environments.

7. 'sex' -> 'desexed status'

Female dogs may be more likely to be desexed as cultural preferences may mean that male dogs are chosen to remain entire. Some community members feel that entire male dogs are more capable of working as hunting partners than their castrated counterparts.

8. 'community' -> 'desexed status'

Communities may have differing management practices of animal populations and differing access to veterinary care and desexing surgery.

9. 'desexed status' -> '*Ancylostoma caninum* positive'

Entire animals may expend more energy on reproductive behaviours and in the case of females, gestation and lactation, which may leave them less capable of combatting infections or more likely to become infected due to increased scavenging

behaviour from increased nutritional demand. Entire dogs may also roam further, exposing them to greater risk of infection from the environment (Molloy et al. 2017).

10. 'community' -> 'number of dogs at house'

Communities may have cultural preferences for keeping certain numbers of dogs and may also have differing management practices of animal populations such as local government restrictions on numbers of animals at a house.

11. 'desexed status' -> 'number of dogs at house'

Entire dogs are more likely to reproduce which may result in larger numbers of dogs at a particular household.

12. 'number of dogs at house' -> '*Ancylostoma caninum* positive'

Larger numbers of free roaming dogs in a household may be linked to an increased chance of any given dog becoming infected during daily movements as well as a larger number of dogs contaminating the local environment to a greater extent.

13. 'age group' -> 'number of dogs at house'

Litters of puppies are likely to be linked to greater numbers of dogs at a house, and young or adult dogs may be more likely to breed and increase numbers than old dogs.

14. 'age group' -> '*Ancylostoma caninum* positive'

Younger animals may explore environments more with their mouths or may explore areas more likely to be contaminated with infectious organisms than more mature animals (Traversa 2012). Puppies may also be subject to vertical transmammary transmission of *A. caninum* from their dam (Shepherd et al. 2018).

Appendix 3: R code for efficacy analyses in Chapter 4

```
library(tidyverse)
library(lme4)
library(readxl)
library(emmeans)
library(performance)
library(broom.mixed)
library(lmerTest)
library(blmecco)
install.packages('epiDisplay')

# Load and define variables
directory <- "C:/Users/..." #Insert directory location of input files

dat_long <-
read_excel(paste0(directory,"TSI_treatment_trial_project_data_long.xlsx"))
dat_wide <-
read_excel(paste0(directory,"TSI_treatment_trial_project_data_wide.xlsx"))

dat_long <- dat_long %>% mutate(treatment = factor(as.numeric(factor(island))),
                             time_point = factor(time_point),
                             flea_yn = as.numeric(flea_count > 0), # Fleas present y/n
                             tick_yn = as.numeric(tick_count > 0), # Ticks present y/n
                             ecto_score = pmax(flea_count, tick_count), # Combining flea and tick
                             score
                             y/n
                             ecto_yn = as.numeric(ecto_score > 0), # Any ectoparasites present
                             age_collapsed = ifelse(age_group == "Puppy" | age_group ==
"Young", "Juvenile", "Adult or Old")) # Collapsing age categories

dat_wide <- dat_wide %>% mutate(treatment = factor(as.numeric(factor(island))),
                             qpcr_cure = t0_qpcr_result_ancylostoma_caninum -
t1_qpcr_result_ancylostoma_caninum,
                             qpcr_cure = ifelse(qpcr_cure < 0, 0, qpcr_cure),
                             epg_perc = ((t0_epg_hookworm -
t1_epg_hookworm)/t0_epg_hookworm) * 100,
                             epg_perc = ifelse(epg_perc < 0 | is.na(epg_perc), 0, epg_perc),
                             epg_90_perc_yn = as.numeric(epg_perc > 90),
```

```

flea_cure = ifelse(t0_flea_count > 0 & t1_flea_count == 0, 1,0),
tick_cure = ifelse(t0_tick_count > 0 & t1_tick_count == 0, 1,0),
t0_ecto_score = pmax(t0_flea_count, t0_tick_count),
t1_ecto_score = pmax(t1_flea_count, t1_tick_count),
ecto_cure = ifelse(t0_ecto_score > 0 & t1_ecto_score == 0, 1,0))

# Overdispersion function
overdisp_fun <- function(model) {
  rdf <- df.residual(model)
  rp <- residuals(model,type="pearson")
  Pearson.chisq <- sum(rp^2)
  prat <- Pearson.chisq/rdf
  pval <- pchisq(Pearson.chisq, df=rdf, lower.tail=FALSE)
  c(chisq=Pearson.chisq,ratio=prat,rdf=rdf,p=pval)
}

# Variance partition coefficient function
VPC <- function(model) {
  VPC <- VarCorr(m1) %>%
  as_data_frame() %>%
  mutate(icc=vcov/sum(vcov))

  VPC[,c(1,6)]
}

# -----

## EPG hookworm
# Poisson final mixed effects model of effects on hookworm eggs per gram of faeces
m1 <- glmer(epg_hookworm ~ treatment*time_point + (1|island) +(1|dog_id) ,
family=poisson, data=dat_long)

summary(m1)
overdisp_fun(m1)
VPC(m1)
model_performance(m1)
joint_tests(m1)
emm1 <- emmeans(m1, specs="time_point", by="treatment")
emm1
pairs(emm1, adjust="none")

```

```
confint(pairs(emm1, adjust="none"))

# -----

# Binomial final mixed effects model of effects on ectoparasite presence
m1_ecto <- glmer(ecto_yn ~ treatment*time_point + (1|island) +(1|dog_id) ,
family=binomial, data=dat_long)

summary(m1_ecto)
VPC(m1_ecto)
model_performance(m1_ecto)
joint_tests(m1_ecto)
emm1 <- emmeans(m1_ecto, specs="time_point", by="treatment")
emm1
pairs(emm1, adjust="none")
confint(pairs(emm1, adjust="none"))
```