Strongyloides stercoralis in Australia

by

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Thesis
submitted to Flinders University
for the degree of

Doctor of Philosophy
College of Science and Engineering
19.08.2019

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LIST OF ABBREVIATIONS

AHDC	Australian Health Development Committee
AHPC	Australian Health Protection Committee
AIDS	acquired immunodeficiency syndrome
*cox*1	cytochrome c oxidase 1
AMRRIC	Animal Management in Rural and Remote Indigenous Communities
BCI	Bayesian confidence interval
BSA	bovine serum albumin
bp	base pair
CDC	Centers for Disease Control and Prevention
CDNA	Communicable Disease Network Australia
CI	confidence interval
CLM	cutaneous larva migrans
Ct	cycle threshold
DALY	disability-adjusted life year
DESS	dimethyl sulfoxide, disodium EDTA, and saturated NaCL
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
EHO	Environmental Health Officer
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
GABA	gamma-aminobutyric acid
GB	GenBank
GluCL	glutamate-gated chloride channel
HIV	human immunodeficiency virus
HTLV-1	human T-lymphotropic virus 1
HVR	hyper variable region
IFAT	immunofluorescence antibody test

IgG4	immunoglobulin G4
ITS	internal transcribed spacer
MDA	mass drug administration
ML	macrocyclic lactone
mtDNA	mitochondrial DNA
NGS	next generation sequencing
NNDL	National Notifiable Disease List
NNDSS	National Notifiable Disease Surveillance System
NPHP	National Public Health Partnership
NSW	New South Wales
NT	North Territory
NTC	non-template control
NTD	neglected tropical disease
OR	odds ratio
PC	physical containment
PCR	polymerase chain reaction
PhD	Doctor of Philosophy
PNG	Papua New Guinea
qPCR	real-time PCR
rDNA	ribosomal DNA
RNA	ribonucleic acid
rpm	rounds per minute
SA	South Australia
SAC HREC	Southern Adelaide Clinical Human Research Ethics Committee
SBREC	Social and Behavioural Research Ethics Committee
SDG	sustainable development goal
SEA	Southeast Asia
SPSS	Statistical Package for the Social Sciences
spp.	species

SSU	small Subunit
STH	soil-transmitted helminth
US	United States
USA	United States of America
USD	US Dollar
USSR	Union of Soviet Socialist Republics
WASH	water, sanitation and hygiene
WHO	World Health Organisation
WWII	World War II

SUMMARY

Strongyloides stercoralis is a parasitic soil-transmitted nematode. It is estimated to infect up to 370 million people worldwide predominantly in tropical and subtropical areas. In Australia, strongyloidiasis is a major public health problem in remote Indigenous communities with up to 60% of people in some areas found to be seropositive to infection. Despite various intervention programs targeted to control strongyloidiasis in remote Australian communities, the disease has never been eradicated and remains endemic in those communities.

The overall aim of this research was to investigate the role dogs might play in transmitting strongyloidiasis, and to explore the knowledge gaps in regards to understanding the transmission, prevalence and distribution of *S. stercoralis* in Australia.

To do this, firstly, an extensive literature review was conducted, which demonstrated that strongyloidiasis is a disease of socioeconomic disadvantage, and improving sanitary and hygiene conditions in the communities should be the primary target in implementing disease control measures. Next, current available treatment options of strongyloidiasis were examined and found that treatment options only included administration of anthelminthic drugs. The literature review demonstrated that there are two issues associated with drug treatment, namely potential resistance development and reinfection. The research proposed a combined approach for controlling strongyloidiasis that includes targeting the parasite in the environment as well as drug treatment.

To get accurate data on the disease distribution and prevalence and to better understand the routes of transmission, there should be a surveillance system in place to record the cases across Australia. For that to happen, the disease needs to be included in the Australian National Notifiable Disease List. This research examined and assessed strongyloidiasis against 12 criteria set by the Australian Legislation and got a score fulfilling the requirements for national notification to be recommended.

Next, the research looked at the dogs' potential to transmit strongyloidiasis to humans. Followed by the literature review, the research looked at the specific markers of *S. stercoralis* DNA that are used for host differentiation. So, three markers (18S rDNA *SSU* HVR-I and HVR-IV regions and mtDNA *cox*1 gene) of the DNA extracted from Australian human and dog faecal samples and one human sputum were genotyped using deep sequencing technique. The results showed that Australian dogs are infected with at least two genetically different strains of *S. stercoralis*, one that is zoonotic infecting dogs and humans, and the other one is dog specific. These findings confirmed that dogs present a potential reservoir for human strongyloidiasis.

And finally, throughout the research 274 dog faecal samples were collected from across 27 communities in the central and northern parts of Australia. The study looked at the zoonotic parasites

in dogs, *S. stercoralis* and hookworm species including *Ancylostoma caninum*, *Ancylsotoma ceylanicum, Ancylostoma braziliense* and *Uncinaria stenocephala*. There was 21.9% (60/274) and 31.4% (86/274) prevalence of *S. stercoralis* and *A. caninum* found in dog faecal samples. The findings of this study emphasise the importance of the One Health initiative, which considers veterinary and public health interventions together. The One Health approach should be central in developing methods to eliminating *S. stercoralis* and hookworms in order to maintain both animal and public health.

This thesis serves to provide a better understanding of strongyloidiasis by examining the disease from an environmental health rather than clinical perspective.

DECLARATION

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

ACKNOWLEDGEMENTS

This research has been supported by the Australian Government Research Training Program Scholarship.

I would like to first thank my supervisors Dr Kirstin Ross and Dr Harriet Whiley. To Kirstin who introduced me to the world of parasitology, which I fell in love with straight away and felt only stronger at the end of my PhD. Your wide knowledge, support and unconditional trust in me made me do truly amazing and interesting things during my PhD for which I am always grateful. You always managed to encourage and motivate me when I needed it, and with your sense of humour you managed to make me laugh and chill when I needed that more. Thank you for not only your academic supervision, but also your enormous support and care that I always felt and that all together let me get through this journey.

To Harriet, your positivity and confidence made me overcome any doubts that I would have during my PhD. Thank you for your invaluable knowledge and knowing exactly how to fix any problem. You always knew how and what words to say to cheer me up. Thank you for your kind spirit and unlimited support.

I would further like to express my sincere gratitude to Dr Rogan Lee, Dr Matthew Watts, John Clancy and Vishal Ahuja at the Westmead Hospital, NSW for providing me with *S. ratti* infected rat faeces throughout my study. Without your help I would not have been able to perform the majority of my tests.

To Professor Robert W. Baird and Dr Richard Sullivan at the Royal Darwin Hospital, Darwin Pathology, NT and Dr Gemma Robertson at the Health Support Queensland, QLD for sending me human *S. stercoralis* DNA samples and infected human poo. Again, faeces of all kind were the key of my research and without it my PhD would not be that fascinating.

Next, I would like to thank Fiona Smith, Aaron Clifford, Kiri Gould, Russel Spargo and Ryan McLean from the Environmental Health Branch at the Department of Health, NT for introducing me to the remote Indigenous communities and helping me dog poo hunting ☺.

My gratitude goes to the staff at the Animal Management in Rural and Remote Indigenous Communities, particularly Dr Jan Allen and Dr. Madeleine Kelso for sending me precious dog faeces. For that I would also like to thank Dr Ted Donelan from the West Arnhem Regional Council, Dr Stephen Cutter, Dr Bob Irving and Associate Professor Elizabeth Tudor.

Another special thanks goes to Dr Richard Bradbury for helping me to perform the work I was dreaming about from the start of my PhD. Thank you for having me as your guest researcher at the Centers for Disease Control and Prevention, helping me to settle down in Atlanta and introducing me to local Aussie delights ☺. Thanks to all very friendly and supportive staff at the CDC.

Further I would like to thank Professor Rebecca Traub for having me in your lab at the University of Melbourne and introducing me to the exciting world of hookworms. I will always remember my short but very enjoyable time in Melbourne working in your lab and being surrounded by very nice and friendly students of yours. Thank you for sharing your deep knowledge with me and your invaluable input in my thesis.

To my fellow post-graduate students and all the peeps at the Environmental Health lab, thank you guys for always being there for me, and your support, help, advice and friendship. I will forever remember our traditional morning tea and delicious snacks, Friday seminars and all round good times throughout the duration of my PhD.

Finally, a very special thanks goes to my family. Mom, dad, thank you for believing in me, without your support I could not be able to even start my PhD. To Olzhas, Ilmira and my sweet nephew Dimash, thank you for your love and support at all times. Thanks to my beautiful friends for listening to my moaning and complaints, for keeping up with me being so antisocial, thank you for your all time encouragement, understanding and friendship. To my other half, Harry, who got to see different me depending on the stage of my PhD. Thank you for being there for me at my wins and losses, sharing my happy moments and supporting me at difficult times. Thank you for teaching me to always think critically and being my main motivator to complete this thesis ☺.

Thank you all so very much!

STATEMENT OF CO-AUTHORSHIP

The following people contributed to the publication of the work undertaken as part of this thesis. The co-authors are listed in the order that the co-authored publications appears in the thesis

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All above listed contributions equated to no more than 25% of the work necessitated for publication of research manuscripts

PUBLICATIONS

Beknazarova, M, Barratt, JLN, Bradbury, RS, Lane, M, Whiley, H and Ross, K (2019), "Detection of classic and cryptic *Strongyloides* genotypes by deep amplicon sequencing: A preliminary survey of dog and human specimens collected from remote Australia communities", accepted for publication in *PLoS Neglected Tropical Diseases*, available as a preprint at [doi: https://doi.org/10.1101/549535]

Beknazarova, M, Whiley, H, Judd, AJ, Shield, J, Page, W, Miller, A, Whittaker, M and Ross, K (2018), "Argument for Inclusion of Strongyloidiasis in the Australian National Notifiable Disease List", *Tropical Medicine and Infectious Disease*, 3(2), 61 [10.3390/tropicalmed3020061]

Beknazarova, M, Whiley, H and Ross, K (2017). "Mass drug administration for the prevention human strongyloidiasis should consider concomitant treatment of dogs", *PLoS Neglected Tropical Diseases*, 11(8), [10.1371/journal.pntd.0005735] [Scopus]

Beknazarova, M, Millsteed, S, Roberston, G, Whiley, H and Ross, K (2017). "Validation of DESS as a DNA preservation methods for the detection of *Strongyloides* spp in canine faeces", *International Journal of Environmental Research and Public Health,* 14(6) pp. Art: 624 [10.3390/ijerph14060624] [Scopus]

Beknazarova, M, Whiley, H and Ross, K (2016). Advocating for both Environmental and Clinical Approaches to Control Human Strongyloidiasis. *Pathogens,* 5(4) pp. aRT: 59. [10.3390/pathogens5040059] [Scopus]

Beknazarova, M, Whiley, H and Ross, K (2016). "Strongyloidiasis: A Disease of Socioeconomic Disadvantage", *International Journal of Environmental Research and Public Health,* 13(5) pp. Art: 517. [10.3390/ijerph13050517] [Scopus]

Whiley, H, Ross, K, **Beknazarova, M**, Strongyloidiasis Is a Deadly Worm Infecting Many Australians, yet Hardly Anybody Had Heard of It. *The Conversation*, 5 September 2017. Available online: https://theconversation.com/strongyloidiasis-is-a-deadly-worm-infecting-many-australians-yet-hardly-anybody-has-heard-of-it-81687

This thesis is based on the published manuscripts, therefore some repetition between chapters occurs

The thesis was written in Australian English; however, words in American English will appear in some chapters that include published manuscripts due to the language style used by the journal.

1. INTRODUCTION

This chapter provides background information on the gastrointestinal parasitic nematode, *Strongyloides stercoralis* and introduces topics explored in more detail throughout the thesis. It includes two articles, which have been published in peer reviewed journals. The first, describes strongyloidiasis prevalence worldwide and factors associated with the disease, including the influence of a community's socioeconomic status and the associated sanitation hardware. The second publication discusses existing treatment options for *S. stercoralis* including anthelminthic drugs and nematicides. The effectiveness, limitations and areas for future research are discussed. This article highlights the benefits of a combined approach for controlling *Strongyloides* spp. that includes both clinical treatment and environmental control methods.

As this study progressed and the zoonotic potential of *S. stercoralis* was explored, the study was expanded to examine hookworms. These zoonotic parasites found in dogs and are introduced in this chapter and explored in more detail in chapter 6.

1.1 *Strongyloides stercoralis*

Strongyloides is a genus of parasitic nematodes that has been estimated to include 52 species of gastrointestinal parasites of different vertebrates (Speare, 1989). *Strongyloides* has a more complex life cycle compared with other nematodes. Its life cycle consists of two phases; a free-living and a parasitic phase. In the free-living phase there are male and female worms, while the parasitic stage has only female larvae (Schad, 1989). Human *Strongyloides* include S*. stercoralis*, *Strongyloides fuelleborni* subsp. *fuelleborni* and *Strongyloides fuelleborni* subsp*. kellyi* (Grove, 1996). While *S. stercoralis* is distributed worldwide, *S. f. fuelleborni* has only been reported in Africa and Southeast Asia and *S. f. kellyi* in Papua New Guinea (PNG) (Thanchomnang et al., 2017, Pampiglione and Ricciardi, 1971, Ashford et al., 1992). It is estimated that *S. stercoralis* infects up to 370 million people globally (Olsen et al., 2009).

1.1.1 Life cycle

S. stercoralis has a unique life cycle comprising of parasitic and free-living phases (Streit, 2008, Schad, 1989) (Figure 1.1). A parasitic phase starts when infective filariform larvae penetrates the skin, enters the circulatory system and travels to the lungs via the blood, from where it is swallowed into the gastrointestinal organs (Ericsson et al., 2001, Mansfield et al., 1995). This is however the traditional pathway of larvae migration. Evidence suggests that random migration of *S. stercoralis* larvae to reach the intestine is also possible even at an early stage of infection (Schad et al., 1989). Inside the small intestine, female larvae moult twice to become adult female worms, which produce eggs via parthenogenesis yielding rhabditiform larvae. These can then be either excreted in stool or become infective filariform larvae in the large intestine through autoinfection (Lim et al., 2004). A

unique feature to *S. stercoralis,* unlike other soil-transmitted helminths, is that larvae can persist indefinitely inside a host by means of asexual reproduction through parthenogenesis and autoinfection (Streit, 2008, Greiner et al., 2008).

The free-living phase of the life cycle occurs when rhabditiform larvae is defecated into the soil and undergoes either direct (homogonic) or indirect (heterogonic) development. In the direct development pathway, excreted rhabditiform larvae moult two times to become infective filariform larvae. In the indirect development pathway rhabditiform larvae moult four times to develop into free-living adult male and female worms. Adult worms reproduce sexually, producing eggs, which hatch into rhabditiform larvae. These then develop into infective filariform larvae. The free-living phase lasts only one generation, where homogonically produced larvae live a short life of 14 days, while heterogonically developed larvae have been shown to survive for longer periods in optimal environment conditions (Streit, 2008, Grove, 1989).

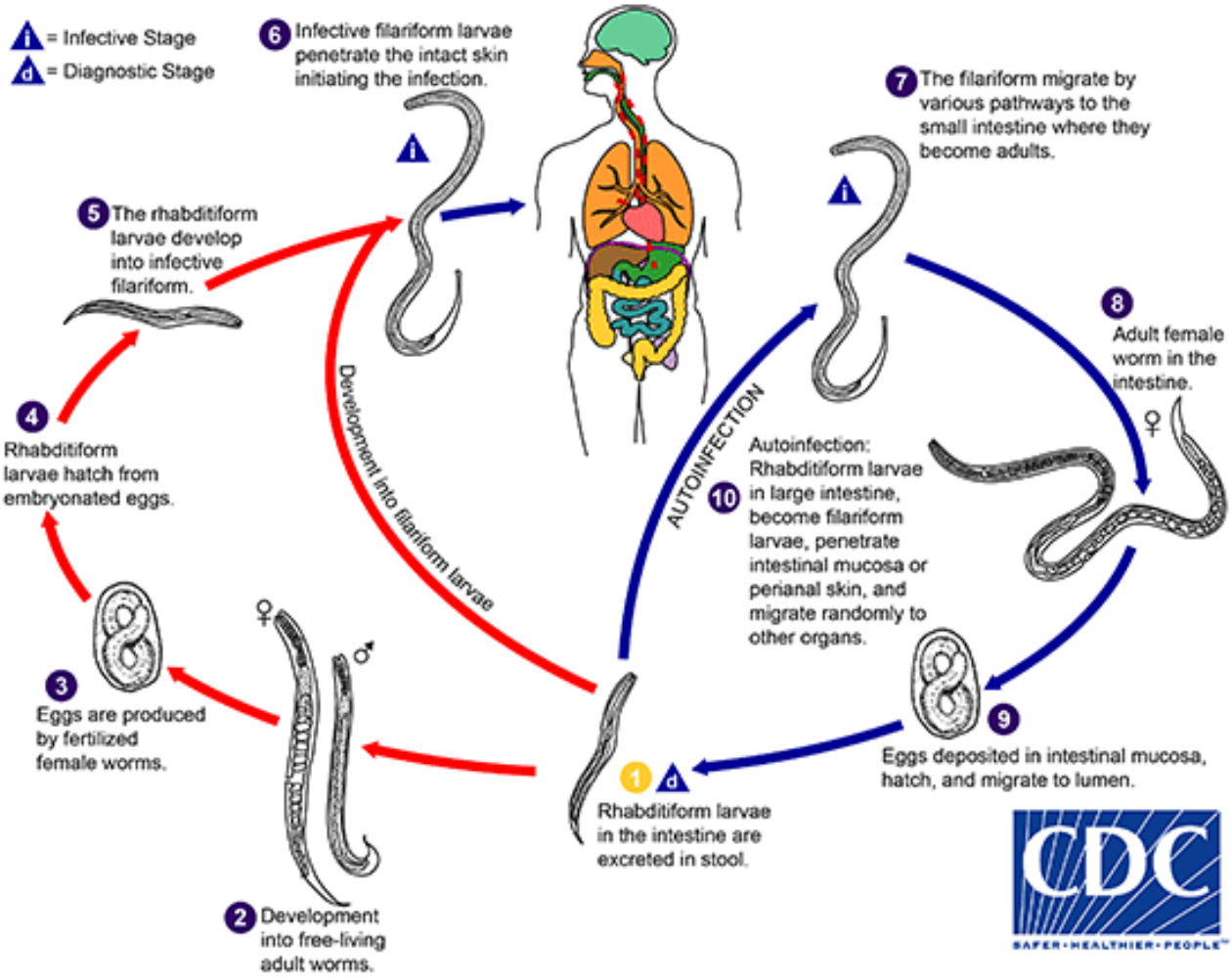

Figure 1.1 The life cycle of *Strongyloides stercoralis* (adapted from the CDC website)

1.1.2 Clinical outcomes and pathology

There are three forms of strongyloidiasis; chronic, hyperinfective, and disseminated. In immunocompetent people, strongyloidiasis takes a chronic form with symptoms ranging from none to mild that include respiratory, gastrointestinal, and skin disorders (Grove, 1995, Caruana et al., 2006). Due to its autoinfective nature, the chronic disease can be lifelong with one remaining larva being able to reproduce and cause recrudescence (Ericsson et al., 2001).

In immunocompromised and/or immunosuppressed people, or those receiving corticosteroid treatment, the infection can develop into more severe forms. Hyperinfective syndrome is where larvae reproduces to high numbers often causing pulmonary, gastrointestinal and/or neurological disorders (Grove, 1996). Disseminated strongyloidiasis is a form where larvae and bacteria spread to other parts of the body causing tissue damage and organ failure. Both severe forms are clinically important and have high mortality rate of up to 90% if not treated (Ericsson et al., 2001, Croker et al., 2010, Fardet et al., 2007, Marcos et al., 2008).

1.2 Strongyloidiasis: A disease of socioeconomic disadvantage

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Academic Editor: Anthony R. Mawson

Received: 19 April 2016; Accepted: 18 May 2016; Published: 20 May 2016

This article has been published in:

International Journal of Environmental Research and Public Health **(2016)** 13(5)

Doi: https://doi.org/10.3390/ijerph13050517

Keywords: *Strongyloides*; *S. stercoralis*; strongyloidiasis prevalence; global; socioeconomic status

1.2.1 Abstract

Strongyloidiasis is a disease caused by soil transmitted helminths of the *Strongyloides* genus. Currently, it is predominately described as a neglected tropical disease. However, this description is misleading as it focuses on the geographical location of the disease and not the primary consideration, which is the socioeconomic conditions and poor infrastructure found within endemic regions. This classification may result in misdiagnosis and mistreatment by physicians, but more importantly, it influences how the disease is fundamentally viewed. Strongyloidiasis must be first and foremost considered as a disease of disadvantage, to ensure the correct strategies and control measures are used to prevent infection. Changing how strongyloidiasis is perceived from a geographic and clinical issue to an environmental health issue represents the first step in identifying appropriate long term control measures. This includes emphasis on environmental health controls, such as better infrastructure, sanitation and living conditions. This review explores the global prevalence of strongyloidiasis in relation to its presence in subtropical, tropical and temperate climate zones with mild and cold winters, but also explores the corresponding socioeconomic conditions of these regions. The evidence shows that strongyloidiasis is primarily determined by the socioeconomic status of the communities rather than geographic or climatic conditions. It demonstrates that strongyloidiasis should no longer be referred to as a "tropical" disease but rather a disease of disadvantage. This philosophical shift will promote the development of correct control strategies for preventing this disease of disadvantage.

1.2.2 Introduction

Strongyloidiasis is an underestimated disease caused by *Strongyloides stercoralis* and *Strongyloides fuelleborni*, two species of soil-transmitted helminths of the genus *Strongyloides* (Grove, 1995, Olsen et al., 2009). While *Strongyloides fuelleborni* is found sporadically in Africa and Papua New Guinea, *Strongyloides stercoralis* is distributed worldwide and clinically important (Grove, 1995). Rhabditiform larvae of *S. stercoralis* are excreted in human feces, from where they develop into infected filariform larvae and can either repenetrate the intestinal mucosa and remain in the human organism, or distribute environmentally to new human hosts. A new host becomes infected with filariform larvae through intact skin penetration (Ericsson et al., 2001). Strongyloidiasis can cause gastrointestinal symptoms, including abdominal pain, diarrhea, nausea and vomiting, skin problems including pruritus and dermatitis or respiratory symptoms such as cough, asthma and dyspnea (Caruana et al., 2006, Hochberg et al., 2011, Mascarello et al., 2011). Hyperinfection or disseminated strongyloidiasis can affect several organs, leading to fatal outcomes (Grove, 1995, Croker et al., 2010). Chronic asymptomatic strongyloidiasis is another significant concern, as when coupled with immunosuppressive treatment, it has potential to develop into disseminated infection (Seybolt et al., 2006).

Currently, strongyloidiasis is predominately described as a neglected tropical disease, found in tropical and subtropical areas (Southeast Asia, Africa, Central and South America) (Genta, 1989a, Lim et al., 2004, Ahmad et al., 2013, Repetto et al., 2013, Toma et al., 2000, Uparanukraw et al., 1999). Whilst these papers do not often include clear climate-area classifications, it seems inappropriate that the primary disease descriptor focuses on geographic and climate conditions. Recent studies have included countries of the temperate zones in the endemic areas for strongyloidiasis (Buonfrate et al., 2012, Einsiedel and Spelman, 2006, Steinmann et al., 2007, Cabezas-Fernández et al., 2015, Valerio et al., 2013).]. There are also cases of strongyloidiasis in some parts of the same climatic zone but not in others (Fisher et al., 1993, Russell et al., 2014, Peeters et al., 2010, Prociv and Luke, 1993). This indicates that climatic conditions are not the primary factors determining the disease presence. Few studies mentioned low sanitation and socioeconomic status of communities as a risk factor for strongyloidiasis, and those that did not examine socioeconomic and sanitation conditions in any depth (Schär et al., 2013b, Russell et al., 2014).

This review explores the global prevalence of strongyloidiasis in relation to its presence in subtropical, tropical and temperate climate zones with mild and cold winters, but also explores the corresponding socioeconomic conditions of these regions. The review demonstrates that classifying the disease as “tropical” is misleading and runs the risk that physicians in other countries may not recognize this pathogen, resulting in misdiagnosis or mistreatment of the disease (Buonfrate et al., 2012, Roberts et al., 2013), but most importantly it influences how the disease is fundamentally viewed. Correct classification and perception of the strongyloidiasis is crucial, as it determines what strategies and control measures are used to prevent the infection. Considering the disease as an environmental health issue than a clinical issue based on geography would provoke a shift from drug administration to environmental health controls. Clinical treatment of strongyloidiasis will not always be effective (Toma et al., 2000, Molento, 2009). Anthelminthic drugs do not prevent reinfection, and can also cause adverse health effects (Zaha et al., 2002, Marti et al., 1996). Additionally, resistance to ivermectin (the primary drug used to treat strongyloidiasis) has already been found in *Strongyloides* spp. infecting ruminants (Maroto et al., 2011),], suggesting that resistance to ivermectin in *S. stercoralis* is likely in the future. Therefore, environmental health interventions represent a safer and more effective way of infection treatment. It was more than twenty years ago that Grove (Grove, 1990) noted that controlling *Strongyloides* in the environment is the most effective way to reduce infection. He pointed out that installation of adequate waste disposal systems was the most effective method to control the nematode(Grove, 1990), although this has not become the primary approach to addressing the disease. A major step towards reducing *Strongyloides* infection is to change the global perception of strongyloidiasis as a neglected tropical disease to recognition that it is primarily a disease of disadvantage and poor sanitation.

The aim of the current review was to assess the global prevalence of *S. stercoralis* to determineprevalence in geographic locations or climate zones, and compare these with

socioeconomic status and poor infrastructure of the communities. The review demonstrates that strongyloidiasis should no longer be referred to as a “tropical” disease but rather a disease of disadvantage. This philosophical shift will promote the development of correct control strategies for preventing the disease.

Studies that collectively demonstrate the global distribution of *S. stercoralis* are presented in Table 1.1 Studies were collated using the Google Scholar and PubMed journal databases and the key words *Strongyloides*, *S. stercoralis*, strongyloidiasis, global, socioeconomic, status. Only studies from 1990–2016, written in English or Russian, with *S. stercoralis* prevalence percentage and details on population studied were included in the review. Reference lists of the collected studies were also examined and relevant articles have been reviewed.

Table 1.1 Global *Strongyloides stercoralis* prevalence distribution

#	Most likely infective source	Climate classification	Population studied	SES	*S. stercoralis* prevalence (%)	Type of detection	Symptoms diagnosed	Comments/details	Reference
1.	East Africa	Group A, C, B*	Immigrants (≥16) lived in the refugee camps, Melbourne community health center and clinic patients	Developing economy 1**	11% (14/124)	Serology	Fever (34%), Stomach pain (30%), weight loss (25%), and diarrhea (13%)	Arrived to Australia, Melbourne between 1997-2000	(Caruana et al., 2006)
2.	Cambodia	Group A	Immigrants and refugees (≥15), Melbourne community health center and clinic patients	Developing economy, 1	42% (97/230)	Serology	Not reported	Arrived to Australia, Melbourne between 1974-2002	(Caruana et al., 2006)
3.	Laos	Group A	Immigrants (≥18)	N/a	24% (22/93)	Serology	75% (60/80) had previously worms, not known symptoms	Arrived to Australia, Melbourne between 1980-1989	(De Silva et al., 2002)
4.	Brazil (North, Northeast, Midwest, Southeast, South)	Group A, C	General population	Developing economy, 3	5.5% 21.7% 29.2%	Stool examination Serology (IFAT) Serology (ELISA)	Not reported	Study conducted from 1990 to 2009	(Paula and Costa-Cruz, 2011)
5.	Mexico, Honduras, Ethiopia, El Salvador, Zambia, Argentina, Congo, Cuba, Grenada, Guatemala,	Group A, C, B	HIV-positive immigrants (≥17)	Developing economy, 1,2,3	26% (33/128)	Serology	Weight loss (53%), diarrhea (48%), fatigue (42%) and abdominal pain (36%).		(Hochberg et al., 2011)

	India, Kenya, Niger, Tanzania, Vietnam								
6.	Africa, Central/South America, Thailand, India, UAE	Group A, B, C	HIV-positive immigrants (≥18), Italian hospital patients	Developing economy, 1,2,3,4	11% (15/138)	Serology	Skin problems (16.7%), gastrointestinal symptoms (15%) respiratory problems (14%)	Study conducted from 2000 to 2009	(Mascarello et al., 2011)
7.	Sub-Saharan Africa	Group A, B, C	Immigrants, Royal Melbourne Hospital, Infectious disease clinic patients	Developing economy, 1	1.4% (2/145) 17.9% (32/179)	Stool examination Serology	Not reported	Study conducted from 2003 to 2006	(Gibney et al., 2009)
8.	China, southern Yunnan province ***	Group A	Local rural inhabitants, random population sample	Developing economy, 3	11.7% (21/180)	Stool examination	Not reported		(Steinmann et al., 2007)
9.	Northern Ghana	Group A	Local inhabitants, random population sample	Developing economy, 2	11.6% (2349/20250)	Stool examination	Not reported	Study conducted from 1995 to 1998	(Yelifari et al., 2005)
10.	Northern Thailand	Group A,C	Local inhabitants excluding pregnant, lactating or with heart diseases	Developing economy, 3	15.9% (114/697)	Stool examination		Study conducted from April 2004 to September 2004	(Nontasut et al., 2005)
11.	Appalachia regions, Kentucky, US ***	Group C,D	Local inhabitants, clinic patients	Developed economy, 4	1.9% (7/378)	Serology	Not reported	All used outdoor toilet	(Russell et al., 2014)
12.	Spain, Barcelona	Group C	Immigrants from endemic areas, few locals	Developed economy, 4	17.7% (33/190) 46% (33/71)	Stool examination Serology	Gastrointestinal symptoms (64%), dermatologic symptoms (32%), neurologic symptoms (1%)	Study conducted from 2003 to 2012	(Valerio et al., 2013)

13.	Cambodia	Group A	Refugees	Developing economy, 1	24.7% (40/162) 77.2% (125/162)	Stool examination Serology	Not reported	Arrived to Canada between 1982 and 1983	(Joseph et al., 1995)
14.	Spain, Valencia, Gandia ***	Group C	Local farm workers, random population sample from the tourist area	Developed economy, 4	12.4 % (31/250)	Stool examination (agar plate culture)	Gastrointestinal symptoms, skin symptoms (no predominance among the infected group)	No information obtained on travelling details	(Roman-Sanchez et al., 2003)
15.	Africa	Group A, B, C	Sudan refugees Somali Bantu refugees	Developing economy, 1	46% (214/462) 23% (23/100)	Serology Serology	Chronic abdominal pain (not associated with the infection prevalence)	Resettled in the US in previous 5 years	(Posey et al., 2007)
16.	Jamaica	Group A	Clinical strongyloidiasis patients and controls (neighboring households)	Developing economy, 3	8.2% (17/207) 30% (62/207)	Stool examination Serology	Not reported		(Robinson et al., 1994)
17.	Far East and Southeast Asia	Group A, C, D	Former WWII Far East prisoners, diagnosed with strongyloidiasis and controls	Developing economy, 2,3	12% (248/2072)	Stool examination and serology	Larva currens rash (70%)	Study conducted from 1968 to 2002, Liverpool, UK	(Gill et al., 2004)
18.	Sub-Saharan Africa, Maghreb and Latin America	Group A, B, C	Immigrants, strongyloidiasis patients	Developing economy, 2,3	90.4% (284/314) 22.9% (67/293)	Serology Stool examination	Gastrointestinal symptoms (abdominal pain, diarrhea, pruritus	Study conducted from 2004 to 2012, Southern Spain	(Cabezas-Fernández et al., 2015)

19.	Africa, Eastern Europe, Southeast Asia, South America, the Caribbean, and the Middle East	Group A, B, C	Refugees	Developing economy, economy in transition, 1,2,3	39% (45/119)	Serology	Asymptomatic	Boston, Massachusetts	(Seybolt et al., 2006)
20.	Southeast Asia (Kampuchea, Laos, Vietnam	Group A,C	Immigrants, random population sample	Developing economy, 2	64.7% (125/193) 25%	Serology Stool examination	Not reported	Quebec, Canada	(Gyorkos et al., 1990)
21.	Spain, Mediterranean coast,	Group C	Strongyloidiasis patients (ex and current farm-workers and family members), local inhabitants	Developed economy 4	0.9% (152/16607)	Stool examination (agar plate culture)	Asymptomatic (77%); Gastrointestinal symptoms (11%); cutaneous symptoms (4%); respiratory symptoms (1%); mixture of all the symptoms (7%)	Study conducted from 1990 to 1997, none travelled to the endemic areas	(Sánchez et al., 2001)
22.	Northeastern Thailand	Group A	Rural and urban population	Developing economy, 3	23.5% (289.8/1233)	Stool examination	Not reported	Study conducted from July to September 2002	(Jongsuks untigul et al., 2003)
23.	Australia, Northern territory ***	Group A	Royal Darwin Hospital patients	Developed economy, 4	33% (68/205)	Stool examination	Gastrointestinal symptoms (72%)	12 month study	(Fisher et al., 1993)
24.	India, Assam	Group A,B, C	Local inhabitants, random population sample	Developing economy, 2	8.5 % (17/198)	Stool examination	Gastrointestinal, respiratory and cutaneous symptoms (29%)	Locals are mostly farm-workers	(Devi et al., 2011)
25.	Malaysia	Group A	Orang Asli community	Developing economy, 3	0% (0/54) 31.5% (17/54) 5.6% (3/54)	Stool examination Serology PCR	Not reported		(Ahmad et al., 2013)

26.	Palestine, Gaza Strip, Beit Lahia	Group B	Local inhabitants, random population sample, 3-18 years	N/a	5.6% (90/1600)	Stool examination	Not reported	Agricultural region	(Alzain, 2006)
27.	Brazil, Bahia	Group A, C	AIDS Clinic patients, HIV positive and negative groups, random population sample	Developing economy, 3	1.05% (59/5608)	Stool examination	Gastrointestinal symptoms among HIV positive	Study conducted from 1997 to 1999	(Feitosa et al., 2001)
28.	Argentina (North) ***	Group C	Local patients at the hospital	Developing economy, 3	29.4% (67/228)	Stool examination	Not reported		(Krolewiecki et al., 2010)
29.	U.S.	Group B, C, D	Cancer treated patients	Developed economy, 4	0.25% (25/10000)	Stool examination	Fever (28%), gastrointestinal symptoms (68%), pruritic skin rash,	Cases between 1971 and 2003 22/25 are US residents	(Safdar et al., 2004)
30.	Northeast Thailand	Group A	Local rural inhabitants	Developing economy, 3	28.9% (96/332) 47.5% (57/120)	Stool examination Serology	Not reported	Study conducted between October-November 2000	(Sithithaworn et al., 2003)
31.	Africa (48%), Asia (34%), Caribbean (20%), South America (3%)	Group A, B, C	Immigrants from endemic countries, travelers, Hospital for Tropical Diseases patients	Developing economy, 1,2,3	53.1% (102/192) 94.6% (157/166)	Stool examination Serology	Bowel upset, gastrointestinal symptoms, skin symptoms	Study conducted between 1991 and 2001, London	(Sudarshi et al., 2003)
32.	Bangladesh, Dhaka	Group A, C	Local inhabitants of a slum	Developing economy, 1	23.1% (34/147) 10.2% (15/147) 61.2% (90/147)	Stool examination Stool examination (agar plate culture) Serology	Diarrhea (19%)	Study conducted from November 2009 to January 2010	(Sultana et al., 2012)
33.	Nigeria, Ilorin	Group A	HIV clinics patients, HIV seropositive and seronegative patients	Developing economy, 2	12.2% (22/180)	Stool examination	Not reported		(Babatunde et al., 2010)

34.	Southeastern Brazil, Uberlandia	Group A, C	Elderly, randomly selected from nursing homes and non-institutionalised	Developing economy, 3	5% (10/200)	Stool examination	Asymptomatic		(Naves and Costa-Cruz, 2013)
35.	Australia, Queensland, Doomadgee ***	Group B, C	Children in aboriginal communities	Developed economy, 4	27.5% (92/334)	Stool examination	Not reported	During the wet season	(Prociv and Luke, 1993)
36.	Northern Cambodia	Group A	Local inhabitants, random population sample	Developing economy, 1	44.7% (1071/2396)	Stool examination	Not reported	Farmers (48.5%), pupils (33%)	(Khieu et al., 2014)
37.	Kazakhstan ***	Group D	Adopted children, lived in orphanage	Economy in transition, 3	42.8 % (3/7)	Serology	Not reported	Study in Belgium	(Peeters et al., 2010)
38.	USSR, North Caucasus ***	Group D	Local inhabitants, random population sample	Developing economy, 2	0.77% (89/11530)	Stool examination	Not reported		(Prokhorov and Golovan, 1983)
39.	Japan, Okinawa ***	Group B	Local hospital patients	Developed economy, 4	3.4% (113/3292)	Stool examination (agar plate culture)	Not reported	*S. stercoralis* is higher in *B.hominis* infected, the last is indicator for poor hygiene	(Hirata et al., 2007)

* Group A - tropical moist climate
Group B - subtropical, dry climate
Group C - subtropical, mediterranean, moist mid-latitude climates with mild winters
Group D - continental, moist mid-latitude climates with cold winters
Group E - polar climate
Group H - highland climate

** 1 - low-income
2 - lower-middle-income
3 - upper-middle-income
4 - high-income

*** showed on a map as a star "*" sign

1.2.3 Global prevalence of strongyloidiasis and climate classification

Table 1.1 summarizes the information available on infection prevalence, population studied, country as the most likely infective source, climate and socioeconomic status of the country, type of the infection detection, presence of symptoms and the study reference details. The table indicates that all cases of strongyloidiasis occur in the following communities: poor communities, former war veterans, immigrants and travelers, immunocompromised populations, or groups occupationally exposed to soil.

The climate classification used in this review is Koppen climate classification system, which divides the world's climate into six major climate groups each containing several subgroups (Pidwirny, 2011). Using the complete range of Koppen climate categories, about 80% of all the world areas falls into either tropical or subtropical zones (Pidwirny, 2011). This justifies the classification of strongyloidiasis as a "tropical" or "subtropical" disease, but lacks any meaning or association. Based on the major Koppen climate categories, the infection is still prevalent in other climate zones apart from tropical or subtropical ones (Figure 1.2). Certain areas of countries with *Strongyloides stercoralis* cases are shown as a "star" sign on the map.

Figure 1.2 presents a world map divided into tropical and subtropical zones with the strongyloidiasis case countries/areas coloured in blue. It can be seen that strongyloidiasis is highly prevalent in subtropical and tropical regions representing mostly developing countries with low socioeconomic status. Cases outside the tropical or subtropical areas correspond to more economically developed countries, but socioeconomically depressed communities (e.g., the Appalachia region population in the U.S., former USSR countries). This emphasizes that socioeconomic factors are more important than climatic conditions in defining the disease. The remaining cases presented are in risk groups of developed economy countries such as former war veterans, refugees, immigrants and travelers, immunosuppressed people or current or ex-farmers and their families, also identified by Schär et al. (Schär et al., 2013b).

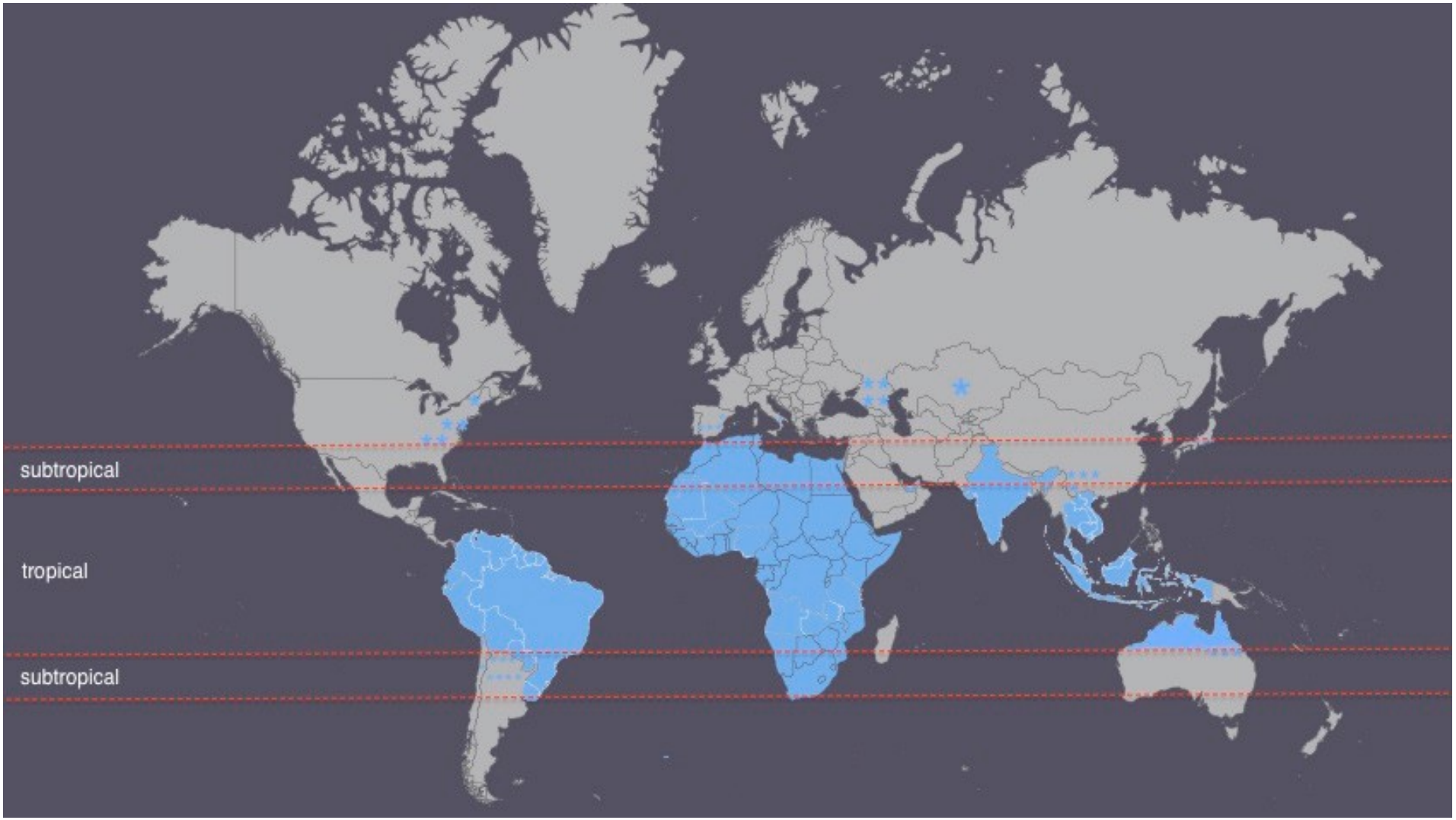

Figure 1.2 Countries with *Strongyloides stercoralis* cases (coloured blue or marked as a “star” sign) on a world map divided into tropical and subtropical zones

1.2.4 Countries of strongyloidiasis prevalence and socioeconomic status

1.2.4.1 Socioeconomic status of the strongyloidiasis case communities in subtropical and tropical zones (hyperendemic)

The socioeconomic status of the countries are presented in Table 1.1, based on their economy status and the income using World Bank data and the United Nations "*World's Economic Situation and Prospects 2016*"report (UN, 2016, Pasquali, 2015). It is globally accepted that an area with *S. stercoralis* prevalence of more than 5% is considered hyperendemic (Grove, 1989). From Table 1.1 it can be seen that almost all the reported countries are shown to be hyperendemic for strongyloidiasis, with exception of the Appalachia region in the U.S., Okinawa in Japan and North Caucasus in the former USSR. The reported endemic areas for strongyloidiasis (Southeast Asia, Africa, Central and South America) are mostly countries with developing economies, as can be seen in Table 1.1. Socioeconomic inequalities result in poor sanitation and hygienic conditions, which act as a triggering factor for the pathogen infection (Steinmann et al., 2007). The lifecycle of *S. stercoralis* and a mode of infection transmission justifies improper sanitation conditions are risk factors for infection (Ericsson et al., 2001). Increased urbanization processes happening in such countries cause inappropriate living conditions for the population such as 5–6 people living in one room and the use of one cubicle shower and a toilet (Paula and Costa-Cruz, 2011). It has been frequently shown that low socioeconomic status communities present higher mortality and morbidity rates compared to higher socioeconomic class population (Adler and Ostrove, 1999, Feinstein, 1993).

1.2.4.2 Socioeconomic status of the strongyloidiasis case communities in temperate zones

Apart from high prevalence strongyloidiasis cases detected in most of the subtropical and tropical countries in the world, cases with strongyloidiasis prevalence were also shown in some continental climate regions (Appalachia, North Caucasus, Kazakhstan). Although the study conducted in the North Caucasus does not meet the current review's criterion for the year of publication of papers, it is still included as not many studies from that area are available. North Caucasus has a continental climate and the study findings highlight that strongyloidiasis is not dependent only on climatic conditions (Prokhorov and Golovan, 1983). While moist and warm soil, enriched with nutrients are favourable conditions for the survival of free-living *S. stercoralis* larvae with further potential to infect a human host, the factors influencing direct or indirect development of infective filariform larvae (L3) are poorly understood (Grove, 1989, Grove, 1995). Previous reports have indicated that larvae cannot survive temperatures below 8 ˝C or above 40 ˝C (Farrar et al., 2013). However, studies have demonstrated *S. stercoralis* larvae surviving at lower temperatures infecting a human (Prokhorov and Golovan, 1983). Considering the parthenogenesis and autoinfection features of this nematode, the likelihood of the larvae remaining and reproducing within the host is high. In conditions of

inadequate sanitary and hygiene environment there is then a high risk of rhabditiform larvae excreted in stools passing to other human hosts.

As seen in Table 1.1, these regions belong to countries or a country with transitional or developed economies with the strongyloidiasis cases identified only in disadvantaged communities (Russell et al., 2014, Peeters et al., 2010, Prokhorov and Golovan, 1983). For example, rural Appalachia regions in Kentucky, West Virginia, Georgia and Tennessee in the United States are identified as areas with high infection prevalence among low socioeconomic status populations (Russell et al., 2014, Walzer et al., 1982). The *Strongyloides* infection case reported in Kazakhstan children were adopted children from orphanages, who probably were exposed to poor sanitary environments (Peeters et al., 2010). The study in the North Caucasus reported different levels of strongyloidiasis prevalence (0.1%–1.4%) in different areas with different temperatures (the lowest being 4ºC). Poor sanitary conditions were however reported in almost all the communities studied (Prokhorov and Golovan, 1983). These single *Strongyloides* infection cases occurred in areas of continental climate, where the precipitation level is low and temperatures go below zero, demonstrating that strongyloidiasis is not primarily influenced by climate conditions but rather sanitary and hygiene factors.

Australia is known to have tropical and subtropical climates, however, strongyloidiasis there is frequently found among indigenous communities and not the general population (Fisher et al., 1993, Prociv and Luke, 1993). Indigenous communities (Aborigines and Torres Strait Islanders) are identified as of a low socioeconomic status populations and are generally reported to live in poorer housing, sanitary and infrastructure conditions, which results in numerous worse health outcomes compared with non-indigenous Australians (Trewin and Madden, 2005).

1.2.4.3 Clinical treatment of and infrastructure, housing, and environmental health.

Currently, anthelminthic drugs (albendazole, mebendazole, and ivermectin) and nemiticides are used to treat the strongyloidiasis in humans (WHO, 2006). Treatment of soil-transmitted helminthiasis is difficult due to the development of resistance and facile reinfection from the environment. Among soil-transmitted helminth infections, strongyloidiasis is the most challenging to treat and clinically important because of a parasite's rhabditiform larvae unique ability of autoinfection (Toma et al., 2000, Molento, 2009, Olsen et al., 2009, Paula and Costa-Cruz, 2011). Moreover, parthenogenesis allows for a single female parasite remaining in a host to reproduce reinfecting that person (Grove, 1995). The drug treatment efficacy depends on number of factors including an individual's immune system status, co-infection with HTLV-1, history of drug use, and bowel ileus (Vadlamudi et al., 2006, Scowden et al., 1978, Carvalho and Da Fonseca Porto, 2004, Sultana et al., 2012). Furthermore, monitoring treatment efficacy has some difficulties associated with the low sensitivity of fecal examination (Toma et al., 2000). Additionally, the drugs can cause adverse effects, including liver disfunction, gastrointestinal symptoms (nausea, vomiting, loose stool, abdominal distension or pain),

chest tightness or pain, itching, fever, cough and wheezing, dizziness, and neurological effects (Shikiya et al., 1994, Zaha et al., 2002, Lichtenberger et al., 2009, Marti et al., 1996).

New anthelminthic drugs and nematicides have to be frequently introduced to the market due to quick resistance development in nematodes and great toxicity they produce to humans (Molento, 2009). Resistance in nematodes to different drugs has been studied and demonstrated frequently in the veterinary field in the last decades (Prichard, 1994, Coles et al., 2006, Kaplan, 2004). This suggests that human-infecting nematodes are also likely, at some stage in the future, to become resistant to the available drugs. Indeed, studies on some drugs used against human nematodes have already reported low drug treatment efficacy, calling for great attention and warnings of possible resistance development (Albonico et al., 2004, De Clercq et al., 1997). Although it is more difficult to study and confirm anthelminthic resistance in human parasites due to number of factors, the potential for resistance is mostly overlooked and should be more carefully examined in drug treatment application (Geerts and Gryseels, 2000).

It is well established that sanitary conditions, including housing and infrastructure, play the most vital role in determining health outcomes (Cassel, 1976, Audy and Dunn, 1974). Overcrowding, poor ventilation, bad living conditions and inadequate sewerage systems create higher risks for infectious and parasitic diseases such as skin infections, respiratory infections and diarrheal diseases (Waters, 2001). Thus, environmental health approaches such as ensuring better infrastructure and sanitation should be the primary approach to controlling *Strongyloides* in the environment. Only this approach will provide the most effective way of infection reduction.

Strongyloidiasis has been also reported in certain groups such as former war veterans, refugees, immigrants and travelers, immunocompromised people and people occupationally exposed to soil (Table 1.1). Poor sanitary and hygiene living conditions are common during times of war, which could explain cases of strongyloidiasis in former war veterans (Einsiedel and Spelman, 2006, Gill et al., 2004). The Okinawa Prefecture area of Japan was reported to have a high prevalence of *S. stercoralis* infections during World War II, which decreased to about 0.5%–1.5% after the war years. This was associated with improved sanitary conditions and systematic monitoring for parasitic diseases after the war (Zaha et al., 2000)[1].

Studies of refugees and immigrants with high *S. stercoralis* infection prevalence have demonstrated an association with inadequate sanitary and hygienic conditions in their home countries, including lack of an access to shower and toilet facilities (Caruana et al., 2006, Seybolt et al., 2006, Valerio et al., 2013, Gyorkos et al., 1990, Joseph et al., 1995, De Silva et al., 2002, Sudarshi et al., 2003, Posey et al., 2007, Gibney et al., 2009).

[1] The prevalence of *S. stercoralis* after World War II was reported as 0.5-1.5%; however the actual prevalence was believed to be up to 10%. Generally,

Individual health condition (immunosuppressed or immunocompromised status) is another risk factor influencing the disease (Hochberg et al., 2011). *S. stercoralis* is especially life-threatening to immunocompromised people due to possible development of the disseminated disease form (Hochberg et al., 2011, Seybolt et al., 2006) which approaches a 90% mortality rate (Igra-Siegman et al., 1981, Hochberg et al., 2011). The study by Zaha *et al.* (Zaha et al., 2000) demonstrated that there was a high prevalence of *S. stercoralis* among Human T-Lymphotropic Virus type I (HTLV-1) positive patients (17.5%) compared to HTLV-1 negative patients (6.7%). Schar *et al.* (Schär et al., 2013b) found an association between strongyloidiasis and HIV infection (OR: 2.17 BCI: 1.18–4.01) and alcoholism (OR: 6.69; BCI: 1.47–33.8). HTLV-1 and HIV infections and alcoholism have been associated with poverty (Rodrigo and Rajapakse, 2010, Fenton, 2004, Adler and Ostrove, 1999, Adler et al., 1994).

High prevalence of strongyloidiasis in subtropical South China has been reported by Wang *et al* (Wang et al., 2013). While the cases reported are within subtropical areas, they are mostly associated with the farming lifestyle in those regions and/or poor hygiene practices. The infection rates in these areas are as high as 11%–14% (Wang et al., 2013). The studies' findings are not included in Table 1.1 as the original papers are only available in Chinese. Similarly, studies in France and Spain (Sánchez et al., 2001, Roman-Sanchez et al., 2003, Magnaval et al., 2000) reported strongyloidiasis cases in local current or ex-farmworkers and their family members who have never travelled to endemic areas. While there is no available information on the income of the studied population, ingestion of non-potable water and possible infection transmission to family members due to unhygienic behavior is reported in one of the studies(Sánchez et al., 2001). This might indicate either inappropriate living conditions due to the depressed socioeconomic status in the area or population unawareness of proper hygienic and sanitary standards. On the other hand, in another study by Roman-Sanchez *et al.*[2] the assessed area (Gandia, Valencia), is reported to have the highest *per capita* income compared to other European Union regions, adequate hygiene-sanitary conditions and high prevalence of the strongyloidiasis. Whether the use of a more sensitive detection method, the agar-plate culture technique, compared to other studies impacted on this result cannot be known until several studies using the same detection tests are conducted. It can, however, be concluded that occupation is likely to contribute to acquiring the infection in this case.

Currently, it is estimated that between 30– 100 million people are infected by *Strongyloides* worldwide (Olsen et al., 2009, Bethony et al., 2006). There is however a general consensus amongst the scientific community that the prevalence is underestimated due to inadequate diagnostic techniques (Bethony et al., 2006), and the lack of sensitivity in tests for *S. stercoralis* and the similarity of its symptoms to other diseases result in great underestimation of the infection and 300 million people infected globally is probably a more accurate estimate (Krolewiecki et al., 2010,

[2] Roman-Sanchez *et al.* 2003

Taylor et al., 2014). Misclassification of the disease may also be contributing to the underestimation of its prevalence. Diagnostic test methods are presented in Table 1.1 for completeness.

1.2.5 Conclusions

It is well established that strongyloidiasis is mainly restricted to tropical and subtropical areas throughout the world. However, within these regions, exposure to infection with the helminth is strongly associated with poor sanitary and living conditions. Thus, immigrants, refugees, travelers, war veterans, immunocompromised and occupationally soil-exposed groups—and their family members—are at especially high risk of strongyloidiasis. This review emphasizes that strongyloidiasis is a disease of disadvantage, and suggests that control measures to prevent the infection should focus as much, or more, on changing the environmental conditions that increase overall risks of the disease, as on the medical treatment of infected persons, especially since the latter is ineffective in preventing reinfection and has the potential for the development of drug resistance.

1.2.6 Acknowledgments

We have received no funds to publish in open access.

1.2.7 Author contributions

BM, HW and KR conceived and participated in review design and coordination. BM drafted manuscript, and HW and KR provided academic input and all authors approved the final manuscript.

1.3 Advocating for both environmental and clinical approaches to control human strongyloidiasis

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Academic Editor: Lawrence Young

Received: 9 June 2016; Accepted: 28 September 2016; Published: 30 September 2016

This article has been published in:

Pathogens **(2016)** 5(4) DOI: https://doi.org/10.3390/pathogens5040059

Keywords: strongyloidiasis; *Strongyloides stercoralis*; anthelminthic drugs; nematicides; ivermectin; resistance

1.3.1 Abstract

Strongyloidiasis is an underestimated disease caused by the soil-transmitted parasite of the genus *Strongyloides*. It is prevalent in socioeconomically disadvantaged communities and it is estimated that global infection could be as high as 370 million people. This paper explores current methods of strongyloidiasis treatment, which rely on administration of anthelminthic drugs. However these drugs cannot prevent reinfection and drug resistance has already been observed in veterinary models. This highlights the need for a combined approach for controlling *Strongyloides* that includes both clinical treatment and environmental control methods. Currently, nematicides are widely used to control plant parasites. The review suggests that due to the species' similarity and similar modes of action, these nematicides could also be used to control animal and human parasitic nematodes in the environment.

1.3.2 Introduction

Strongyloidiasis is a disease caused by two soil-transmitted helminths of the genus *Strongyloides*, *Strongyloides stercoralis* and to a lesser extent *Strongyloides fuelleborni*. *S. fuelleborni* is only found in Africa and the Southeast Asian countries of Papua New Guinea[3], while *S. stercoralis* is globally distributed and is clinically more important (Grove, 1995, Olsen et al., 2009). Strongyloidiasis is an underestimated disease highly prevalent in socioeconomic disadvantage communities (Beknazarova et al., 2016b, Schär et al., 2013b). Although it is currently estimated that about 30-100 million people are infected globally, a more accurate estimate is thought to be around 300 million (Bethony et al., 2006, Olsen et al., 2009) or up to 370 million people (Bisoffi et al., 2013). Due to the ability of *S. stercoralis* to remain in a host organism as autoinfective filariform larvae (L3)[4], a person can stay asymptomatically infected for decades (Lim et al., 2004, Ericsson et al., 2001). Parthenogenesis allows a single remaining female parasite present in a host to reproduce and cause reinfection, which has serious ramifications for effective treatment (Grove, 1995, Streit, 2008, Keiser and Nutman, 2004). The survival of asexually produced infective larvae (L3) is estimated to be less than 14 days. However, heterogonically developed infective larvae (L3) have been shown to survive indefinitely in the soil of optimal environment conditions until they find a host (Grove, 1989, Toledo et al., 2015, Streit, 2008). The complicated *S. stercoralis* life-cycle, insensitivity of detection methods and social factors challenge strongyloidiasis identification, diagnosis and treatment (Beknazarova et al., 2016b, Requena-Méndez et al., 2013). If not diagnosed in time it can lead to fatal outcomes (Grove, 1995, Croker et al., 2010), and given that we see high disease rates in population sub-groups, strongyloidiasis is not only a personal but a public health issue (Muennig et al., 1999, Olsen et al., 2009). *Strongyloides* genus species have both parasitic and free-living life cycles. The infection of a human starts from infective larvae (filariform larvae L3), which penetrate the host and are

[3] Southeast Asian countries and Papua New Guinea
[4] Due to the ability of *S. stercoralis* to remain in a host organism continuing autoinfection

transported via blood to the lungs, from where the larvae migrate to the gastrointestinal tract. In the intestine, larvae moult two times to become adult female worms, which hatch eggs through parthenogenesis and produce rhabditiform larvae. The rhabditiform larvae can either be excreted in feces or become infective filariform larvae autoinfecting a host (Lim et al., 2004). Certain respiratory conditions; however, are believed to affect the filariform larvae transition through the lungs and cause its development into adult egg laying female worms in the lungs (Chu et al., 1990, Woodring et al., 1994). The repeated cycle of this leads to pulmonary strongyloidiasis (Oka et al., 2009). There are no further studies done showing the filariform larvae maturing into adult worm in the lungs. The pulmonary strongyloidiasis is believed to occur due to autoinfection and filariform larvae disseminating to respiratory system (Namisato et al., 2004). Currently, the nematode's environmental stage has not been extensively studied or controlled. However, exploring mechanisms to control the nematode in the environment should be made a priority. Biocontrol has been described as a promising way of environmental control of agricultural, animal and human soil-transmitted nematodes (Garrard et al., 2016), and the use of commercially available nematicides should be considered and explored.

At the World Health Organization global parasite control meeting in 2004 it was recommended that *S. stercoralis* control measures should be included in the health package for endemic areas (WHO, 2005). However, to date, there has been no progress made mostly due to the gaps in knowledge regarding *S. stercoralis* treatment and control (Taylor et al., 2014). Investigation into transmission hot-spots is currently being undertaken (Knopp et al., 2008). To address the transmission, the best management approaches need to be identified and this discussion represents a step in this process. For example, wastewater overflow in septic tanks, solid waste including diapers or other animal feces might be areas to target.

This paper reviews currently commercially available drugs used to treat human strongyloidiasis, and explores the main issues associated with drug application. In addition, this paper looks at nematicides registered in Australia, their use, main constituents, mode of action and toxic effects. To date, strongyloidiasis treatment has tended to be viewed only from a clinical perspective, which is an inevitable part of treatment once infection has occurred. However, drug treatment cannot prevent reinfection and there is the potential for drug resistance. Here we suggest a combined approach of strongyloidiasis treatment; through clinical intervention with drugs once infection has occurred, but supplemented with nematode control in the environment. The advantages and concerns with both approaches are discussed.

1.3.3 Anthelminthic drugs

The World Health Organization currently recommends albendazole and ivermectin as suitable drugs against strongyloidiasis. Mebendazole is not recommended anymore, as it has been demonstrated

to have a suboptimal effect against strongyloidiasis (Table 1.2) (WHO, 2006). Ivermectin has been shown to be the most effective and therefore the first choice drug in strongyloidiasis treatment (Toma et al., 2000), especially for chronic strongyloidiasis (Nontasut et al., 2005, Igual-Adell et al., 2004).

Table 1.2 WHO recommended anthelminthic drugs to treat strongyloidiasis

#	Drug name	Class (drench group)	Mode of action*	Resistance of gastrointestinal nematodes (veterinary studies)	Resistance of *Strongyloides* spp (veterinary studies)
1a	Albendazole	Benzimidazole, BZ, "white" (introduced in 1961)	Interaction with β-tubulin impairing cytoskeleton	1. Horse (97.7%) (Kaplan et al., 2004) 2. Sheep (71%) (Maroto et al., 2011)	1. Sheep (57%) (Maroto et al., 2011), Sheep (66.7%) (Mohamed and Al-Farwachi, 2008) 2. Horse (Molento, 2009)
1b	Mebendazole				
2	Ivermectin	Macrocyclic lactone, ML, "ectin" (introduced in 1980s)	Paralysis of pharyngeal and body wall musculature	1. Sheep (29%) (Maroto et al., 2011)	1. Sheep (43%) (Maroto et al., 2011)

* Source: (Holden-Dye and Walker, 2005)

1.3.3.1 Benzimidazoles (albendazole and mebendazole)

Benzimidazole is a group of anthelminthic drugs, which includes albendazole and mebendazole. They are shown to affect parasite locomotion and reproduction through action on the β-tubulin, compromising nematode's cytoskeleton by impairing glucose uptake (Venkatesan, 1998). Albendazole is poorly absorbed and a single dose is shown to have an efficacy rate of 62.2 % (Venkatesan, 1998, Horton, 2000).

1.3.3.2 Macrocyclic lactones (ivermectin)

Macrocyclic lactones (MLs), in which ivermectin is the only approved drug for use in humans, act on nematodes residing in mammals' gastrointestinal tract or lungs, inhibiting their capacity to move and feed, which results in their death (Geary et al., 2010). Ivermectin is a very effective drug against early and adult stages of gastrointestinal parasites, and less effective against adult stages of filarial nematodes (Geary et al., 2010). Macrocyclic lactones including ivermectin are known to react with a range of ligand-gated ion channels (α7 nACh receptors, acetylcholine-gated chloride channels, GABA-gated chloride channels, histamine-gated chloride channels, glycine receptors, and P2X4 receptors). The anthelminthic activity is shown by ivermectin interacting with glutamate-gated chloride channels (GluCl) in nematodes, increasing chloride permeability, which results in nematode paralysis (Cully et al., 1994, Ottesen and Campbell, 1994). Ivermectin is currently the best treatment for onchocerciasis and administered at intervals of one year in highly prevalent countries. While it is

also effective at treating other helminth infections, it is not available in the onchocerciasis-free areas and recommended to be substituted with diethylcarbamazine (WHO, 2006, Hotez, 2009). Due to the strong protein binding ability of ivermectin, its oral administration can be impaired in strongyloidiasis disseminated patients. There is, however, no parenteral administration of ivermectin licensed currently, which is essential in cases of disseminated strongyloidiasis (Hauber et al., 2005, Turner et al., 2005).

1.3.3.3 Anthelminthic drugs associated issues

Treatment of soil-transmitted helminthiasis is challenging due to development of resistance, as demonstrated in veterinary practice, and reinfection occurrence (Coles et al., 2006, Kaplan, 2004). Among soil-transmitted helminth infections, strongyloidiasis is the most difficult to treat because of its unique ability of autoinfection, especially in cases of hyperinfection or disseminated diseases (Toma et al., 2000, Molento, 2009, Olsen et al., 2009, Paula and Costa-Cruz, 2011, Grove, 1995). The drug treatment efficacy depends also on number of factors including an individual's immune system, co-infection with HTLV-1 and history of drug intake (Vadlamudi et al., 2006, Scowden et al., 1978, Carvalho and Da Fonseca Porto, 2004, Sultana et al., 2012). Fecal examination, traditionally used for monitoring treatment efficacy, is associated with low sensitivity. Although less available in low resource settings, serology tests are known for higher sensitivity and accuracy, and should be used for not only strongyloidiasis diagnosis but also follow-up tests (Buonfrate et al., 2015a, Buonfrate et al., 2015b).

The drugs, while reasonably well tolerated, can cause adverse effects including liver disfunction, gastrointestinal symptoms (nausea, vomiting, loose stool, abdominal distension or pain), chest tightness or pain, itching, fever, cough and wheezing, dizziness, and neurological effects (Shikiya et al., 1994, Zaha et al., 2002, Lichtenberger et al., 2009, Marti et al., 1996). Another issue with anthelminthic drugs is their teratogenicity potential in pregnant women who have a high risk of developing iron-deficiency anemia (de Silva et al., 1997).

Animal-infecting nematode resistance development results in need for new anthelminthic drugs to be introduced to the market. Nematode resistance to different drugs has been widely studied and demonstrated frequently in animal studies (Molento, 2009, Waghorn et al., 2006, Sutherland and Leathwick, 2011, Kaplan and Vidyashankar, 2012, Coles et al., 2006, Kaplan, 2004, Prichard, 1994). Resistance to the benzimidazole class of drugs has been shown to be up to 97.7% and 71% in gastrointestinal nematodes parasitizing horse and sheep respectively (Kaplan et al., 2004, Maroto et al., 2011). Resistance (66.7%) to benzimidazoles has been also determined in sheep *Strongyloides* spp. (Mohamed and Al-Farwachi, 2008). The most recently introduced anthelminthic drug, ivermectin, has been shown to be the most successful in helminth infection treatment with less resistance development compared with the benzimidazole drugs. Nevertheless, resistance has been

demonstrated in the last few years in gastrointestinal nematodes (29%), and sheep *Strongyloides* spp. (40%) (Maroto et al., 2011). Treatment of sheep parasites two times per year caused a rapid drug resistance development demonstrating that resistance can occur even in low frequency drug application (Besier and Love, 2004). This suggests that human-infecting nematodes are also likely, at some stage in the future, to become resistant to the available drugs. This is also induced by continuous use of a one drug family over the years, as in case of ivermectin against strongyloidiasis (Satoh et al., 1999). Studies on benzimidazole drugs against human nematodes have reported low efficacy of drug treatment, calling for great attention and warning for possible resistance development (Albonico et al., 2004, De Clercq et al., 1997). To date, human nematode studies with ivermectin have shown no resistance to the drug (Shikiya et al., 1994).

A little is understood in the mechanism of resistance development in *S. stercoralis* or other human parasites to anthelminthic drugs. Satoh at al. (1999) have found that *S. stercoralis* specific antibody, IgG4, is associated with both resistance to albendazole and elevated level of HLA-DRBI*0901, suggesting that patients should be tested for this antibody prior to drug treatment to check for their therapeutic effect on them. However, no other reports are available showing the association between increased level of IgG4 and resistance in a parasite (Satoh et al., 1999). A human immune system changes in response to strongyloidiasis infection, in particular T and B cells. The immune system has two responses to infective filariform larvae and host adapted larvae, which start autoinfection (Vadlamudi et al., 2006). There are two mechanisms of ivermectin resistance identified so far: alteration of the membrane transport protein called P-glycoprotein, which is responsible for the drug delivery to the cell membrane, and alteration of the Cl channel receptor (Xu et al., 1998, Prichard, 1994, James and Davey, 2009, Gilleard and Beech, 2011).

It is a risk for resistance development in response to large scale drug administration programs within the parasite control programs. The presence of a free-living stage of *S. stercoralis,* sexual reproduction, and relatively short lifespan and generation time could contribute to quicker drug resistance development in nematodes. Generally it is thought that if different drugs target and involve different receptors, their combined use will delay resistance development (Prichard, 2007). However, if resistance in two drugs involves same mechanism, combined drug treatment may be overlooked. ABC transporters have been shown to be involved in both ivermectin and albendazole resistance, which can potentially enhance the resistance development if both drugs are used for treatment (James and Davey, 2009). It has been shown in some nematodes that ivermectin selects on β-tubulin, which is a primarily receptor for albendazole (Prichard, 2007).

Although it is more difficult to study and confirm anthelminthic resistance in human parasites due to number of factors, the potential for resistance is mostly overlooked and should be more carefully examined in drug treatment application (Geerts and Gryseels, 2000). Notably, there are many gaps

identified in our understanding of the pharmacology of anthelminthic drugs despite the fact that that millions of people around the world are treated by these drugs (Geary et al., 2010).

Mass drug administration (MDA) is the main clinical approach to controlling highly prevalent neglected tropical diseases. Ivermectin, along with benzimidazole drugs, have been shown to be effective against intestinal helminth and schistosome infections. Coadministration of different anthelminthic drugs allows integrating control programs for intestinal helminth infections, lymphatic filariasis and onchocerciasis with schistosomiasis and food-borne trematode infections. However, MDA could be associated with a higher risk for resistance development, as more people are given the drug more often, including those that are no carrying disease. More research is required to study the long-term effects of repeated drug doses (Hotez, 2009, Smits, 2009).

1.3.4 Nematicides

Nematicides are used to control plant parasite nematodes, which are ubiquitous and globally cause costly yield loses in agriculture (Holden-Dye and Walker, 2005, Askary, 2015). To date, there have been limited studies demonstrating nematicides use on non-plant nematodes, as they are mostly treated by anthelminthic drugs. However, the mode of their action and species' similarity might allow using them on animal and human parasites.

According to the Australian Pesticides and Veterinary Medicines Authority there are currently around 20 registered nematicides to use in Australia with the four active compounds fenamiphos, fluensulfone, oxamyl and carbofuran (Table 1.3) (Authority, 2016). The active constituents of used nematicides are of organophosphorus, carbamate and thiazole chemical groups.

Table 1.3 Registered in Australia nematicides and their active constituents

#	Active constituent	Chemical group	No of registered nematicides	Mode of action
1 2 3	Fenamiphos Oxamyl Carbofuran	Organophosphorus Carbamate- methylcarbamate	14 1 2	Inhibition of cholinesterase
4	Fluensulfone	Thiazole	1	

1.3.4.1 Organophosphorus and carbamate nematicides (fenamiphos, oxamyl and carbofuran) mode of action

Organophosphates and carbamates are non-fumigant nematicides. Organophosphorus and carbamate nematicides (fenamiphos, oxamyl and carbofuran) cause the paralysis of nematodes through inhibition of cholinesterase enzymes, which are responsible for acetylcholine

neurotransmitter breakdown. Organophosphates and carbamates cause either irreversible or reversible inhibition of a cholinesterase enzyme blocking its function (Husain et al., 2010).

Not much research has been done on human parasitic nematodes including *S. stercoralis*; however, in *C. elegans*, acetylcholine is the neurotransmitter that controls nematode's movement, pharyngeal pumping, and egg laying. When acetylcholinesterase/cholinesterase suppressed, acetylcholine builds up, transmitting nerve impulses and causing constant muscle and nerve contraction leading to the nematode's exhaustion and tetany (Gupta, 2011). Oxamyl is known as a more effective nematicide than fenamiphos (Giannakou and Karpouzas, 2003).

1.3.4.2 Thiazole (fluensulfone) mode of action

Fluensulfone, a fluoroalkenyl thioether group drug, has different mode of action and effect on nematodes from those of organophosphorus and carbamate nematicides and also anthelminthic drugs such as ivermectin (Kearn et al., 2014). There have not been studies done describing its mode of action on nematodes. However, fluensulfone has shown to be highly effective against a number of plant nematodes (Kearn et al., 2014, Oka et al., 2009). In their study, Kearn et al (2014) have studied fluensulfone effect on *C. elegans,* a genetic nematode model to study effects of different anthelminthic drugs and nematicides that are used against animal and human parasites (Holden-Dye and Walker, 2005). It has been shown that a slightly higher dose of fluensulfone is required to have a similar effect on *C. elegans* as on plant parasite nematodes, inhibiting egg laying, hatching, development, feeding and moving stages of the nematode (Kearn et al., 2014).

1.3.4.3 Nematicides associated issues (toxic effects and resistance to nematicides).

Most cholinesterase inhibiting nematicides have been banned or restricted for use due to their adverse toxic effects on non-target organisms including humans, and the environment, which is associated with absence of species' selectivity (Husain et al., 2010, Holden-Dye and Walker, 2005). Another disadvantage of non-fumigant nematicides is their mobility in soil which can potentially cause widespread non-target toxic effects. Oxamyl and fenamiphos are known for leaching from the site of application (Zaki et al., 1982, Bilkert and Rao, 1985). Carbofuran has been banned for use in European Union in 2009 (Regulation 1107/2009), Canada and U.S. due to its adverse side-effects (Otieno et al., 2010).

A study on fenamiphos, oxamyl and carbofuran effects on *C. elegans* has shown AChE recovery ability by nematodes in response to all the three nematicides. It has been also shown that only small recovery of the enzyme is required for nematode moving restoration and normal behaviour (Opperman and Chang, 1991).

There are currently no studies available on the non-specific toxicity of fluensulfone. However, the acute LD_{50} value for rats via for oral administration of fluensulfone is much lower compared with organophosphate nematicides (Oka et al., 2009, WHO, 2013).

It is commonly thought that nematicide resistance for plant nematodes is not as great a concern as for animal nematodes, hence there are limited studies exploring potential plant nematodes' resistance compared to the numerous studies on animal nematodes' resistance. It is thought that there is a lesser potential for the development of plant nematicide resistance due to number of factors. These include: nematicides altering the selection pressure on plant parasitic nematodes, mitotic parthenogenesis in plant nematodes leading to less genetic diversity, and biodegradation of nematicides by soil bacteria (Silvestre and Cabaret, 2004). However, these factors can probably delay but not prevent resistance development. It is known that resistance is more likely to develop with persistent compounds such as organophosphorus and carbamate substances rather than short-lived molecules (Dobson et al., 1996). Plant nematodes have been shown to be quite adaptive to chemical treatment. *Rhabditis oxycerca, Criconemella xenoplax, Xiphinema index, Meloidogyne incognita and Pratylenchus vulnus* have developed high resistance to organophosphates and carbomates after long-term exposure (Below et al., 1987, Glazer et al., 1997).

1.3.5 Conclusions

While nematicides are extensively used against plant nematodes, their use is limited or non-existent in human parasite control. An overlooked environmental approach in strongyloidiasis control is to kill free-living parasites in environment before they get into a human host.

Above we have assessed commercially available drugs used to treat strongyloidiasis and explored the main issues associated with these drug treatments. This includes the emergence of drug resistance in numerous animal nematodes when applied in veterinary practice. This highlights the potential for resistance in human helminths, which is a particular problem for *S. stercoralis* as currently there is only two drugs approved for human treatment. Other issues with treatment include drugs' inability to prevent reinfection, and potential for problems associated with drug administration during pregnancy. Nematicides have potential to be used on free-living *Strongyloides* nematodes. A combined approach to fight strongyloidiasis should consider environmental control as well as drug treatment. Future studies could consider focusing initial efforts on the nematicide fluensulfone, which has been shown to have the least toxic effect on the environment and non-target species, and desirable effects on all the stages of a nematode, as demonstrated by a model parasite, *C. elegans.*

1.3.6 Acknowledgments

We have received no funds to publish in open access.

1.3.7 Author contributions

Meruyert Beknazarova, Harriet Whiley and Kirstin Ross conceived and participated in review design and coordination. Meruyert Beknazarova drafted manuscript, and Harriet Whiley and Kirstin Ross provided academic input and all authors approved the final manuscript.

1.4 *Strongyloides stercoralis* in Australia

Strongyloies stercoralis is believed to have been present in Australia prior to European settlement (Johnston, 1916, Nicoll, 1917, Willis, 1920). The first cases of infection were reported in the early 1900s in North Queensland (Johnston, 1916, Nicoll, 1917). Improved sanitation and healthcare conditions eliminated strongyloidiasis from the mainstream population; however, it currently remains an issue in remote Indigenous communities (Heydon and Green, 1931, Shield et al., 2015, Page et al., 2016). *S. stercoralis* has also been shown in high numbers in immigrants and refugees from South East Asia, Vietnam veterans, those exposed occupationally to *S. stercoralis,* and returned international travellers from endemic areas (Caruana et al., 2006, De Silva et al., 2002, Rahmanian et al., 2015, Swaminathan et al., 2009, Soulsby et al., 2012). More information on *S. stercoralis* status in Australia including its prevalence and current surveillance system is described in chapter 3 of this thesis.

1.5 *Strongyloides stercoralis* in dogs

Dogs are known to be infected with the same species as humans, *S. stercoralis* based on the morphological analysis. However, there is no genetic evidence confirming that dogs are infected with the same *S. stercoralis* strains as humans. The prevalence of dog *S.stercoralis* infection ranges from 0% to 50% around the world with the highest rates being reported in the developing countries (Júnior et al., 2006, Thamsborg et al., 2017, Palmer et al., 2008, Riggio et al., 2013, Paulos et al., 2012). Depending on the immune system of dogs symptoms range from none to dermatological, gastrointestinal and/or respiratory symptoms (Robertson and Thompson, 2002, Umur et al., 2017). Autoinfection is also present in dogs and can evolve from hyperinfective syndrome causing disseminated strongyloidiasis (Schad et al., 1984).

There was a single survey performed a decade ago looking at the gastrointestinal parasites including *S. stercoralis* in dogs from the veterinary clinics and refugees in Australia (Palmer et al., 2008). There is however no information and data available on the presence and prevalence of canine strongyloidiasis in dogs living in the remote communities in Australia. More information on strongyloidiasis prevalence and distribution in dogs in remote communities in Australia is described in chapter 6 of this thesis.

1.6 Genetic aspects of *Strongyloides stercoralis*

Genetic studies are useful in understanding genetic diversity within the genus and possible transmission routes of *S. stercoralis*, especially between humans and animals. There are two main markers of *S. stercoralis* DNA that are highly conserved and thus have been studied for genotyping purposes. These are small subunit (SSU) in 18S ribosomal DNA (rDNA) and cytochrome c oxidase subunit 1 gene (*cox*1) in the mitochondrial DNA (mtDNA). Both regions are considered to be intra

and interspecific, and used for studying *S. stercoralis* population variations of different geographic locations and/or hosts (Hasegawa et al., 2009, Hasegawa et al., 2010).

Currently, there is no knowledge on the genetic diversity of *S. stercoralis* around the world and in Australia. There have been no studies done looking at the human and/or dog *S. stercoralis* strains. More information on the human and dog *S. stercoralis* haplotypes existing in Australia and dogs' zoonotic potential is provided in the chapter 5 of this thesis.

1.7 Hookworms

Hookworm is another group of soil-transmitted helminths (STH) (Forouzanfar et al., 2016). Human infective hookworms include *Necator americanus* and *Ancylostoma duodenale* (Prociv and Luke, 1995). The most common hookworm species of dogs worldwide and in Australia are *Ancylostoma caninum, Ancylostoma ceylanicum*, *Ancylostoma braziliense* and *Uncinaria stenocephala* (Bowman et al., 2010, Palmer et al., 2008). Hookworms are endemic in the remote communities in north of Western Australia and North Territory (Hopkins et al., 1997, Davies et al., 2013, Bradbury and Traub, 2016). A national study on the prevalence of gastrointestinal parasites in dogs and cats around Australia showed that hookworms were the second most common parasites in dogs (6.7% CI 5.4-8.0) after *Giardia* (Palmer et al., 2008). These hookworms are also known to have zoonotic potential (Prociv and Croese, 1996, Smout et al., 2017, Koehler et al., 2013, Robertson and Thompson, 2002). Infection in humans occurs through percutaneous exposure to infective larvae, or oral exposure to contaminated water and/or food, mainly uncooked meat (Bowman et al., 2010). Hookworm infections with *A. caninum* in humans can lead to cutaneous larva migrans (CLM) caused by prolonged subcutaneous migration of hookworm larvae (Prociv, 1998). Other symptoms might include eosinophilic pneumonitis (Del Giudice et al., 2002, Schaub et al., 2002), erythema multiforme (Vaughan, 1998), folliculitis (Opie et al., 2003, Rivera-Roig et al., 2008), and localised myositis ((Little et al., 1983, Bowman et al., 2010). *A. caninum* infection in humans is associated with eosinophilic enteritis (Prociv and Croese, 1990), while *A. ceylanicum* larvae can develop into adult stage causing abdominal pain (Prociv, 1998). Thus effective control measures should be undertaken to control hookworm infections in dogs as they represent not only veterinary but public health significance. There is currently no data available on the prevalence of hookworm in dogs living in the remote Indigenous communities. More information on hookworm prevalence and distribution in dogs living in the remote communities in Australia is described in chapter 6 of this thesis.

1.8 One Health and environmental health approaches

The first mention of the term "One Health" in a context of improving human and animal health dates back to ancient times (Dhammika, 1993). Nowadays, "One Health" is used in various contexts. According to the "One Health Initiative" and "One Health Sweden", the "One Health Umbrella" covers

multiple disciplines including biology, ecology, human medicine, veterinary medicine, public health, health economics, environmental sciences etc (http://www.onehealthinitiative.com) targeting to improve human health (Lerner and Berg, 2015, Rock et al., 2009, Gibbs, 2014).

It has been suggested that collaborative interinstitutional and interdisciplinary responses to emerging diseases is needed, especially to combat neglected zoonoses (Gibbs, 2014, Jaleta et al., 2017).

Environmental health is a part of public health discipline that looks at the physical, chemical, biological, social and psychological factors in the environment that can harm human health. Its aim is to identify, assess, and control these environmental aspects in order to prevent disease by creating health-supportive environments (Cameron et al., 2004).

The research presented in this thesis was approached using an environmental health perspective. The focus was on the environmental sources that can contribute to human strongyloidiasis transmission. This led to studying dogs living in Indigenous communities and the potential for dog-human transmission of *S. stercoralis*.. Here, we argue for applying "One Health" approach to control *S. stercoralis* infection by linking and incorporating human and animal health disciplines together.

1.9 Methods

In order to avoid repetition, methods used throughout the PhD research are described in the subsequent chapters and method development and optimisation is presented in Appendix A.

For this project, microbiological risk assessment was prepared and biosafety approval No 2018-05 "Isolation of *Strongyloides stercoralis* from faeces" was issued by the Flinders University Institutional Biosafety Committee to use risk group 2 or higher microorganisms (Appendix B).

Additionally, human ethical approvals were obtained from the Social and Behavioural Research Ethics Committee (SBREC), Project Number 6852, titled as "Determining the environmental sources of *Strongyloides stercoralis*: the first step in preventing infection and reinfection" (Appendix C), and the Southern Adelaide Clinical Human Research Ethics Committee (SAC HREC), OFR Number 309.17 (Appendix D). The project was also added to the Register of the Animal Welfare Committee as a project using scavenged animal tissue but not requiring animal ethics approval (Appendix E).

2. AIM AND OBJECTIVES

2.1 Aim

The initial aim of this research was to explore the environmental reservoirs for strongyloidiasis, specifically the role dogs might play in disease transmission.

However as the research was conducted, it appeared that there were other more basic knowledge gaps with reference to *Strongyloides stercoralis* in Australia. These include a lack of understanding of the transmission, prevalence and distribution of *S. stercoralis*. Therefore, this research focussed on exploring these gaps, followed by assessing the role of dogs in transmission of *S. stercoralis*.

2.2 Main objectives

- To identify the main factors influencing the distribution of strongyloidiasis
  - review the literature and summarise the cases of *S. stercoralis* around the world
  - map the global *S. stercoralis* prevalence distribution
  - examine the climatic conditions, socioeconomic factors and corresponding sanitary and hygiene conditions in areas where *S. stercoralis* is prevalent
- To explore a combined approach for controlling strongyloidiasis
  - evaluate drug treatment and associated issues
  - explore the options of targeting *S. stercoralis* in the environment
- To argue for strongyloidiasis to be included in the Australian National Notifiable Disease List
  - determine strongyloidiasis status in Australia
  - examine the reasons strongyloidiasis remains neglected and endemic in the remote communities in Australia
  - prepare the case for strongyloidiasis nomination in Australia
- To identify the role of dogs in human strongyloidiasis in remote communities in Australia
  - develop and validate a DNA preservation method to preserve *Strongyloides* DNA in faecal samples
  - determine the presence of *S. stercoralis* in dogs living in remote communities
  - explore the hypothesis that dogs present a potential zoonotic reservoir for human strongyloidiasis by comparing Australian human and dog *S. stercoralis* haplotypes
- To understand the prevalence and distribution of the zoonotic parasites, *S. stercoralis* and hookworms, in dogs living in the remote communities in Australia
  - determine the prevalence of *S. stercoralis* and hookworm in dogs
  - map the distribution of *S. stercoralis* and hookworm in dogs

2.3 Thesis structure

The thesis consists of 7 chapters; a general introductory chapter, an aim and objectives chapter, four research chapters and a general discussion chapter. There is a detailed methods section included in the Appendix A.

3. ARGUMENT FOR INCLUSION OF STRONGYLOIDIASIS IN THE AUSTRALIAN NATIONAL NOTIFIABLE DISEASE LIST – IN MEMORY OF EMERITUS PROFESSOR RICK SPEARE

This chapter provides an evidence-based argument for the inclusion of strongyloidiasis in the Australian National Notifiable Disease List. It addresses the criteria for inclusion as outlined by the National Health Security Act 2007 (Commonwealth). This work was done to raise the profile of strongyloidiasis at a national level and highlight the need to gather more information about strongyloidiasis in Australia.

The manuscript was written in collaboration with the National Strongyloidiasis Working Group in Australia. It was dedicated to Emeritus Professor Rick Speare and as such was submitted to a special issue of Tropical Medicine and Infectious Diseases Journal, entitled "Control of Communicable Diseases in Human and in Animal Populations: 70th Anniversary Year of the Birth of Professor Rick Speare (2 August 1947 – 5 June 2016)".

Argument for inclusion of strongyloidiasis in the Australian National Notifiable Disease List (NNDL)

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Received: 7 May 2018; Revised: 23 May 2018; Accepted: 31 May 2018; Published: 5 June 2018

This article has been published in:

Tropical Medicine and Infectious Diseases **(2018)** 3(2), 61

DOI: https://doi.org/10.3390/tropicalmed3020061

*(This article belongs to the Special Issue Control of Communicable Diseases in Human and in Animal Populations: 70th Anniversary Year of the Birth of Professor Rick Speare (2 August 1947 – 5 June 2016))

Keywords: strongyloidiasis; *Strongyloides stercoralis*; notifiable; Australia

3.1 Abstract

Strongyloidiasis is an infection caused by the helminth, *Strongyloides stercoralis.* Up to 370 million people are infected with the parasite globally, and it remains endemic in the Indigenous Australian population for many decades. Strongyloidiasis has been also reported in other Australian populations. Ignorance of this disease has caused unnecessary costs to the government health system, and been detrimental to the Australian people's health. This manuscript addresses the 12 criteria required for a disease to be included in the Australian National Notifiable Disease List (NNDL) under the *National Health Security Act 2007* (Commonwealth). There are six main arguments that provide compelling justification for strongyloidiasis to be made nationally notifiable and added to the Australian NNDL. These are: The disease is important to Indigenous health, and closing the health inequity gap between Indigenous and non-Indigenous Australians is a priority; a public health response is required to detect cases of strongyloidiasis and to establish the true incidence and prevalence of the disease; there is no alternative national surveillance system to gather data on the disease; there are preventive measures with high efficacy and low side effects; data collection is feasible as cases are definable by microscopy, PCR or serological diagnostics; and achievement of the Sustainable Development Goal (SDG) # 6 on clean water and sanitation.

3.2 Introduction

Strongyloidiasis is an infection caused by the intestinal and tissue helminth, *Strongyloides stercoralis* (Grove, 1989). *S. stercoralis* has been estimated to infect up to 370 million people worldwide (Bisoffi et al., 2013). In Australia, strongyloidiasis remains endemic in Indigenous populations, infecting communities in Queensland (Prociv and Luke, 1993), the Northern Territory (Mounsey et al., 2014, Shield et al., 2015, Einsiedel and Fernandes, 2008, Kearns et al., 2017, Flannery et al., 1993, Johnston et al., 2005), Western Australia (Jones, 1980), northern South Australia (Einsiedel et al., 2014) and northern New South Wales (Walker-Smith et al., 1969). Seroprevalence in some communities reaches 60% (Mounsey et al., 2014, Shield et al., 2015, Einsiedel and Fernandes, 2008, Kearns et al., 2017, Page et al., 2006, Flannery et al., 1993, Johnston et al., 2005). Despite the prevalence and potential for morbidity and mortality posed by this disease, the true incidence in Australia remains unknown (Bisoffi et al., 2013) as a consequence of both under-diagnosis of the disease and the absence of mechanisms to capture surveillance data (Olsen et al., 2009). The absence of reliable national data of the geographic extent and rate of transmission of this disease blinds medical and public health professionals attempting to institute effective control. This knowledge gap is not unique to Australia. Schar et al. (2013) noted that adequate information on *S. stercoralis* prevalence is still lacking from many countries, but their review found that the information that does exist points out to it being an infection that must not be neglected. They recommended information needs to be collected in a range of socio-economic and ecological settings and that in

many settings the integration of control and treatment of *S. stercoralis* into a holistic helminth control program is warranted (Schär et al., 2013b).

S. stercoralis is a soil-transmitted helminth, infecting a human when infective stage larvae penetrate the skin, enter the circulation, and subsequently travel to the lungs via the blood, from where it is swallowed into the gut (Ericsson et al., 2001). This is the traditional ordered pathway, though evidence exists that random migration through the body to reach the intestine is also likely, even in primary infection (Schad et al., 1989, Grove, 1995, Mansfield et al., 1995). A free-living phase of the parasitic life cycle occurs in the soil after host defaecation in the open, but this can only last one generation, and thus a soil reservoir of the parasite is not a factor in long term control after implementation. Symptoms are protean, including respiratory, gastrointestinal and skin disorders (Grove, 1995, Caruana et al., 2006). Unlike most other soil-transmitted helminth infections, *S. stercoralis* larvae can persist indefinitely inside the host through asexual reproduction by parthenogenesis and subsequent autoinfection (Streit, 2008, Greiner et al., 2008).

After initial infection, there is a rapid increase in numbers as the result of an autoinfective burst (Schad et al., 1997). This causes acute disease. This typically abates in immunocompetent people, becoming asymptomatic, or mildly symptomatic, often mimicking the symptoms of other diseases (Johnston et al., 2005, Montes et al., 2010). Due to its peculiar autoinfective nature, disease is often lifelong and a single remaining larva of *S. stercoralis* post-treatment can cause recrudescence of disease. In immunocompetent persons, the disease is chronic and long-lasting. In immunocompromised/immunosuppressed persons, or those receiving corticosteroid treatment, the infection may transform to a hyperinfective or disseminated disease syndrome, with up to 90% mortality (Croker et al., 2010, Ericsson et al., 2001, Fardet et al., 2007, Marcos et al., 2008, Igra-Siegman et al., 1981, Page and Speare, 2016).

Strongyloides is typically found in tropical and subtropical zones, but is mainly associated with areas of low socioeconomic status as a consequence of inadequate sanitary conditions (Beknazarova et al., 2016b). This is supported by evidence of strongyloidiasis being found in desert communities (Einsiedel and Fernandes, 2008, Einsiedel et al., 2014). Strongyloidiasis is described as the most neglected of the Neglected Tropical Diseases (NTDs) (Olsen et al., 2009), and it is important to make strongyloidiasis notifiable so that epidemiological and prevalence data can be obtained to inform appropriate strategies for controlling the disease.

3.3 The Australian National Notifiable Disease Surveillance System (NNDSS)

The Quarantine Act (NSW) of 1832 was the first legislative document to cover public health issues and included mandatory reporting of diseases to local health authorities in Australia (the Quarantine Act 1832). The Communicable Disease Network Australia (CDNA) was established in 1989 to

enhance national communicable disease surveillance reporting to the then National Public Health Partnership (NPHP) (Australia, 2017). In 2006, NPHP split into the Australian Health Protection Committee (AHPC) and the Australian Health Development Committee (AHDC). CDNA now operates under the AHPC.

The Australian National Notifiable Diseases Surveillance System (NNDSS) was first introduced in 1990 and serves as a platform to collate and report data on nationally-approved notifiable diseases from all jurisdictions to the Commonwealth (Australia, 2017, Miller et al., 2004). The National Notifiable Disease List (NNDL) was created in 2008 under the National Health Security Act 2007 (Commonwealth), a document that contains a list of notifiable communicable diseases to the NNDSS.

3.4 Criteria for inclusion on the National Notifiable Disease List

To determine whether a disease should be notifiable there are currently 12 criteria against which a disease is ranked. These criteria were established by the CDNA in 2014 (Australia, 2017). Table 3.1 presents an assessment of strongyloidiasis against each of these criteria. A score of 28 (if conservative estimates are used) to 30 (if less conservative estimates are used) was calculated for strongyloidiasis based on CDNA descriptors. The CDNA criteria state that if a disease scores less than 15, national notification is not recommended; if it falls between 15 to 25, national notification is to be considered further; and if it is higher than 25, national notification is recommended. As such, even with a conservative estimate of 28, strongyloidiasis fulfils the requirements for national notification to be recommended (Australia, 2017). The criteria can be found at: (http://www.health.gov.au/internet/main/publishing.nsf/Content/8DF6148BCAC589D6CA257EE5001D0DF7/$File/Protocol-change-NNDL.pdf)

Based on Table 3.1 there are six key arguments for making strongyloidiasis notifiable:

- The disease is important to Indigenous health, and closing the health inequity gap between Indigenous and non-Indigenous Australians is a priority.
- A public health response is required to detect cases of strongyloidiasis and to establish the true incidence and prevalence of the disease.
- There is no alternative national surveillance system to gather data on the disease.
- There are preventive measures with high efficacy and low side effects.
- Data collection is feasible as cases are definable by microscopy, PCR, or serological diagnostics.
- Achievement of the Sustainable Development Goal (SDG) # 6 on clean water and sanitation.

3.5 Prevalence of strongyloidiasis in Australia

Strongyloidiasis may have been present in Australia prior to the arrival of Europeans. Due to improvements in sanitation and healthcare, it is no longer typically seen in non-Indigenous Australian communities (Heydon and Green, 1931), but remains a major health problem for Indigenous communities, particularly in remote areas. Despite its long-term persistence in Australia, it is difficult to determine the true distribution and prevalence of the disease. The first confirmed reports of strongyloidiasis in Australia date back to the early 1900s in north Queensland (Johnston, 1916, Nicoll, 1917, Willis, 1920). The first reports of it specifically affecting Aboriginal communities (in Atherton Tablelands) date back to the early 1900s, at which time it was noted to affect Aboriginal people at almost 30 times that of non-Aboriginal (Heydon and Green, 1931). To date, infection-related mortality rate in Indigenous people is much higher compared with that in non-Indigenous population. Strongyloidiasis is one of the causes of deaths (Einsiedel et al., 2008). Current estimates of strongyloidiasis incidence are limited and based on opportunistic testing in hotspot areas and diagnostic pathology laboratory data (Page et al., 2016). The former is biased towards high prevalence communities and the latter towards subjects with easy access to healthcare and laboratory services and having sufficiently symptomatic disease to require diagnostic evaluation. There is also no standard detection method available. A study conducted in north Queensland in 2006 showed a strongyloidiasis prevalence in Indigenous and non-Indigenous populations of 24% and 10% respectively (Eager, 14 July 2011). An epidemiological study with Aboriginal communities in Northern Australia conducted over 2010-2011 showed a strongyloidiasis seroprevalence of 21% (Kearns et al., 2017). Overall, based on studies completed in different localised endemic areas from 1980 to 2010, strongyloidiasis prevalence is estimated to range from 2% to 41% based on faecal microscopy surveys (Prociv and Luke, 1993, Jones, 1980, Shield et al., 2015, Holt et al., 2017) and from 5% to 60% based on serology survey tests (Sampson et al., 25-26 June 2003, Lord, 10-11 June 2005). Furthermore, infected people are known to live elsewhere in Australia apart from endemic areas (Soulsby et al., 2012).

Strongyloidiasis also affects other populations. GeoSentinel Surveillance Network site holds a database for returned international travelers with infectious gastrointestinal disease. Based on analysis of their international database during the period 1996-2005, *S. stercoralis* was rated the fifth most common pathogen (Swaminathan et al., 2009). Screening and treatment is now policy for refugees coming to Australia.

Table 3.1 *Strongyloides stercoralis* against 12 criteria for NNDL assessment

#	Criterion	Score	Notes on Strongyloidiasis
Priority setting			
1	Necessity for public health response	2/4 = case reporting important for detecting outbreaks that require investigating or contacts require routine intervention	A public health response and immediate intervention is required based on the following: 1. Inadequate hygiene and sanitary conditions are the main factors for human strongyloidiasis. A person can get infected when coming into contact with or near infected human or dog faeces. In low socioeconomic status communities, such as some Indigenous communities, sanitation conditions present a high risk for strongyloidiasis transmission, contamination, re-infection, and recurrence (Beknazarova et al., 2016b). Therefore, it is crucial to get a public health response to create and maintain adequate sanitary and hygiene conditions in the communities to prevent the disease. Culturally comprehensive health education for understanding the nature of infectious diseases and how they are transmitted is fundamental for maintaining hygienic conditions (Shield et al., 2018). 2. There is the opportunity to highlight environmental health role in the public health response. There is an opportunity to make a difference in endemic communities and specific families/communities with high need targeting the SDG # 6 on clean water and sanitation. 3. Intervention programs such as targeted mass drug administration (MDA) have shown to be very effective in reducing the reservoir of human infection, and need to be implemented regularly on a local and national level in endemic communities (Kearns et al., 2017). 4. Another intervention program in an endemic Indigenous community incorporated *S. stercoralis* screening into the adult health check, and positive cases were treated and followed up. This selective chemotherapy intervention resulted in a decreased risk of potentially fatal hyperinfection and decreased prevalence in the community (Page et al., 2006, Fearon and Wilson, 23 September 2017). 5. Strongyloidiasis has been shown to prevent weight gain in children, and therefore it is critical to identify and treat *S. stercoralis* infection to avoid intervention by social services. This intervention can result in child removal from parents into care if the child shows signs of malnutrition (Fearon and Wilson, 23 September 2017). An environmental health response to geographic hot spots would also bring in the SDG # 6 on clean water and sanitation.
2	Utility and significance of notification for prevention programs	1/4 = Need to establish burden of illness for monitoring or research purposes / priority setting	The geographic prevalence of *S. stercoralis* within Australia is essential to understand and map the hotspots. Notification and establishing the true burden of infection will improve monitoring, prevention and research, for assessing the effectiveness of prevention and control programs at the local and regional levels. Currently, there are no true disability-adjusted life years (DALYs) identified for strongyloidiasis, mainly because of poor estimates of disease prevalence.
3	Vaccine preventability	0/4 = No vaccine available	No vaccine available
4	Importance for Indigenous health	4/4 = Very high	Strongyloidiasis is endemic in the Indigenous population, affecting up to 60% of the population in some remote communities.

			Strongyloidiasis has been and continues to be an issue in the Australian Indigenous population, causing unnecessary morbidity and mortality in all age groups (Page et al., 2016). Many in the Australian Indigenous population, as a result of socioeconomic conditions and compromised/ suppressed immunity due to chronic disease, are unusually susceptible to both acute strongyloidiasis, and life-threatening disseminated and/ or hyperinfective strongyloidiasis.
5	Emerging or re-emerging disease	2/4 = slowly re-emerging or increasing incidence/prevalence disease over the past 5 years	Strongyloidiasis has been called 'the most neglected of Neglected Tropical Diseases' (Olsen et al., 2009). Cases have been reported since the early 1900s. The literature shows that the prevalence of the disease trend declined following mass drug administration (MDA) of ivermectin (2010) and albendazole (1995) in these communities (Page et al., 2006, Kearns et al., 2017). However, the disease has never been eliminated and tend to reappear (Shield et al., 2015, Holt et al., 2017) . The disease has been neglected, and the real prevalence of the disease is underestimated due to lack of disease surveillance. Due to the unique autoinfective cycle of *S. stercoralis*, chronic strongyloidiasis lasts for a lifetime if not effectively diagnosed and treated. Cases of hyperinfection and iatrogenic fatal dissemination are predicted to increase as the infected populations age and are at a higher risk of being immunosuppressed. Corticosteroids have been considered a factor in 65% of fatalities from hyperinfection (Genta, 1992). Another factor contributing to this emerging disease status with increasing cases of severe, complicated strongyloidiasis, has been the lack of awareness of strongyloidiasis in medical personnel who have been trained in Australia.
6	Communicability and potential for outbreaks	2/4 = Medium	There is a potential for outbreaks in poor-infrastructure settings with low sanitary and hygiene conditions, which together produce a high risk for strongyloidiasis transmission from person to person via faecal-skin and faecal-oral routes (Grove, 1982).
7	Severity and socioeconomic impacts	1/4 = low severity and socioeconomic impacts in chronic strongyloidiasis (strongyloidiasis in healthy person) **or** 2/4 = medium severity and socioeconomic impacts in disseminated or hyperinfective strongyloidiasis	In healthy people, chronic strongyloidiasis may have only mild, intermittent, and non-specific symptoms. However, the autoinfection feature of this helminth and parthenogenesis, allows single larvae reproducing within the host leading to a chronic, long-lasting disease. If not diagnosed and treated, the disease can take a more serious form as the person becomes immunocompromised/ immunosuppressed, with an often-fatal outcome. A case fatality rate of almost 90% has been reported (Einsiedel and Fernandes, 2008). Strongyloidiasis presents unnecessary cost to the health systems, as strongyloidiasis is both preventable and treatable if diagnosed early, and in the chronic stage. The diagnostic and treatment costs, including selective chemotherapy, targeted MDA and water, sanitation and hygiene (WASH) have been estimated in previous research and shown to be affordable (Gordon et al., 2017, Beknazarova et al., 2017b). It was estimated in US citizens that presumptive preventive intervention would decrease DALYs caused by intestinal parasites, including *Strongyloides,* by up to 1976 - saving USD 16.4 million (Muennig et al., 1999).
8	Preventability	4/4 = preventive measure with high efficacy/low side	Adequate sanitary and hygiene conditions including safe water supply, proper toileting and hygiene facilities would provide long term sustainable prevention and elimination of strongyloidiasis (Grove,

		effects/high acceptability and uptake	1982). This should be combined with health education and research to determine the gold standard for strongyloidiasis diagnosis. Treatment of chronic strongyloidiasis prevents hyperinfection. Currently, ivermectin is the drug of first choice to treat human strongyloidiasis, followed by albendazole (Henriquez-Camacho et al., 2016). Ivermectin and albendazole, given according to therapeutic guidelines for strongyloidiasis (Holt et al., 2017), have been shown to eliminate the disease in 70% to 85% of those with chronic strongyloidiasis. Both drugs have negligible side effects. Ivermectin requires only one to two administrations. Albendazole requires two courses of daily doses for three days. A single dose is ineffective (Kearns et al., 2017, Page et al., 2006).
9	Level of public concern and/or political interest	2/4 = low to medium public concern or political interest or 3/4 = medium to high public concern or political interest	Strongyloidiasis is an overlooked, neglected disease (Olsen et al., 2009). However, when people are made aware of the disease, there is high public concern. This is illustrated by a recently published article on strongyloidiasis in "The Conversation" which received a large number of responses by the general public showing their interest and concern about the disease (Whiley et al., 2017). Closing the Gap (the health inequity gap between Indigenous and non-Indigenous Australians) is a high priority in mainstream Australia (Hoy, 2009). The fact that locally-acquired infection in Australia is almost exclusively seen in Indigenous communities should be of great public and political concern.
Feasibility of collection			
10	A case is definable	4/4 = Case has an acceptable laboratory definition with or without a clinical definition	A strongyloidiasis case is definable and we propose to notify strongyloidiasis by the laboratories based on positive serology or parasitological diagnosis (Speare et al., 2014). In disseminated and hyperinfective strongyloidiasis, faecal examination has higher sensitivity due to large numbers of viable larvae and the patient is usually in a hospital setting at the time of diagnosis. In immunocompetent persons, chronic strongyloidiasis might not always be detected by microscopy due to low and irregular larval load, and serology has the highest sensitivity and is recommended (Page and Speare, 2016).
11	Data completeness is likely to be acceptable	2/4 = Data represent a proportion of community cases with a known undercount	Data on the prevalence of strongyloidiasis is limited. Studies suggest that up to 60% of the population in Indigenous rural or remote communities is infected with strongyloidiasis. A study in North Queensland found that 10% of the non-Indigenous population has strongyloidiasis (Eager, 14 July 2011). It is believed that the disease is likely to be more widespread in Australia that the current data suggest. .
12	Alternative surveillance mechanisms	4/4 = No alternative surveillance mechanisms in place.	There is no surveillance mechanism available to monitor and report on strongyloidiasis.

Total score: 28-30

However, screening has not yet been introduced to policy or systems for endemic Aboriginal communities in Australia. Immigrants and refugees from South East Asia also have high prevalence rates as do returned travelers (Caruana et al., 2006, De Silva et al., 2002). In a South Australian study, 11.6% of Vietnam veterans tested seropositive for *S. stercoralis* (Rahmanian et al., 2015). Four non-Indigenous cases of strongyloidiasis acquired through occupational exposure were reported in Central Australia (Soulsby et al., 2012).

It is difficult to estimate mortality rates associated with strongyloidiasis as it is responsible for several fatal clinical manifestations, each of which may be attributed to other causes (Hutchinson, 25-26 June, 2003). For example, during hyperinfective strongyloidiasis, larvae migrate from the gastrointestinal system to other organs, transporting enteric bacteria with them. This can result in community-acquired septicaemia or meningitis, or local sepsis, which are then registered as cause of death on death certificates, despite the underlying cause of death being strongyloidiasis (Einsiedel and Fernandes, 2008). Additionally, acute strongyloidiasis can also cause severe gastrointestinal (intestinal obstruction), or respiratory (pulmonary strongyloidiasis) disease that can be potentially fatal if the strongyloidiasis is not diagnosed and treated (Shields et al., 2014, Mukerjee et al., 2003, Byard et al., 1993).

3.6 Socioeconomic impact caused by strongyloidiasis

Due to the chronic nature of most NTDs, their burden is usually estimated using disability-adjusted life years (DALYs) lost. One DALY equals one year of life lost by a healthy person due to a disease. A study in the United States of America compared the costs and benefits of no preventive intervention and preventive intervention of 1996 people that were at risk of intestinal parasite infections, including *S. stercoralis* (Muennig et al., 1999). Preventive intervention included presumptive treatment with 400 mg of albendazole daily for five days and data was analysed using a decision-analysis model. It was estimated that presumptive preventive intervention against human parasites would decrease DALYs by 1976 and save up to 16.4 million USD. While it is difficult to estimate treatment efficacy for an individual parasite due to complexity of the tests used in this study, strongyloidiasis was shown to cause the highest number of deaths and hospitalization costs (Muennig et al., 1999). Notably, DALYs do not describe the complete story of harmful consequences of strongyloidiasis, such as an economic impact from productivity loses, and social impact on individuals and the community (Hotez et al., 2014). DALYs are also not appropriate to use when estimating the burden of strongyloidiasis due to the underestimated prevalence and in cases of asymptomatic strongyloidiasis.

Under-diagnosis, under-treatment and a neglected approach to this disease cause chronic strongyloidiasis cases to develop, which is costly to the health system with expensive imaging and investigations being undertaken before diagnosis. Currently, effective treatment of strongyloidiasis is available and available information technology can be used to establish a

notification database. Health promotion and community engagement are also required and need to be incorporated into public health and population health strategies, which can make a difference in disease detection and treatment and ultimately, closing the gap.

3.7 Recommendation to make strongyloidiasis a notifiable disease

Notifiable disease data are collected to estimate the prevalence of the disease, identify hotspots of infection, and determine any susceptible populations. These data will then be used by the public health institutions and authorities to implement prevention and control measures and/ or interventions at local, regional and national levels. Notification of the disease allows estimates of the effectiveness of the treatment and/ or control strategies that would result in a systematic evidence-based approach to addressing this public health issue (Speare, July 2011). Strongyloidiasis represents a chronic, possibly widespread, potentially debilitating, and life-threatening disease endemic in Australia, which affects Indigenous communities and new Australian populations at a rate far in excess of the general population. Extra-intestinal strongyloidiasis is now listed as a notifiable disease by the Centre for Disease Control in the Northern Territory. The logical next step of national notification, and thus registration, of cases of strongyloidiasis would allow public health authorities the critical information they need to implement relevant prevention, control actions, and regulations.

Globally, it has been noted that strongyloidiasis is an underreported disease and information on at-risk and affected populations is missing (Schär et al., 2013b). This review found that information on incidence is virtually non-existent, and without this information we lack insight into "how often and how quickly people are re-infected after successful treatment", "how often first-time infections are sustained over a longer period", and knowledge of risks for infection for children and adults is missing. They argued for supporting longitudinal studies, especially at a community level, to address these knowledge gaps about an important infectious disease.
There is a compelling justification for strongyloidiasis to be made notifiable in order to establish prevalence data, identify the most severely affected regions and groups and subsequently implement and monitor public health interventions to control this important disease. Based on this, it is recommended that strongyloidiasis is made nationally notifiable and added to the Australian NNDL as a matter of priority.

3.8 Author contributions

M.B. conceived in a review design, put the ideas together, and drafted the manuscript. H.W. and K.R. provided academic input to the draft. K.R., J.J., J.S. and W.P. provided their extensive knowledge and expertise of the topic to the manuscript. A.M. and M.W. provided their knowledge to some aspects of the manuscript. M.B. incorporated all the authors' comments, K.R. reviewed the final version of the manuscript. All authors approved the final manuscript.

3.9 Acknowledgments

The work has been supported by the Australian Government Research Training Program Scholarship. Authors received no funds to publish in open access.

3.10 Conflicts of interest

The authors declare no conflict of interest

3.11 Dedication

The authors would like to dedicate this work to Emeritus Professor Rick Speare. He spent much of his life understanding *S. stercoralis*, preventing and managing strongyloidiasis in Indigenous communities. He argued for inclusion of strongyloidiasis to the Australian National Notifiable Disease List as means of controlling the disease.

4. VALIDATION OF DESS AS A DNA PRESERVATION METHOD FOR THE DETECTION OF *STRONGYLOIDES* SPP. IN CANINE FAECES

This chapter describes the development, validation and optimisation of the *Strongyloides* DNA preservation method in faecal samples. The research conducted throughout the PhD required the collection of faecal samples from remote communities and transportation interstate for further processing. The main aim was to prevent DNA from degrading using methods that are applicable for field-collected samples.

Validation of DESS as a DNA preservation method for the detection of *Strongyloides* spp. in canine feces

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Academic Editor: Anthony R. Mawson

Received: 12 May 2017; Accepted: 8 June 2017; Published: 9 June 2017

This article has been published in:

International Journal of Environmental Research and Public Health **(2017)** 14(6)

DOI: https://doi.org/10.3390/ijerph14060624

Keywords: *Strongyloides stercoralis*; *Strongyloides ratti*; *Strongyloides*; real-time PCR; DESS; DNA preservation; DNA degradation; canine feces

4.1 Abstract

Strongyloides stercoralis is a gastrointestinal parasitic nematode with a life cycle that includes free-living and parasitic forms. For both clinical (diagnostic) and environmental evaluation, it is important that we can detect *Strongyloides* spp. in both human and non-human fecal samples. Real-time PCR is the most feasible method for detecting the parasite in both clinical and environmental samples that have been preserved. However, one of the biggest challenges with PCR detection is DNA degradation during the postage time from rural and remote areas to the laboratory. This study included a laboratory assessment and field validation of DESS (dimethyl sulfoxide, disodium EDTA, and saturated NaCL) preservation of *Strongyloides* spp. DNA in fecal samples. The laboratory study investigated the capacity of 1:1 and 1:3 sample to DESS ratios to preserve *Strongyloides ratti* in spike canine feces. It was found that both ratios of DESS significantly prevented DNA degradation compared to the untreated sample. This method was then validated by applying it to the field-collected canine feces and detecting *Strongyloides* DNA using PCR. A total of 37 canine feces samples were collected and preserved in the 1:3 ration (sample: DESS) and of these, 17 were positive for *Strongyloides*. spp. The study shows that both 1:1 and 1:3 sample to DESS ratios were able to preserve the *Strongyloides* spp. DNA in canine feces samples stored at room temperature for up to 56 days. This DESS preservation method presents the most applicable and feasible method for the *Strongyloides* DNA preservation in field-collected feces.

4.2 Introduction

Strongyloides stercoralis is a gastrointestinal human parasitic nematode whose lifecycle includes both free-living and parasitic forms (Toledo et al., 2015). The environmental phase comprises of one free-living generation with long-lived infective larvae (L3) (Toledo et al., 2015). Globally, it is estimated that there are 370 million people infected with *Strongyloides* spp. (Bisoffi et al., 2013). However, there is limited knowledge regarding the parasites' survival in the environment and limited public health strategies for controlling the disease (Beknazarova et al., 2016a). For both clinical (diagnostic) and environmental evaluation, it is essential to have a reliable method for *Strongyloides* spp. detection in both human and non-human fecal samples.

The real-time polymerase chain reaction (PCR) is a nucleic acid detection-based technique which has been shown to have high sensitivity and specificity for pathogen detection (e.g., *Strongyloides* spp.) in fecal samples (Verweij et al., 2009, Sultana et al., 2013). The ability to use preserved or frozen samples for PCR makes it a convenient method for field-based samples (Steinmann et al., 2007, Verweij et al., 2009) and preferable to culture detection which relies on live organisms (Sato et al., 1995). The success of PCR as a detection method is generally dependant on whether nuclear or mitochondrial DNA is amplified, the number of base pairs targeted by PCR, the presence of PCR inhibitors, and the preservation of the targeted DNA in the samples (Frantzen et al., 1998). As such, it is imperative to

preserve the DNA and increase the feasibility of using fecal DNA. One of the biggest challenges to this method is DNA degradation during postage time from rural and remote areas to the laboratory. This degradation is due to components of feces that rapidly degrade DNA (Schrader et al., 2012), and is compounded by the fact it is unfeasible to refrigerate samples during the travel time from rural locations and it is often impossible to post liquids with alcohol-based preservatives.

DNA in biological samples degrades as cells lyse due to decomposition processes, and free DNA is hydrolyzed by DNAses produced by microorganisms in the sample (Alaeddini, 2012, Nsubuga et al., 2004). Studies suggest that positive PCR results in fecal samples significantly decline after three days from collection, which is well below the expected collection and transport time for field-collected samples (Santini et al., 2007). While a number of reasonably successful DNA preservation methods currently exist, their applicability to field-based collection and transportation from rural areas is lacking. An ideal DNA preservative will be easy, safe, and inexpensive to utilize, transport, and store (Yoder et al., 2006). Cryopreservation is considered to be the most effective method for long-term DNA preservation, but it is difficult to acquire the equipment to achieve this in rural areas, and strict regulations prevent cryopreserved samples being transported by air (Yoder et al., 2006, Gray et al., 2013). Ethanol is cheap and readily available, but does not preserve optimally at room temperature (Santini et al., 2007, Kilpatrick, 2002), and may require additional processing such as sample homogenization, subsampling, and changing the ethanol after a few days of storage, which may be unfeasible when dealing with large numbers of samples (Yoder et al., 2006). Furthermore, ethanol is a hazardous substance and restrictions apply when transporting by air (Kilpatrick, 2002). Other methods are either ineffective, or prohibitively expensive—as is the case with proprietary solutions (Gray et al., 2013). A field-applicable method, which is effective, easy, safe, and inexpensive should be developed for studies involving environmental samples collected from rural areas.

DESS (dimethyl sulfoxide, disodium EDTA, and saturated NaCl) has been previously shown to be highly effective in long-term DNA preservation of different tissues at room temperature (Frantzen et al., 1998, Yoder et al., 2006, Kilpatrick, 2002, Gray et al., 2013, Seutin et al., 1991). However, this is the first study that has developed and validated a method of DNA preservation using DESS for the subsequent detection of *Strongyloides* spp. in fecal samples. The method was first tested in the laboratory using fresh non-infected dog feces spiked with *Strongyloides ratti,* as a model organism used to determine the effectiveness of 1:1 and 1:3 sample: DESS ratios for DNA preservation at room temperature. This was then validated by using the method on field-collected dog fecal samples for *Strongyloides* spp. detection.

4.3 Materials and methods

DESS protocol

100 mL of deionized water was added to 46.53 g EDTA disodium salt and stirred. Then, 1 M NaOH was added while heating at 30 °C until the pH reached 7.5 and the EDTA disodium salt had dissolved. A 400-mL aliquot of the solution was created and 100 mL of DMSO was added for a final DMSO concentration of 20% and EDTA disodium salt concentration of 0.25 M. Finally, 50 g NaCl was added to the solution

Assessing the ability of DESS to preserve *Strongyloides ratti* DNA in faeces

Fresh dog fecal samples were collected from a non-infected dog and utilized within 24 h of collection. Fresh feces from rats infected with *S. ratti* via subcutaneous penetration were collected 7–10 days post infection and donated by the Center for Infectious Diseases and Microbiology, Westmead Hospital, NSW, Australia; this was used to spike the canine feces at a 1:9 rat to canine feces ratio.

The spiked feces (canine and rat combined) was then aliquoted and either treated with 1:1 or 1:3 sample to DESS ratio or no DESS (for the no-treatment control). All samples were conducted in duplicate for each treatment/control and DNA extraction time point (day 0, 3, 7, 14, 28 and 56). A negative control was also included containing non-infected fresh canine feces dissolved in DESS solution at a 1:1 ratio. The negative controls were conducted in duplicate for each DNA extraction time point (day 3, 14 and 56). All samples were stored in sealed containers at room temperature prior to DNA extraction using the method described below.

PCR positive and negative controls

PCR positive controls were created using fresh dog fecal samples spiked with feces from rats infected with *S. ratti*, and PCR negative controls were created using non-infected canine feces. The DNA from these positive and negative controls were extracted immediately.

DNA extraction

Prior to DNA extraction, samples containing DESS were centrifuged for 3 min at 3000× g rpm at the Orbital 400 Clements (Phoenix, Lidcombe, NSW, Australia). The supernatant was removed, and approximately half a milligram of the remaining pellet was exposed to further DNA extraction using the PowerSoil DNA extraction kit as per the manufacturer's protocol (QIAGEN, Hilden, Germany). A slight modification to the methods was introduced, comprising an incubation of the sample at 56 °C overnight, after the cell lysis step (Sitta et al., 2014, Alonso et al., 2011).

Field collection of canine faeces

A total of 37 canine samples were collected from eight different locations across Australia. Two grams of dog feces were collected into the plastic tube containing 6 mL of DESS solution. The samples were kept at room temperature up to 30 days until being processed at the laboratory.
The sample collection was approved by the Social and Behavioral Research Ethics Committee # 6852.

Real-time PCR conditions for *Strongyloides* spp.

The real-time PCR assay was adopted from Verweij et al. (2009) and Sultana et al. (2013), using the *S. stercoralis* species-specific primers and probes (the F primer is 100% homologous to other *Strongyloides* species) targeting a 101 base pair region of 18S rRNA gene (GenBank accession No. AF279916) (Verweij et al., 2009, Sultana et al., 2013). The 20 µL reaction contained 10 µL of Supermix (SSoAdvanced, Universal Probes Supermix, Foster City, CA, USA), 1 µL of primers and probe mixture (Stro18S-1530F 5′-GAATTCCAAGTAAACGTAAGTCATTAGC-3′; Stro18S-1630R 5′-TGCCTCTGGATATTGCTCAGTTC-3′ and probe Stro18S-1586T FAM-5′-ACACACCGGCCGTCGCTGC03′-BHQ1) (Verweij et al., 2009), 4 µL of deionised H_2O, and 5 µL of DNA template. The cycling conditions included an initial hold at 95 °C for 15 min, followed by 40 cycles consisting of 95 °C for 15 s and 60 °C for 30 s. All PCR reactions were performed in triplicate using the Corbett Rotor-Gene 6000 machine (QIAGEN, Germany). Each PCR run contained a positive, negative, and non-template control (NTC). To determine the presence of environmental inhibitors, all samples were tested at pure and 1 in 10 dilution of the DNA extract into nuclease-free water. If the cycle threshold (Ct) value for the pure DNA extract was 3.3 higher the Ct value of the 1 to 10 dilution of DNA extract, then the pure DNA was assumed to be inhibited by the environmental inhibitors and the diluted DNA extracts were used (Livak and Schmittgen, 2001).

A sample was considered positive when the Ct value was lower the mean negative Ct minus 2.6 standard deviations of a mean negative control Ct. Positive samples were amplified in every PCR reaction (Ct 20.50–24.65).

Statistical analysis

Statistical analysis was performed using SPSS (SPSS Inc., Chicago, IL, USA). Related Samples Wilcoxon Signed Rank Test was performed to compare the means of replicates within each treatment. One-Sample Kolmogorov-Smirnov Test was used to check the data distribution. Paired-sample *t*-test was performed to compare the DESS-treated samples with control samples, and DESS 1:1 to DESS 1:3 treatments.
The means of duplicates of each treatment sample (DESS 1:1, DESS 1:3, and control) were not statistically significantly different ($p > 0.05$). This shows the replicability of the PCR runs and rules out

experimental errors associated with pipetting. One-Sample Kolmogorov-Smirnov Test was further performed for each of the treatments (duplicates) at each measured day. The data distribution was found normal within the treatments and means of the treatments were further used for the graphs.

4.4 Results

Effect of DESS solution on the preservation of *Strongyloides ratti* DNA over time

DESS-treated samples preserved DNA better than no-treatment controls for 56 days. Control and DESS-treated groups were statistically significantly different ($p = 0.000$[5]), while there was no significant difference between DESS 1:1 and DESS 1:3 treated samples ($p = 0.752$). Figure 4.1 shows the mean Ct values for 1:1 and 1:3 sample to DESS ratios and a no-treatment control for each DNA extraction time point.

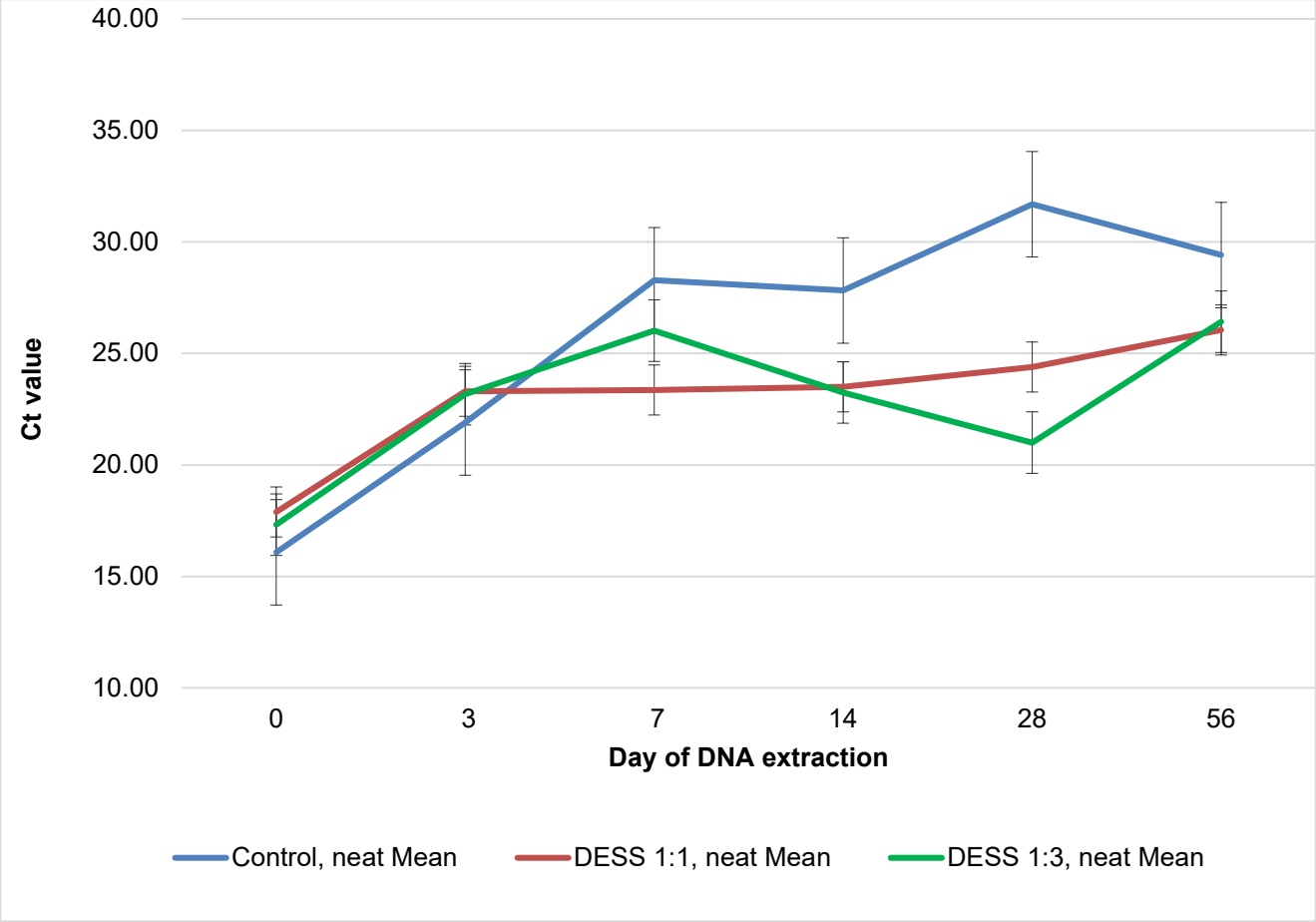

Figure 4.1 Performance of 1:1 and 1:3 ratios of *Strongyloides ratti* spiked fecal sample to DESS (dimethyl sulfoxide, disodium EDTA, and saturated NaCl) and a no-treatment control over time.

[5] p<0.0001

Undiluted DNA extracts were consistently amplified better (corresponding to lower Ct values) compared to 1 to 10 DNA dilutions, indicating that there was no inhibition observed. DESS negative control samples appeared to be negative consistently over the incubation period, indicating that there was no cross-contamination between the samples during the incubation period.

Significant degradation of the *S. ratti* DNA in no-treatment samples was observed after two weeks of storage at room temperature. The 1:3 treated samples showed Ct values slightly and steadily decreasing from day 7 to day 28. The heterogeneity of the feces matter with uneven distribution of different inhibitors (bilirubins, bile salts, complex carbohydrates) (Schrader et al., 2012) might affect the DNA amplification by PCR.

DESS preservation effect in field-collected canine faeces

From the 37 canine fecal samples collected and stored in a 1:3 sample: DESS ratio for up to 30 days at room temperature, there were 17 positive for *Strongyloides* DNA.

4.5 Discussion

This study demonstrated that DESS in both 1:1 and 1:3 sample to DESS ratios could preserve *S. ratti* DNA in canine feces for up to 56 days at room temperature. It was important to identify the optimum concentration of DESS, as higher concentrations might result in its active components releasing inhibiting properties and interfering with PCR amplification. However, no statistically significant difference was observed with the two different DESS concentrations. Being a nonproprietary solution, cost-effective, not resource intensive, and non-flammable, DESS presents a feasible option for DNA preservation in field-based samples, particularly from rural areas (Gray et al., 2013). This study tested the DESS and optimum sample to DESS ratios to preserve *S. ratti* DNA in canine feces for up to 56 days in the laboratory setting.

The 1:3 sample to DESS ratio was further validated through the preservation of field-collected canine fecal samples stored at room temperature for a maximum of 30 days until being processed. The subsequent PCR results identified 17 canine fecal samples positive for *Strongyloides* spp. out of a total of 37 field-collected samples. The study demonstrated that DESS can preserve DNA in fecal samples, which presents a more challenging matrix containing complex polysaccharides, bile salts, urate, and lipids compared to soil or other tissues (Schrader et al., 2012).

One of the limitation to this study was that it was not feasible to have comparable field-collected samples not treated with DESS for comparison of DNA degradation. However, the laboratory based study demonstrated the DESS DNA preservation effect compared to no-treatment, *S. ratti* spiked fecal

samples. In the preliminary study, no feces were autoclaved to prevent any change in the amount of DNAses and RNAses present in the sample which would impact on the total amount of DNA degradation, thus making these samples comparable to the field-collected non-autoclaved fecal samples.

DESS presents a non-toxic preservative method for *Strongyloides* spp. DNA, and can be used for the detection of *Strongyloides* spp. DNA in fecal samples in both animal and human models. Studying the environmental phase of the parasite is important to be able to apply a combined approach of disease treatment, including environmental as well clinical control. The first step would be to look at the potential environmental sources of *Strongyloides* spp. such as canine feces, wastewater, or sewage. As such, it is important to be able to use DESS, a non-toxic, easy to apply method, to preserve the *Strongyloides* spp. DNA in the samples collected from remote or rural areas.

The DESS method for the storage of fecal samples to prevent the degradation of DNA could also be applied to clinical samples to improve sensitivity. Given that microscopy tests are not always sensitive enough (Ericsson et al., 2001, De Silva et al., 2002), and serology can result in false positives due to the risk of cross-reactivity with other antibodies (Ahmad et al., 2013, Buonfrate et al., 2012), PCR detection should be conducted to avoid misdiagnosis. This is particularly important in settings where culture techniques are not possible or impractical. In fact, in a personal communication with a clinical diagnostic laboratory worker, there was a hyperinfection strongyloidiasis case where a human stool sample was collected and transported from a rural area, resulting in DNA degradation. The subsequent laboratory results for PCR returned high Ct values that would not have been treated as a positive result, were it not for the fact that the serology was also positive and there was significant eosinophilia, but negative microscopy, which can be due to low larval output in feces. This suggests that DNA degradation in human feces as a result of long postage times may result in potential false negative results and misdiagnosis (Robertson, 2016).

4.6 Conclusions

S. stercoralis is a parasitic nematode of public health concern, which is able to reproduce in both the environment and in a host. Real-time PCR is the most feasible method for its detection in both clinical and environmental samples. However, the sensitivity of the PCR method can be affected by DNA degradation, which is particularly an issue when the majority of samples are coming from rural and remote locations. This study demonstrated that both 1:1 and 1:3 sample to DESS ratios can preserve DNA in fecal samples for up to 56 days at room temperature prior to the subsequent real-time PCR detection of *Strongyloides* spp. The method was validated using field-collected canine fecal samples, which were stored at room temperature for up to 30 days. The DESS preservation method requires no

additional kits or certain storage temperatures and is cost-effective, which presents a feasible method for DNA preservation in field-collected animal feces, and potentially can be applied in detecting *S. stercoralis* in human feces for clinical diagnostics.

4.7 Acknowledgments

The work has been supported by the Australian Government Research Training Program Scholarship. Authors would also like to thank the Center for Infectious Diseases and Microbiology, Westmead Hospital, NSW, Australia for kindly donating fresh feces from rats infected with *Strongyloides ratti.*

4.8 Author contributions

Meruyert Beknazarova, Harriet Whiley and Kirstin Ross conceived and designed the experiments; Meruyert Beknazarova performed the experiments, analyzed the data and drafted manuscript. Shelby Millsteed participated in the discussion. Gemma Robertson, Harriet Whiley and Kirstin Ross provided academic input and all authors approved the final manuscript.

4.9 Conflicts of interest

The authors declare no conflicts of interest.

5. *STRONGYLOIDES STERCORALIS* GENOTYPES IN HUMANS AND DOGS IN AUSTRALIA

This chapter describes the role of dogs in human strongyloidiasis. It presents work that used molecular genotypes of Australian human and dog *Strongyloides stercoralis* to assess the potential of dogs to

transmit strongyloidiasis to humans. This work was done in response to the high prevalence of *S. stercoralis* infections in Australia and a recently growing interest in the genetics of *S. stercoralis.* This is the first molecular study conducted on Australian human and dogs *S. stercoralis*. It is the third study on genotyping human and dog *S. stercoralis* strains following studies conducted in Cambodia and Japan. Furthermore, for the first time this study has employed deep sequencing techniques to sequence DNA extracted from the faecal samples and discover classic and cryptic *Strongyloides* genotypes.

The chapter contains two papers published during the course of the PhD.

5.1 Mass drug administration for the prevention human strongyloidiasis should consider concomitant treatment of dogs

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Academic Editor: James B. Lok

Published: 24 August 2017

This article has been published in:

***PLoS Neglected Tropical Di*seases (2017)** 11(8)

Doi: https://doi.org/10.1371/journal.pntd.0005735

Strongyloides stercoralis is the causative agent of strongyloidiasis, which affects more than 300 million people worldwide (Bisoffi et al., 2013). Canine strongyloidiasis is also caused by the same species, *S. stercoralis* of an animal origin with up to 50% worldwide prevalence (Júnior et al., 2006, Umur et al., 2017, Wang et al., 2015, Thamsborg et al., 2017). It is known that human *S. stercoralis* strains can infect dogs in laboratory settings (dogs have to be immunosuppressed to maintain infection) (Grove and Northern, 1982, Genta et al., 1986, Lok, 2006). Increasing evidence suggests that *S. stercoralis* could be a potential zoonotic pathogen (Júnior et al., 2006, Goncalves et al., 2007, Georgi and Sprinkle, 1974, Schär et al., 2014b, Thamsborg et al., 2017).

A study by Goncalves et al. (Goncalves et al., 2007) examined 181 kennels and 11 dog keepers responsible for kennel cleaning in the southeastern region of Brazil. The serological analysis (ELISA) found that 24.3% (44 out of 181) and 33% (3 out of 9) of dogs and humans were infected with *S. stercoralis*, respectively (Goncalves et al., 2007). In another study, stool examination identified rhabditiform larvae in the feces of an animal handler. Examination of his wife and his domesticated pet dog found no larvae present in their stools. However, one-third of the dogs under his charge had *S. stercoralis* larvae in their feces (Georgi and Sprinkle, 1974). Another recent study identified *S. stercoralis* in dog using the *S. stercoralis* species-specific primers and probes commonly used to identify human *S. stercoralis* identification (Buonfrate et al., 2017). These studies suggest that dogs may play a role in human strongyloidiasis and/or that humans may play a role in canine strongyloidiasis.

There are a few studies describing the role of dogs in the spread of human strongyloidiasis. A study from Anima Islands, Japan, assessed more than 600 humans and their dogs and found no strongyloidiasis cross-contamination (Takano et al., 2009). However, further studies are needed to explore the effect of differing interactions and behaviours between humans and dogs in different communities and cultures.

Genetic studies are useful for exploring the differences between dog and human *S. stercoralis* strains and provide insight into the potential for cross infection. The whole genome *S. stercoralis* sequence (accession number PRJEB528) described by Hunt et al. (Hunt et al., 2016) was from a canine fecal sample. There are 2 regions of *S. stercoralis* that have been of a particular interest for nucleotide sequencing. Hyper-variable regions ([HVR] I-IV) in 18s ribosomal DNA and a cytochrome c-oxidase subunit 1 gene (cox1) region in mitochondrial DNA (mtDNA) are generally considered to be inter and intraspecific and are used to examine *S. stercoralis* populations of different geographic locations or hosts (Hasegawa et al., 2009, Hasegawa et al., 2010). A study by Hasegawa et al. (Hasegawa et al., 2009) evaluated the HVR-I-IV regions among different species of *Strongyloides*. They found that for the HVR-I-III regions, multiple species frequently shared the same sequence. In HVR-I they describe minor

sequence variations within *S. stercoralis* sampled from different hosts and locations. These differences, however, did not indicate the host of origin of the particular worm. HVR-IV appeared more species-specific, but did not show intra-specific variations in some *Strongyloides* spp., including *S. stercoralis*. In a following study Hasegawa *et al.* (Hasegawa et al., 2010)[6] sequenced the HVR-IV and the mtDNA cox1 gene in *S. stercoralis* and *S. fuelleborni* isolated from different hosts (humans, dogs, apes, and monkeys) and from different geographical locations. The HVR-IV region was again found species-specific. In the study by Hasegawa *et al.* [16], [7]the authors described 1 within species polymorphism, which, however, occurred within the worms isolated from humans and, therefore, provided no indication of a separation of human and dog derived *S. stercoralis*. In contrast to this, in their earlier study, Hasegawa *et al.* (Hasegawa et al., 2009) had noticed that based on a preliminary genetic analysis of the mtDNA, dog-derived *S. stercoralis* appeared phylogenetically distant from those of primate (including human) derived *S. stercoralis*. In the second study, (Hasegawa et al., 2010) the mtDNA cox1 gene was found to be more conserved within *S. stercoralis* compared to *S. fuelleborni*. Nevertheless, based on the cox1 nucleotide sequences, *S. stercoralis* from dogs appeared phylogenetically separated from those isolated from humans. Further, there was 1 amino acid substitution identified, which consistently separated the admittedly rather small number of dog derived *S. stercoralis* from human derived *S. stercoralis* (Hasegawa et al., 2010). Genetic studies of human *S. stercoralis* from different geographic zones suggested that climatic conditions, such as temperature and moisture, may coincide with genetic changes (Pakdee et al., 2012, Kikuchi et al., 2016, Schär et al., 2014a). More research examining the DNA sequences of dog and human *S. stercoralis* has to be done in order to improve our understanding of animal *S. stercoralis* infectivity to humans.

Presently, there is limited evidence regarding the role of dogs in human strongyloidiasis; however, there is enough to suggest that further research is needed to investigate this potential route of infection. Dogs carrying *S. stercoralis* in communities could possibly explain the limitations of previous Mass drug administration (MDA) programs; this is another area requiring further research. A study by Kearns et al. (Kearns et al., 2015) investigated the prevalence of human strongyloidiasis and scabies in remote Australian Aboriginal communities to evaluate the efficacy of ivermectin MDA. The study demonstrated that ivermectin MDA reduced prevalence but failed to eliminate strongyloidiasis and scabies in the community (Kearns et al., 2015). Reappearance of strongyloidiasis could potentially be due to helminth resistance development or reinfection from environmental reservoirs (such as dogs, soil, etc.). Control of environmental reservoirs would also reduce reliance on MDA targeting humans as *S. stercoralis* ability to autoinfect in humans can compromise the success of the MDA, leading to potential helminth resistance development to a drug (Beknazarova et al., 2016a). However, this would not be a concern

[6] In a following study Hasegawa et al. (2010)
[7] Hasegawa et al (2010)

for MDA of dogs as *S. stercoralis* tend to lose autoinfection ability in healthy dogs (Thamsborg et al., 2017, Genta, 1989b).

Anecdotally, animal management strategies are already being undertaken in many remote communities, and as such, concurrent treatment with anthelmintic drugs would minimise costs. Mass vaccination of dogs, including oral drug treatment targeting stray dogs, have been successfully practiced over the years to significantly decrease the rabies prevalence among humans (Chomel et al., 1988, Cleaveland et al., 2006). The rabies elimination model has been estimated to cost between US$2 to $5 for a single dog vaccination, suggesting that treatment of dogs presents an economically suitable option (Fishbein et al., 1991, Cleaveland et al., 2006). Drug treatment of water buffaloes is another successful example of MDA when applied to a potential animal reservoir to control human schistosomiasis (Guo et al., 2006). Future MDA programs should consider treating both humans and dogs to fight strongyloidiasis. Given that the relative cost of treating dogs is low, this potentially could provide a low cost and low-risk mechanisms to reduce the risk of reinfection.

5.2 Detection of classic and cryptic *Strongyloides* genotypes by deep amplicon sequencing: A preliminary survey of dog and human specimens collected from remote Australian communities

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This article has been accepted for publication in:

***PLoS Neglected Tropical Diseases* (2019)**

Keywords: *Strongyloides*, genotypes, dogs, Australia, zoonoses, hookworms, *Ancylostoma*

5.2.1 Abstract

Strongyloidiasis is caused by the human infective nematodes *Strongyloides stercoralis, Strongyloides fuelleborni* subsp. *fuelleborni* and *Strongyloides fuelleborni* subsp. *kellyi*. The zoonotic potential of *S. stercoralis* and the potential role of dogs in the maintenance of strongyloidiasis transmission has been a topic of interest and discussion for many years. In Australia, strongyloidiasis is prevalent in remote socioeconomically disadvantaged communities in the north of the continent. Being an isolated continent that has been separated from other regions for a long geological period, description of diversity of Australian *Strongyloides* genotypes adds to our understanding of the genetic diversity within the genus. Using PCR and amplicon sequencing (Illumina sequencing technology), we sequenced the *Strongyloides SSU* 18S rDNA hyper-variable I and hyper-variable IV regions using *Strongyloides*-specific primers, and a fragment of the mtDNA *cox*1 gene using primers that are broadly specific for *Strongyloides* sp. and hookworms. These loci were amplified from DNA extracted from Australian human and dog faeces, and one human sputum sample. Using this approach, we confirm for the first time that potentially zoonotic *S. stercoralis* populations are present in Australia, suggesting that dogs represent a potential reservoir of human strongyloidiasis in remote Australian communities.

5.2.2 Author summary

Strongyloides stercoralis is a soil-transmitted nematode that causes the disease strongyloidiasis. Due to the autoinfective nature of this parasite, it can re-infect a host causing chronic infection. If not diagnosed and treated it can be highly detrimental to human health and has a high mortality rate. Strongyloidiasis is common in remote communities in the north of Australia and has been an issue for decades. Despite various successful intervention programs to treat human strongyloidiasis, the disease remains endemic in those communities. Here for the first time we looked at the Australian dogs' potential to infect humans and found that they carry two genetically distinct strains of *Strongyloides* spp., one of which also infects humans. This supports the hypothesis that dogs are a potential source for human strongyloidiasis. We also found that dogs in Australia might be carrying unique haplotypes. Whether these new haplotypes are also human infective is to be confirmed by further research.

5.2.3 Introduction

Strongyloidiasis is caused by the human infective nematodes *Strongyloides stercoralis, Strongyloides fuelleborni* subsp. *fuelleborni* and *Strongyloides fuelleborni* subsp. *kellyi* (Grove, 1996). Worldwide, *Strongyloides* spp. are estimated to infect up to 370 million people, predominately in socioeconomically disadvantaged communities (Olsen et al., 2009, Bisoffi et al., 2013, Beknazarova et al., 2016b). While *S. stercoralis* is a globally distributed nematode, *S. f. fuelleborni* has thus far only reported in Africa and

Southeast Asia and *S. f. kellyi* from Papua New Guinea (PNG) (Thanchomnang et al., 2017, Pampiglione and Ricciardi, 1971, Ashford et al., 1992)[8].

In Australia, strongyloidiasis is prevalent in remote communities located across the Northern Territory, Queensland, Western Australia, northern South Australia and northern New South Wales (Page et al., 2016). Current estimates of infection rates in some communities are up to 41% or 60% based on microscopy or serology respectively (Heydon and Green, 1931, Sampson et al., 25-26 June 2003, [9]Miller et al., 2018, Holt et al., 2017). Despite initially successful intervention programs targeting treatment to eliminate human strongyloidiasis in remote Australian communities, the disease remains endemic (Kearns et al., 2017, Page et al., 2006). Reappearance of the infection could possibly be as a result of zoonotic transmission from dog reservoirs given that dogs and humans share a close and intimate cultural bond in rural and remote Indigenous communities of Australia (Constable et al., 2010).

The zoonotic potential of *S. stercoralis* infected dogs and their potential role in the maintenance of strongyloidiasis transmission has been a topic of interest and discussion for many years (Beknazarova et al., 2017b, Goncalves et al., 2007, Takano et al., 2009). Molecular investigation of human and dog derived *S. stercoralis* is useful for understanding the nature of cross infection. There are two regions of the *S. stercoralis* nuclear and mitochondrial genome that are considered to be conserved within the *Strongyloides* genus and can be used as markers for molecular typing of *S. stercoralis*. Hyper-variable regions (HVR) I and IV of the small Subunit (*SSU*) 18S ribosomal DNA and the cytochrome c-oxidase subunit 1 (*cox*1) gene of the mitochondrial DNA (mtDNA) have been widely used to study relationships between *S. stercoralis* from different hosts and different geographic locations (Hasegawa et al., 2009, Hasegawa et al., 2010, Jaleta et al., 2017, Nagayasu et al., 2017, Basso et al., 2018). Based on genetic analysis of these loci, it has been recently found that there are two genetically different *S. stercoralis* strains, one is dog and human infective, and the other is dog specific. These data were collected from dogs and humans in Cambodia and Japan[10] (Jaleta et al., 2017, Nagayasu et al., 2017).

Being an isolated continent that has been separated from other regions for a long geological period, Australia could represent an interesting addition to our understanding of the genetic diversity within *S. stercoralis* and the *Strongyloides* genus more generally. Indigenous Australians have inhabited the continent for at least 40,000 years and dogs (in the form of dingoes) were likely introduced up to 12,000 years ago (Clutton-Brock, 1995). Given this long period of relative isolation, it might be expected that

[8] Pampiglione and Ricciardi, 1971, Ashford et al., 1992, Thanchomnang et al., 2017
[9] Sampson et al., 2003.
[10] dogs and humans in Cambodia and Myanmar (not Japan)

Australia could harbor unique endemic genotypes or unique sub-species of *S. stercoralis* that have evolved within dog and human populations over this period.

Using PCR and amplicon sequencing (Illumina sequencing technology), we sequenced the *Strongyloides SSU* 18S rDNA hyper-variable I and hyper-variable IV regions using *Strongyloides*-specific primers, and a fragment of the mtDNA *cox*1 gene using primers that are broadly specific for *Strongyloides* sp. and hookworms. This approach was applied to DNA extracted from human and dog faeces, and one human sputum sample. The main focus of this study was to genotype Australian human and dog *S. stercoralis* strains to see whether dogs carry human *S. stercoralis* strains and/or vice versa. To our knowledge this is the first time human and dog *S. stercoralis* have been studied in Australia on a molecular level.

5.2.4 Methods

Study area and faeces collection

Dog faecal samples were collected from communities in the Northern Territory, Australia. Dog faeces were collected from the environment (i.e., the ground) in the selected communities and stored in the DESS (dimethyl sulfoxide, disodium EDTA, and saturated NaCL) solution to preserve the DNA (Beknazarova et al., 2017a). For those dog faeces that were collected from privately owned land, the consent forms were received to collect the samples. The preserved faecal samples were express posted to the Environmental Health laboratory, Flinders University, South Australia, for further analysis.

Human faecal and sputum samples were provided by our colleagues at the Royal Darwin Hospital, NT, AusDiagnostics Pty Ltd, NSW, and Townsville Hospital, Queensland. While the personally-identifying information of the patients was de-linked from our analyses, their infections are known to have been locally acquired. Ethics approval from the Social and Behavioural Research Ethics Committee (SBREC) No 6852 dated 1st June 2015 was obtained for collecting dog faeces from the remote communities. Human ethical approval from the Southern Adelaide Clinical Human Research Ethics Committee (SAC HREC) No 309.17 dated 24th January 2018 was obtained for comparing *S. stercoralis* DNA extracted from human and dog tissues. CDC investigators were not engaged with sample collection and their participation did not include engagement with human or animal subjects.

DNA extraction

Prior to DNA extraction, faecal samples containing DESS were centrifuged for three minutes at 3000 x g rpm using an Orbital 400 Clements (Phoenix, Lidcombe, Australia). The supernatant consisting of the preservative solution was removed. The remaining faecal sample was washed with sterile saline

solution. DNA was extracted using the Power Soil DNA isolation kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions with slight modifications that included incubating samples at 56ºC overnight after the cell lysis step, followed by vortexing of samples for three minutes. Approximately 250 milligrams of the pellet was placed into a PowerBead tube containing lysis buffer (included in the Power Soil DNA extraction kit). The remainder of the extraction process was performed according to the manufacturer's instructions. Approximately 100 µL of extracted DNA was stored at -20°C prior to real-time PCR (qPCR) analysis.

Real-time PCR

The samples (273 dog and 4 human DNA samples) were first screened for *Strongyloides* spp. using qPCR in the Environmental Health laboratory at Flinders University, SA, Australia. The real-time PCR assay was adopted from Verweij *et al.* (2009) using *S. stercoralis* - specific primers targeting a 101 base pair region of 18S rRNA. The 20 µL reaction contained 10 µL Supermix (SSoAdvanced, Universal Probes Supermix, Foster City, Bio-Rad Laboratories, CA, USA), 1 µL primers and probe mixture (1x) (Stro18S-1530F, Stro18S-1630R and Stro18S-1586T FAM) (Sto 18S PrimePCR probe assay, Bio-Rad Laboratories, CA, USA), 4 µL deionised H_2O, and 5 µL DNA template. All qPCR reactions were performed in triplicate on the Corbett Rotor-Gene 6000 machine (QIAGEN, Hilden, Germany) (Verweij et al., 2009). *S. stercoralis* primers and probes and qPCR conditions used are shown in Table 5.1. A sample was considered positive when the Ct value was lower than the mean negative Ct minus 2.6 standard deviations of a mean negative control Ct. Positive samples were amplified in every qPCR reaction.

Conventional PCR for amplification of *SSU* HVR-I and HVR-IV, and *cox*1 sequences

Extracted DNA from samples that were qPCR positive for *Strongyloides* spp. (47 dog and four human DNA samples) was shipped on dry ice to the Centers for Disease Control and Prevention (CDC), Georgia, USA for conventional PCR, sequencing and bioinformatics analysis. Hyper-variable regions (HVR) I and IV in the small Subunit (*SSU*) 18S ribosomal DNA and a fragment of the mitochondrial cytochrome c-oxidase subunit 1 (*cox*1) gene were amplified using conventional PCR and then sequenced using Illumina technology. All PCR reactions were performed on a GeneAmp PCR System 9700 Thermo Cycler, version 3.12 (Applied Biosystems, USA). *S. stercoralis* primers and PCR conditions used for qPCR and conventional PCR are shown in Table 5.1. For the *cox*1 gene, PCR reactions were performed in a total volume of 50 µL containing 10 µL NEB 5X Q5® Buffer (New England BioLabs, USA), 10 µL NEB 5X Q5® High GC Enhancer (New England BioLabs, USA), 4 µL NEB Deoxynucleotide Solution Mix (10 mM each nt) (New England BioLabs, USA), 1 µL Q5® High-Fidelity DNA Polymerase (New England BioLabs, USA), 2.5 µL 10 µM forward primer (SSP_COX1_F), 2.5 µL

10 µM reverse primer (SSP_COX1_R), 18 µL deionised H_2O, and 2 µL DNA template. For the HVR-I and HVR-IV regions, PCR reactions were performed in a 25 µL reaction containing 12.5 µL of NEBNext® Q5® Hot Start HiFI PCR Mastermix, MO543L (New England BioLabs, USA), 1.5 µL 10 µM forward primer (NEW_HVR_I_F or NEW_HVR_IV_F), 1.5 µL 10 µM reverse primer (NEW_HVR_I_R or NEW_HVR_IV_R), 7.5 µL deionised H_2O, and 2 µL DNA template.

The amplified PCR products were separated by 1.5% agarose gel electrophoresis and stained with ethidium bromide. The stained DNA bands were visualised by UV illumination using a Ugenious 3 (SYNGENE, Japan). For quality control, each PCR run included a positive control containing *Strongyloides* genomic DNA as template, a non-template control containing autoclaved sterile water instead of template, and a negative control containing DNA extracted from a parasite-free specimen. Amplicons for each of the three markers were also generated for *Strongyloides ratti* as an additional control for the sequencing and *in silico* analysis steps.

Next Generation Sequencing (NGS)

Ten microliters of PCR amplicon was purified and normalized for concentration prior to library preparation using a SequalPrep Normalization Plate Kit (Thermo Fisher Scientific, USA). DNA libraries were prepared using the NEBNext Ultra DNA Library Prep Kit for Illumina (New England BioLabs, USA), and NEBNext Multiplex Oligos for Illumina Index kit (New England BioLabs, USA). The NEBNext Ultra DNA Library Prep Kit was used as it does not include a tagmentation step. Consequently, adapters and indices are added to the ends of the amplicon without fragmenting the DNA. This produces paired reads with each mate spanning approximately 250 bases up each end of the amplicon, so that when overlapping reads are merged, the entire length of our short amplicons is covered (Table 5.1). The sequencing reactions were prepared using the MiSeq reagent Nano Kit v2 (PE250bp), and performed on the Illumina MiSeq platform (Illumina). The Illumina MiSeq reads generated for successfully sequenced specimens were uploaded to NCBI and are available under BioProject accession number PRJNA531959.

Table 5.1 Primers and probes and PCR conditions

Primer	Amplicon	Sequence	Reaction conditions
Stro18S-1530F Stro18S-1630R Stro18S-1586T FAM	rDNA 101 bp	5'-GAATTCCAAGTAAACGTAAGTCATTAGC-3' 5'-TGCCTCTGGATATTGCTCAGTTC-3' 5'-ACACACCGGCCGTCGCTGC-3'-BHQ1	**Step 1:** 95°C for 15 min, **Step 2:** 95°C for 15 s, **Step 3:** 60°C for 30 s. Repeat steps two and three 40 times.
SSP_COX1_F * SSP_COX1_R *	mtDNA 270 bp	5'-TTTGATCCTAGTTCTGGTGGTAATCC-3' 5'-GTAGCAGCAGTAAAATAAGCACGAGA-3'	**Step 1:** 98°C for 30 s, **Step 2:** 98°C for 10 s, **Step 3:** 60°C for 10 s, **Step 4:** 72°C for 10 s, **Step 5:** 72 °C for 2 min. Repeat steps two to four 45 times.
NEW_HVR_I_F NEW_HVR_I_R	rDNA 500 bp	5'-GCTCATTATAACAGCTATAGACTACACGGTA-3' 5'-CCACAACAATCATTTTATGCACTTGG-3'	**Step 1:** 98°C for 30 s, **Step 2:** 98°C for 10 s, **Step 3:** 60°C for 10 s, **Step 4:** 72°C for 10 s, **Step 5:** 72 °C for 2 min. Repeat steps two to four 45 times.
NEW_HVR_IV_F NEW_HVR_IV_R	rDNA 320 bp	5'-CGGGCCGGACACTATAAGG-3' 5'-ATCTCTAAACAGGAACATAATGATCACTAC-3'	**Step 1:** 98°C for 30 s, **Step 2:** 98°C for 10 s, **Step 3:** 63°C for 10 s, **Step 4:** 72°C for 10 s, **Step 5:** 72 °C for 2 min. Repeat steps two to four 45 times.

In silico analysis

The Illumina reads were analyzed using Geneious (www.geneious.com) by means of a workflow that performed read quality control, assembly of contigs and genotype assignment. As part of this workflow, quality trimming to a minimum phred score of 20 and removal of adapter sequence was performed using BBDuk (v 37.64). Reads less than 50 bases in length were discarded. Paired reads were then merged using BBMerge (v 37.64) and all reads (merged and unmerged) were mapped to a reference sequence. For *cox*1 amplicons, reads were mapped to a *S. stercoralis* sequence with the GenBank (GB) accession number LC050212.1. For *SSU* HVR-I and *SSU* HVR-IV, reads were mapped to a *S. stercoralis* with the accession AF279916.2. Prior to mapping, each reference sequence was trimmed to the length of the amplicon, excluding the primer sequences. Mapping was performed using the Geneious mapper under the Medium sensitivity / Fast default settings. This mapping served as a read filtering step to exclude reads derived from spurious PCR artifacts, off target amplifications, or DNA from the fecal extract. Merged reads (and their corresponding mates for reads that could not be merged) that successfully mapped were retained for *de novo* assembly. These reads were assembled using the Geneious *de novo* assembler with the following customized parameters; minimum overlap: 50 bases, minimum overlap identity: 100%, maximum number of mismatches per read: 0%, and the maximum number of ambiguities: 1. Contigs were split if coverage fell below 50 bases and sub-variants with coverage less than 50 were not considered. The short amplicon lengths facilitated generation of merged reads that spanned the entire amplicon in most cases. Consequently, these *de novo* assembled contigs represent a consensus of large numbers of identical reads. To identify multiple haplotypes within a single specimen using these reads, the option to merge contigs with coverage less than 100 was selected during *de novo* assembly. According to Geneious documentation, setting this value to 100 requires that at least 100 reads, each with a Phred base quality score of 30 at a potential variant site, map to that variant site before a new variant is considered real and used to construct a new contig (i.e., a new haplotype). Consequently, variant bases were supported by a cumulative Phred quality score of greater than 3,000 before a new haplotype was generated. If some reads supporting a variant site had a Phred quality score less than 30 at this site, more reads were required to support the variant and call the new haplotype (i.e., until the cumulative Phred quality score exceeded 3,000). Contigs were trimmed to the primer sequence using the 'Trim Ends' function in Geneious. These high-quality contigs were validated further by taking the trimmed and merged reads and mapping them back to the contigs generated using the Geneious mapper, using the following custom parameters: minimum mapping quality: 30 bases, minimum overlap: 150 bases, minimum overlap identity: 100%, maximum number of mismatches: 0%, and the maximum number of ambiguities: 1. Contigs were split or discarded if the coverage fell below approximately 300 to 500 depending on the depth obtained for a particular specimen (~500 for specimens that obtained >10k reads, ~300 bases for specimens with <10k reads). Following mapping, each alignment was manually examined in Geneious for misalignments and gaps. Contigs

were considered valid if the coverage obtained approached or exceeded these thresholds, and if misalignments and gaps were absent upon manual examination. The number of merged reads that mapped back to each contig during this final validation step are listed in Table 5.S1. Finally, haplotypes were assigned by performing a local BLASTN search (within Geneious) against a database constructed from all unique *Strongyloides* sp. 18S and *cox*1 sequences available in GenBank and the DNA Databank of Japan. The *cox*1 sequences used to construct this BLAST database are provided in Table 5.S2 and GenBank accession numbers for sequences used to construct the 18S database are listed in Tables 5.3 and 5.4. A set of homologous sequences from several other roundworms (parasitic and free-living) were also included in this database. Sequences were only added to the BLASTN database if they overlapped our 217 bp *cox*1 amplicon by more than 95%. This included several previously published *cox*1 haplotypes that had matching *SSU* HVR-I and HVR-IV haplotypes assigned (Jaleta et al., 2017).

Any specimens for which *Strongyloides ratti* sequences were detected as part of this workflow were considered to be at risk of contamination from our positive control and potentially from other specimens included in the study. Any contaminated specimens were excluded from further analysis.

Construction of a *cox*1 cluster dendrogram

Sequences of *cox*1 were exported from Geneious as a fasta file and were aligned using the 'msa' package in R. The 'dist.alignment' function from the 'seqinr' package was used to compute a pairwise identity matrix, considering gaps in the identity measure. The resulting matrix was clustered using the agglomerative nested clustering approach performed with the 'agnes' R package, using "euclidean" distances and the "average" clustering method. A cluster dendrogram was generated using the 'ggtree' R package. Vector images (i.e. graphics) used for annotation of the dendrogram were either generated in house at CDC or obtained from PhyloPic (http://phylopic.org).

5.2.5 Results

Real-time PCR, conventional PCR and sequencing

We screened 273 dog and four human samples using real-time PCR. Forty seven (47) dog and four human samples that were positive or presumed positive (higher Ct values) by qPCR were selected for conventional PCR with further sequencing of their *cox*1, *SSU* HVR-I and HVR-IV regions. Out of 47 dog samples, there were samples with lower CT values, and in some cases, useable sequences were not generated due to poor amplification of the PCR product. Sequence data was obtained for 24 specimens including four human specimens and twenty dog specimens. The complete set of amplified sequence variants (ASVs) of all specimens analyzed in this study is shown in Figure 5.1 and summarized in Table 5.2.

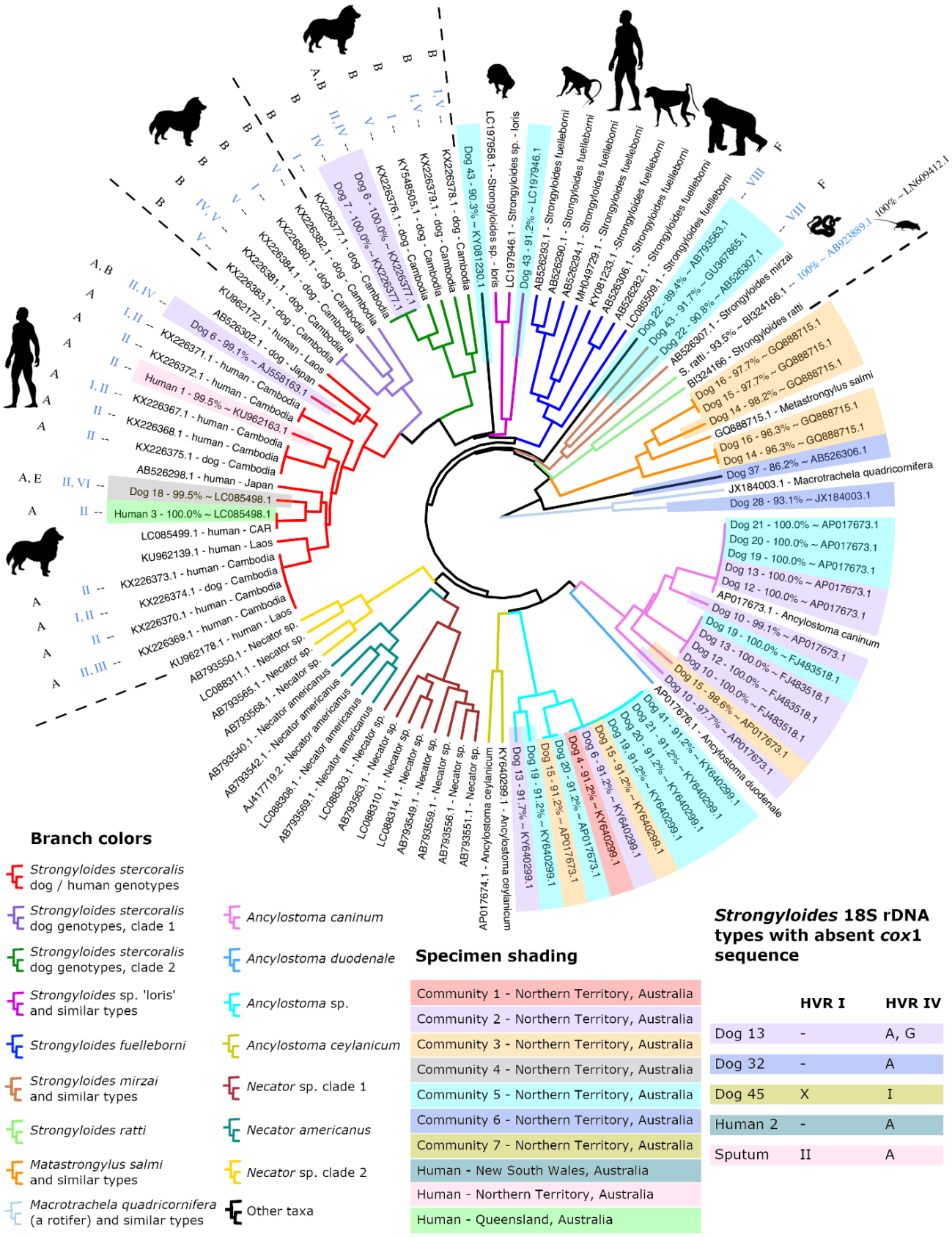

Figure 5.1 Dendrogram of clustered *cox*1 amplicons from Australian dog and human specimens

This dendrogram includes *cox*1 sequences generated in this study and a selection of previously published *cox*1 sequences that overlap with our 217 base pair *cox*1 amplicon by 100%. Specimens analyzed as part of this study are shaded according to their site of collection. Branches are color coded according to their identity; either a species assignment, a proposed genus assignment, or their *S. stercoralis* genotype. When available, *Strongyloides* sp. *cox*1 sequences are annotated with their associated *SSU* haplotypes, with their HVR-I type shown in blue and their HVR-IV type shown in black. Specimens for which a *cox*1 sequence was not obtained are shown in a table embedded in the figure (bottom right), which includes two specimens possessing unique 18S haplotypes; dog 13 (HVR-IV, type G) and dog 45 (HVR-I, type X). A dash (-) shown in this table indicates failed amplification and/or sequencing of that marker. 'Sputum' refers to the sole sputum sample from a human patient (human 4) included in this study. Sequences published in previous studies that are not from *S. stercoralis* are labelled with their GenBank and/or DNA Data Bank of Japan accession numbers followed by their species name. *Strongyloides stercoralis* sequences from previous studies are labelled with their accession number, host species, and country of origin. Note that 'CAR' means Central African Republic. Names of specimens collected as part of this study begin with a host name and a unique number assigned in this study, followed by a percentage similarity to (~) a near BLASTN hit identifiable by its accession number.

Table 5.2 Human and dog samples analyzed in this study and their haplotypes

Sample	HVR-I haplotype	HVR-IV haplotype	*Cox*1 Accessions
Human 1	Haplotype II	Haplotype A	MK434219†
Human 2	NA (excluded due to *S. ratti* contamination)	Haplotype A	NA
Human 3	Haplotype II	Haplotype A	MK434218
Sputum (Human 4)	Haplotype II	Haplotype A	NA
Dog 4	NA	NA	MK434258†
Dog 6	Haplotype II and Haplotype IV	Haplotype A and Haplotype B	MK434255 MK434256† MK434257†
Dog 7	Haplotype IV	Haplotype B	MK434254
Dog 10	NA	NA	MK434251 MK434252† MK434253†
Dog 12	NA	NA	MK434249 MK434250
Dog 13	NA	Haplotype A and Haplotype G*	MK434246 MK434247 MK434248†
Dog 14	NA (excluded due to *S. ratti* contamination)	NA	MK434244† MK434245†
Dog 15	NA	NA	MK434240† MK434241† MK434242† MK434243†
Dog 16	NA	NA	MK434238† MK434239†
Dog 18	Haplotype II and Haplotype VI	Haplotype A and Haplotype E*	MK434237†
Dog 19	NA	NA	MK434233 MK434234 MK434235† MK434236†
Dog 20	NA	NA	MK434230 MK434231† MK434232†
Dog 21	NA	NA	MK434228 MK434229†

Dog 22	Haplotype VIII*	Haplotype F*	MK434226† MK434227†
Dog 28	NA	NA	MK434225†
Dog 32	NA	Haplotype A	NA
Dog 37	NA	NA	MK434224†
Dog 41	NA	NA	MK434223†
Dog 43	NA	NA	MK434220† MK434221† MK434222†
Dog 45	Haplotype X*	Haplotype I*	NA

* Novel *SSU* haplotypes, †Novel *cox*1 sequences
Note: All GenBank Accession numbers associated with the sequences generated in this study are provided in Table 5.S1

SSU HVR-I haplotypes detected among *S. stercoralis* from Australian dogs and humans

Jaleta *et al.* (2017) previously sequenced the *SSU* HVR-I region of *S. stercoralis* worms from Cambodian dog and human specimens and identified five different haplotypes (HVR-I haplotypes I-V) (Jaleta et al., 2017). A recent study of European dogs identified a new haplotype from the HVR-I region (haplotype VI) (Basso et al., 2018), which was previously mentioned in the Hasegawa *et al.* (2009) study (AB453316.1 and AB453314.1) (Hasegawa et al., 2009) (Table 5.3). In our Australian samples we found haplotype II in both human and dog samples and haplotype IV in dog samples only, which is consistent with the findings from Jaleta *et al.*(Jaleta et al., 2017). We also identified haplotype VI in a single Australian dog. Following the haplotype nomenclature developed by Jaleta *et al.* (2017) and Basso *et al.* (2018), we discovered two new HVR-I haplotypes; haplotypes VIII and X (Figure 5.2, Table 5.2), in addition to these six haplotypes previously described (Jaleta et al., 2017, Basso et al., 2018), Due to the existence of noteworthy similarities (> 99% in all cases) between sequences of *S. stercoralis*, *Strongyloides procyonis*, a sequence assigned to *Strongyloides* sp. Okayama (GB: LC038066.1), and our novel dog sequences, we expanded the Jaleta *et al*. typing scheme to include these sequences (Jaleta et al., 2017). This involved inclusion of haplotypes that could not be confidently assigned to *S. stercoralis* given the information on hand, yet are highly similar to known *S. stercoralis* 18S haplotypes. This adjustment was also required because a sequence attributed to *S. procyonis* (GB: AB272234.1) possesses HVR-I haplotype IV, which is identical to an *S. stercoralis* haplotype assigned to a Cambodian dog (GB: KU724124.1). For details, refer to Figure 5.2, and Tables 5.2 and 5.3.

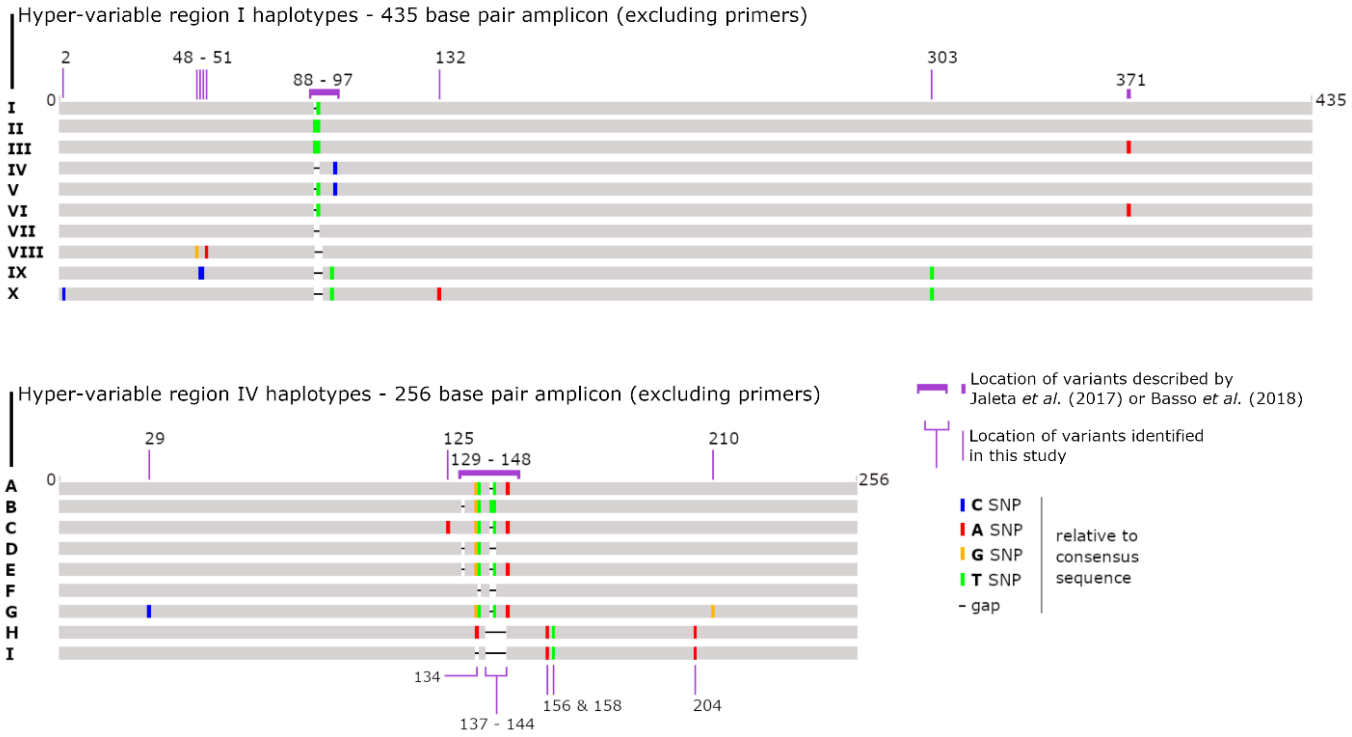

Figure 5.2 Schematic detailing the proposed modifications to the previously *described Strongyloides stercoralis* genotyping scheme

A graphical representation of the novel *Strongyloides* haplotypes discovered in this study compared to haplotypes identified in previous reports. The location of sites that were genotypically informative based on the original genotyping method described by Jaletta *et al.* (2017) and Basso *et al.* (2018) are indicated, as well as new SNP/indel sites that have been incorporated into the typing scheme based on the results of this study (Jaleta et al., 2017, Basso et al., 2018). For hypervariable region I, we introduce two novel types (VIII and X), and assign new haplotype names to published sequences that had not been previously considered in this typing scheme (VII and IX). For hypervariable region IV, we introduce three novel types (E, F, G and I), and assign new haplotype names to published sequences that had not been previously considered in this typing scheme (C, D and H) (see Tables 5.3 and 5.4 for details).

Table 5.3 HVR I haplotypes assigned to *Strongyloides* sp. based on current data

Haplotypes	GenBank Accession/s	Notes
I	AB923888.1, AF279916.2, AJ417023.1	Found in dogs and humans.
II	KF926659.1, MK468655, MK468656, MK468657, MK468658, MK778085	Found in dogs and humans.
III	AB453315.1	Found in dogs and humans.
IV	AB272234.1, KU724124.1, MK468663	This haplotype has been detected in dogs though not in humans. It has also been described in a badger where it was assigned to *S. procyonis.*
V	Jaleta *et al*. (2017)	Described by Jaleta *et al*. (2017) but the sequence of this type was not available in GenBank prior to this study. This is potentially a dog-specific haplotype.
VI	AB453316.1, AB453314.1, MH932098.1, MH932099.1, MH932100.1, MK468660	Assigned only to *S. stercoralis*. Identified in dog 18 from this study. Described predominantly in dogs but also in a chimpanzee (AB453314.1).
VII	AB205054.1	A *S. procyonis* sequence greater than 99% similar to *S. stercoralis* Haplotype I and Haplotype IV.
VIII	MK468661	Novel *Strongyloides* sp. sequence from Dog 22 most similar to Haplotype VII.
IX	LC038066.1	*Strongyloides* sp. Okayama isolated from a Japanese striped snake. This sequence is greater than 99% identical to sequences of *S. stercoralis* and *S. procyonis*. Similar to Haplotype X (see below) identified in an Australian dog.
X	MK468662	Sequence identified in dog 45 in present study. Most similar to *Strongyloides* sp. Okayama (Haplotype IX).

SSU HVR-IV haplotypes detected among *S. stercoralis* from Australian dogs and humans

In the Australian samples we identified HVR-IV haplotype A in both humans and dogs, and haplotype B only in dogs, as previously observed (Jaleta et al., 2017). Supporting the findings of Jaleta *et al*. (2017), our results also showed that haplotype II of HVR-I is found in combination with haplotype A of HVR-IV, and haplotype IV of the HVR-I region is only found in combination with haplotype B of the HVR-IV region (Jaleta et al., 2017). We observed that a unique sequence attributed to *S. stercoralis* had been submitted to GenBank in 1993, and this was assigned to haplotype C (GB: M84229.1). Next, given the strong similarity between HVR-IV sequences of *S. procyonis* and *S. stercoralis* and the fact that HVR-I haplotype IV (Jaleta *et al*. 2017) is also found in *S. procyonis SSU* sequences, we assigned the HVR-IV sequence from *S. procyonis SSU* DNA to haplotype D (GB: AB272234.1 and AB205054.1) (Table 5.4). A HVR-IV haplotype 99% similar to the *Strongyloides* sp. Okayama (GB: LC038066.1) was detected in dog 45. Therefore, the HVR-IV sequence of *Strongyloides* sp. Okayama was assigned to haplotype H, and the sequence from dog 45 was assigned to Haplotype I. Consequently, four new haplotypes detected in four Australian dog samples were assigned to HVR-IV haplotypes E, F, G and I (Figure 5.1, Table 5.2 and 5.4).

Clustering of *Strongyloides stercoralis* based on *cox*1 sequences

A 217 base pair fragment of *cox*1 was sequenced from 20 Australian specimens including those from 18 dogs and two humans, plus the *S. ratti* control (21 *cox*1 sequences in total). Multiple *cox*1 types were obtained from a single specimen in many cases, revealing infections caused by multiple helminth species and multiple *S. stercoralis* genotypes in a single host (Figure 5.1). Dendrogram construction by agglomerative nested clustering revealed three distinct *S. stercoralis* clades, including one occupied predominantly by worms possessing the II/A *SSU* genotype, which constituted sequences obtained from dogs and humans. Four *cox*1 sequences obtained in this study (one from each of human 1, human 3, dog 6, and dog 18), were assigned to the dog and human-infecting *S. stercoralis* clade. A *S. stercoralis* clade occupied mostly by specimens possessing the I/B and V/B *SSU* genotypes was also apparent (one specimen possessed the IV/B genotype), representing dog infections only (dog clade 1). None of the Australian specimens were assigned to this clade (Figure 5.1). A final *S. stercoralis* clade containing *cox*1 sequences obtained from only dogs (dog clade 2) was also dominated by specimens possessing the I/B and V/B genotypes, though two specimens were also assigned the IV/B. A single *cox*1 sequence from each of dogs 6 and 7 was assigned to this clade (Figure 5.1)

Table 5.4 HVR IV haplotypes assigned to *Strongyloides* sp. based on current data

Haplotypes	GenBank Accession/s	Notes
A	KY081221.1, KU724128.1, KU724125.1, LC085483.1, LC085482.1, LC085481.1, KU962182.1, KU962181.1, KU962180.1, KU962179.1, AB923888.1, KF926662.1, KF926661.1, AF279916.2, MH932097.1, MH932097.1, MH932095.1, KY081223.1, AB526826.1, AB453316.1, AB453315.1, AB453314.1, MK468664 - MK468671	Identified in dogs, humans and chimpanzees. A *S. stercoralis*- specific haplotype
B	KU724129.1	Identified only in dogs. Consistently assigned to *S. stercoralis*
C	M84229.1	This sequence was published in GenBank in 1993 and assigned to *S. stercoralis*. It has not appeared in the literature since based on our knowledge, though we assigned to haplotype C for its historic value. It shares one SNP difference to type A (Figure 2).
D	AB272234.1, AB205054.1	Includes two sequences assigned to *S. procyonis*. This type is 99% similar to Haplotype B.
E	MK468674	Novel haplotype identified in dog 18. Most similar to Haplotype A.
F	MK468675	Novel haplotype identified in dog 22. Most similar to Haplotype D.
G	MK468676	Novel haplotype identified in dog 13. Most similar to Haplotype A.
H	LC038066.1	*Strongyloides* sp. Okayama isolated from a Japanese striped snake.
I	MK468677	Novel haplotype identified in dog 45. Most similar to Haplotype H.

Cryptic *cox*1 sequences potentially from *Strongyloides* sp. helminths that could not be assigned to a genus or species

Sequences were obtained from two dog fecal specimens (dogs 22 and 43) that potentially belong to a *Strongyloides* sp. helminth but could not be confidently assigned to a species given the information available. Two *cox*1 sequences were obtained for dog 22. One of these clustered with a *cox*1 sequences from *Strongyloides mirzai* (GB: AB526307.1), a helminth that infects a Japanese pit viper. The second sequence from dog 22 clustered between the *S. mirzai* clade and the *S. fuelleborni* clade yet also clustered in a position immediately basal to all hookworms (Figure 5.1). This sequence also obtained a nearest BLASTN hit to a *Necator* sp. sequence (GB: AB793563.1), though its next best hit based on an online BLASTN search was to a sequence from *S. stercoralis* (GB: LC179452.1). Three unique *cox*1 sequences were obtained from dog 43 (GB: MK434220, MK434221; MK434222), one clustering with *S. mirzai* and a second clustering with two sequences of the *Strongyloides* sp. 'loris' clade (GB: LC197958.1, LC197946.1). When submitted to an online BLASTN search, the sequence clustering alongside *S. mirzai* also obtained top hits to free living nematodes (*Ektaphelenchus* sp. and *Bursaphelenchus populi*, GB: JX979197.1 and HQ699854.1 respectively), yet several of its top hits were also to *S. fuelleborni cox*1 sequences. The third sequence from dog 43 clustered between the *Strongyloides* sp. 'loris' clade and a clade containing all *S. stercoralis* sequences (Figure 5.1).

Mixed genotype infections with *Strongyloides stercoralis*

In two samples, dog 6 and dog 18, a complete set of ASVs was obtained (a sequence for *cox*1, HVR-I and HVR-IV), indicating mixed *S. stercoralis* infections. When examining the number of reads that mapped to each haplotype for these specimens (Table 5.S1), for dog 6 approximately 20% of reads were assigned to haplotype II and 80% to haplotype IV for the HVR-I region. For the HVR-IV region, approximately 20% of reads were assigned to haplotype A and 80% to haplotype B. With this information it can be assumed that this dog was infected with two strains of *S. stercoralis*, one from the human / dog clade (genotype II/A) and another from a dog-specific clade (genotype IV/B). However, it should be noted that the assays for amplification of HVR-I and HVR-IV could possess different amplification efficiencies, though this is difficult to confirm. Interestingly, two *cox*1 sequences were obtained from this dog, one assigned to the human / dog clade and another assigned to dog clade 2, supporting our deduction. Dog 18 was also infected with two types of *S. stercoralis*, with a genotype of II + VI / A + E assigned to this specimen. Given that the number of reads assigned to each of these types fell between 40% and 50%, it is difficult to link the HVR-I types identified here to their corresponding HVR IV type. While the specimen from dog 18 possessed two 18S haplotypes for HVR-I and HVR-IV, evidence was only found for a single *cox*1 sequence. There were two *S. stercoralis*

strains found in the HVR-IV region of the dog 13. Approximately 50% of reads were assigned to the haplotype A and 50% to a new haplotype G.

Detection of non-*Strongyloides* sp. *cox*1 sequences in remote Australian communities

Dog samples were collected from seven remote communities in Australia. While all specimens included in this study tested positive or presumed positive for *S. stercoralis* using a published real-time PCR assay (Verweij et al., 2009), deep sequencing found no evidence of *S. stercoralis* infection in several cases where instead, an infection with another helminth (usually *Ancylostoma* spp.) was confirmed based on *cox*1 sequences. Some of these sequences clustered closely to *A. ceylanicum* yet appeared to be distinct. However, it should be noted that these sequences were identical to a published sequence assigned to *A. caninum* (GB: AJ407962.1) which did not overlap completely with our amplicon (50 bases short), so we cannot be sure they possess the same sequence type. This necessitates the conservative assignment of these sequences to *Ancylostoma* sp.

Twenty-three *cox*1 sequences were attributed to *Ancylostoma* spp. and one dog (dog 6) was infected with an *Ancylostoma* sp. clustering closely with *Anyclostoma ceylanicum* (Figure 5.1, cyan clade), and two distinct types of *S. stercoralis*. Sequences were obtained from dogs 14, 15 and 16 that belong to a *Metastrongylus*-like helminth, possibly *Metastrongylus salmi*. Two sequences attributed to *Ancylostoma caninum* were also obtained from dog 15, and a fourth sequence belonging to an *Ancylostoma* sp. was also detected in this dog. A sequence was obtained from dog 37 that obtained BLASTN hits to *S. fuelleborni* sequences (e.g., GB: AB526303.1, 86.2% identity). Agglomerative nested clustering placed this sequence in a position basal to all *Strongyloides* and hookworm sequences included in this analysis. This *cox*1 sequence also obtained close BLASTN hits (87% identity) to *Aphelenchoides* sp. (GB: KX356839.1) and *Bursaphelenchus luxuriosae* (AB097863.1) which are free living mycophagous and/or potentially plant parasitic nematodes. The *cox*1 sequence from dog 28 does not appear to be helminth in origin and most closely resembles a *cox*1 sequence from a rotifer; *Macrotrachela quadricornifera* (GB: JX184003.1), which served as a convenient outgroup for the clustering analysis (Figure 5.1).

Two dogs from community 2 were also infected with *S. stercoralis* while in one dog from community 5, a cryptic *Strongyloides* sp. possessing a unique *SSU* haplotype for both HVR-I and HVR-IV was detected (dog 22, genotype VIII/F). Interestingly, a *Metastrongylus*-like *cox*1 sequence was detected in all dogs from community 3 that were tested, and a single dog (dog 15) from this same community was infected with *A. caninum* and at least one other *Ancylostoma* sp. (Figure 5.2, cyan clade). We propose that sequences obtained from community 6 are from environmental organisms, possibly representing extraneous contaminants given that one represents a rotifer-like sequence (dog 28) (GB:

MK434225) and the other obtained BLASTN hits to free-living nematodes (dog 37) (GB: MK434224). Community 4 and community 7 are each represented by a single typed specimen each (dog 18 and dog 45 respectively), that include unique sequences from a helminth that we can confidently assign to the genus *Strongyloides* (Figure 5.1).

5.2.6 Discussion and conclusions

In our study we observed that HVR-IV haplotype A is associated with strains infective for both humans and dogs, while HVR-IV haplotype B is restricted to strains that are only infectious to dogs. The same was discovered in a recent study on *S. stercoralis* from humans and dogs in Cambodia, where two genetically distinguishable *S. stercoralis* populations were identified based on the HVR-IV region. The HVR-IV haplotype A strain was found to be dog and human infective, while HVR-IV haplotype B strain was shown to be dog specific (Jaleta et al., 2017). Supporting earlier findings, our results also showed that haplotype II of HVR-I is found in combination with haplotype A of the HVR-IV region, and haplotype IV of the HVR I region is only found in combination with haplotype B, which is specific to dogs. One dog had a mixed *S. stercoralis* infection, presumably with worms of the genotype II/A (a type infectious to dogs and humans) and others with the genotype IV/B (a dog specific type). The detection of two *cox*1 sequences that cluster in the dog / human and dog specific clades respectively supports this assessment. In an Australian dog, we also identified HVR-I haplotype VI which has only been previously reported in European dogs. Interestingly, this dog (dog 18) also had a mixed gentoype infection that included a novel *S. stercoralis* HVR-IV haplotype (haplotype E), that was linked to a *cox*1 sequence clustering in the dog / human *S. stercoralis* clade.

In agreement with previous reports, the current study demonstrated that the *SSU* HVR IV region in the *SSU* rDNA can be used to detect within species differences that correspond with the genetic clades that appear when the same specimens are analyzed at the *cox*1 locus (Hasegawa et al., 2016, Jaleta et al., 2017) (Figure 5.1). To support analysis of the *cox*1 locus by deep sequencing, the *cox*1 PCR assay described here was designed so that merged paired-end Illumina reads span the entire length of the amplicon. This greatly reduces the complexity of *in silico* analysis when mixed *cox*1 haplotypes are encountered. A trade-off of using a short amplicon for this analysis is that it may capture less diversity. Additionally, short sequences are of limited use in phylogenetic analysis. However, phylogenies are only truly relevant for constructing the evolutionary history of taxa and because evolutionary analysis was not the objective of this study, agglomerative nested clustering was used to group *cox*1 sequences based on their pairwise sequence identity. Despite its limitations, our *cox*1 assay clearly resolved the dog and human infective *S. stercoralis* types into a clade that is distinct from the dog-specific types, which was our primary objective, and allowed us to compare our results to those obtained in previous studies. Furthermore, we show that the *cox*1 fragment amplified here clearly

distinguishes *S. stercoralis*-derived *cox*1 amplicons from other helminth species including *S. fuelleborni* and multiple species of hookworm.

As part of this study, we included certain *SSU* sequences previously published in GenBank in order to accommodate our novel haplotypes. A sequence published in 1993 by Putland *et al.* (1993) (GB: M84229.1) has been assigned Haplotype C at its HVR-IV region in this study, differing from HVR-IV haplotype A by one SNP at position 125 (Figure 5.2) (Putland et al., 1993). This sequence was later found to be a PCR induced hybrid sequence with the HVR-I region derived from a fungal contaminant (Dorris et al., 2002, Putland et al., 1993). Our analysis confirmed that its HVR-I sequence is so drastically different to that of other *S. stercoralis* haplotypes (and to that of any other *Strongyloides* spp. in general), and was not added to the typing scheme. Next, we observed that *S. stercoralis* HVR-I haplotype IV (reportedly a dog-specific type) is also found in sequences assigned to *S. procyonis* from a Japanese badger (GB: AB272234.1). To reconcile this observation, we incorporated the HVR-IV region of sequences assigned to *S. procyonis* into the typing scheme, referring to them as haplotype D (Table 5.4, GB: AB272234.1 and AB205054.1). This also meant that the HVR-I sequence of the *S. procyonis SSU* (GB: AB205054.1) became HVR-I haplotype VII. Hence, the new *SSU* HVR-I haplotype from dog 22 (GB: MK468661) became *SSU* HVR-I haplotype VIII. A novel *SSU* HVR-I haplotype from dog 45 was also discovered as part of this study (GB: MK468662), and its sequence was most similar to the *SSU* HVR-I region from *Strongyloides* sp. Okayama, isolated from a Japanese striped snake (GB: LC038066.1). As this sequence was already in GenBank prior to the commencement of this study, the HVR-I region of LC038066.1 was assigned to haplotype IX, while the novel sequence obtained from dog 45 was assigned to haplotype X (GB: MK468662). As haplotypes A to D for HVR-IV had been assigned to other sequences, the novel haplotypes discovered in dogs 18, 22 and 13 were assigned to HVR-IV haplotype E, F and G respectively (GB: MK468674, MK468675, and MK468676). Finally, the HVR-IV sequence from *Strongyloides* sp. Okayama (GB:LC038066.1) was assigned to haplotype H because it obtained a nearest match to the HVR-IV regions sequenced from dog 45, which was consequently assigned to haplotype I (GB: MK468677). Also note that all HVR-I and HVR-IV types discussed above (both novel and previously published) are more similar to each other than they are to the corresponding *SSU* regions from *S. ratti*. Consequently, they do not provide enough information on their own to make confident species assignments (Figure 5.2).

While the typing scheme developed by Jaleta *et al.* (2017) was originally designed to consider *S. stercoralis* haplotypes alone, the detection of several novel cryptic haplotypes that: (1) cannot be confidently assigned to a species, (2) are nonetheless greater than 99% similar to each other and to known *S. stercoralis* haplotypes and (3), are haplotypes detected in the same host (dogs), means that the adjustments made here represent the most straightforward solution to the issue at hand (Jaleta et

al., 2017). Being an isolated continent, it is possible that Australian dogs might be infected with genetically distinct *S. stercoralis* strains (Cawood and Korsch, 2008). Consequently, it is not unreasonable to suggest that some of the cryptic dog *Strongyloides* types described herein (i.e., from dogs 18, 22, 13 and 45) might be attributable to truly novel *S. stercoralis* genotypes restricted to Australia. We also propose that some of the cryptic *Strongyloides* sp. haplotypes we discovered in this study are potentially unique to the Australian continent and may have diverged from Southeast Asian *Strongyloides* populations as a result of vicariance. Discovery of a *cox*1 sequence that clusters most closely to a *Strongyloides* sp. identified from a slow loris might support this (dog 43, GB: MK434221), given that lorises are endemic to southeast Asia, yet the lack of any *SSU* sequences associated with this specimen makes it difficult to draw any solid conclusions in that regard. A larger sample number is needed along with additional sequences and morphological analysis of multiple specimens before these sequences can be assigned to a species of helminth.

This study employed an alteration of the Jaleta *et al.* (2017) genotyping assay developed at the Centers for Disease Control and Prevention for adaptation to NGS technologies (Jaleta et al., 2017). The assay was designed to genotype *Strongyloides* sp. and potentially detect mixed helminth infections (e.g., hookworm and *Strongyloides*) when applied directly to DNA extracted from faeces and other biological specimens. This method has great advantages over previous genotyping techniques in that it can be undertaken directly from faecal DNA extracts and does not require culture of larvae for extraction of DNA from individual worms. Furthermore, the depth of sequencing provided by NGS allows the detection of all genotypes in a single sample (Zahedi et al., 2017). However, it is also worth mentioning that no information about the genotype of individual worms is obtained using this method. If two 18S haplotypes are found in one sample it remains unclear if they occur in the same individuals (heterozygous) or if they represent different populations. Also, if multiple variants are detected for more than one of the genotyping loci analysed here (i.e., SSU and *cox*1), it remains unclear which *SSU* sequence belongs to which *cox*1 sequence.

This study had a number of limitations including the collection of dog stool samples from the environment where they could have possibly become contaminated with extraneous environmental organisms or their DNA. As noted in Table 5.1, the *cox*1 PCR employed in this study also detects multiple hookworm species, and was even found to amplify the *cox*1 sequence of a *Metastrongylus*-like helminth. This may represent an advantage of the method if simultaneous detection and genotyping of multiple pathogenic intestinal nematodes from dogs and humans is required. However, these results should be viewed with caution. Given the sensitivity of deep sequencing, we suspect that detection of *cox*1 sequences resembling those of *Metastrongylus salmi* could be attributable to the consumption of pig offal by dogs in community 3. While *Metastrongylus* sp. are known to occasionally infect other

species including humans (Calvopina et al., 2016), the genus is generally thought to be specific to pigs. We also note that the haplotypes from dog 45 closely resembles that of a reptile-infecting *Strongyloides* sp. and its presence in a dog could be due to consumption of reptiles or reptile feces by the dog. Consequently, these cases may represent incidental findings rather than true infections with a *Metastrongylus*-like helminth or a *Strongyloides* sp. resembling those found in reptiles. Similarly, the *cox*1 assay described here detected DNA from potentially free – living nematodes. A sequence obtained from dog 37 received a BLASTN hit to *S. fuelleborni* (GB: AB526306.1), though with only 86.2% identity. However, agglomerative nested clustering placed this sequence in a position basal to all *Strongyloides* and hookworm sequences included in this analysis. This sequence also obtained close BLASTN hits (87% identity) to *Aphelenchoides* sp. (GB: KX356839.1) and *Bursaphelenchus luxuriosae* (AB097863.1) which are mycophagous and/or potentially plant parasitic nematodes. This sequence could therefore represent a free-living nematode that came into contact with the fecal specimens in the environment between when the stool was passed and collected. Surprisingly, a sequence similar to one obtained from a rotifer; a free-living, extremely distant relative of nematodes, was detected in the specimen from dog 28 using this assay. This also likely represents contamination of the stool specimen from the local environment prior to its collection.

Our study was able to independently support previous reports of at least two genetically distinct groups of *S. stercoralis*; one infecting both dogs and humans and another group that is specific to dogs. While this study does not demonstrate direct transmission of the *S. stercoralis* from dogs to humans or vice versa, it supports the hypothesis of zoonotic transmission in remote Australian communities. As discussed previously and with respect to the One Health approach (Rock et al., 2009), we suggest that humans and dogs should be treated concomitantly in these communities to control strongyloidiasis (Beknazarova et al., 2017b, Jaleta et al., 2017). Ultimately, we confirm for the first time that potentially zoonotic *S. stercoralis* populations are present in Australia and suggest that dogs might represent a potential reservoir of human strongyloidiasis in remote Australian communities.

5.2.7 Author Contributions

MB: Designed study, obtained ethics approvals, collected specimens, extracted DNA, ran real-time PCR, optimized and ran PCR assays for *SSU* HVR-I, HVR-IV and *cox*1, performed Illumina library preparation, performed *in-silico* analysis, data analysis and interpretation, wrote and reviewed manuscript drafts; JB: Designed PCR assay for *SSU* HVR-I, HVR-IV and *cox*1, performed *in silico* analysis and data interpretation, constructed cluster dendrogram, wrote and reviewed manuscript drafts; RB: Designed study, obtained funding, reviewed manuscript drafts; ML: Carried out PCR product normalization, prepared Illumina libraries for deep sequencing, and sequenced amplicons; HW: Provided PhD supervision of MB, designed study, obtained ethics approvals, obtained funding,

reviewed manuscript drafts; KR: Provided PhD supervision of MB, designed study, obtained ethics approvals, obtained funding, reviewed manuscript drafts.

5.2.8 Acknowledgments

The authors would like to thank Professor Robert W. Baird and Dr Richard Sullivan at the Royal Darwin Hospital, Darwin Pathology, NT and Dr Gemma Robertson at the Health Support Queensland, QLD for providing us human *S. stercoralis* DNA samples We would also like to acknowledge the contribution of Animal Management in Rural and Remote Indigenous Communities, Environmental Health Branch at the Department of Health, NT and all the other lovely veterinarians across Australia for helping us collecting dog faecal samples in the remote communities. We acknowledge the help of Dr Rogan Lee, Dr Matthew Watts, John Clancy and Vishal Ahuja at the Westmead Hospital, NSW with sending us *S. ratti* infected rat faeces. We wish to thank staff at the Centers for Disease Control and Prevention in Atlanta, USA.

The work has been supported by the Australian Government Research Training Program Scholarship and Flinders University Overseas Travelling Fellowship.

Supporting Information Legends

Table 5.S1 GenBank accession numbers for the *SSU* and *cox*1 haplotypes and their mapping statistics.

The GenBank accession numbers are provided for the *SSU* HVR I and IV haplotypes generated in this study (MK468654-MK468677). The GenBank accession numbers are provided for the *cox*1 haplotypes generated in this study (MK434217- MK434258). Read mapping statistics are also provided to support the validity of each sequence.

***Cox*I sequencing metrics**

Specimen Name	# Paired End Reads Generated	# Reads after adapter and quality trimming	# Reads merged	# Reads that could not be merged	# Merged and unmearged reads mapped back to contig (100% identity, overlap of 150 bases)	Name on Dendrogram	GenBank Accession	Organism
Human 3	28930	25210	12405	400	6561	Human 3 - 100.0% ~ LC085498.1	MK434218	*Strongyloides stercoralis*
Human 1	11270	9314	2847	3620	929	Human 1 - 99.5% ~ KU962163.1	MK434219	*Strongyloides stercoralis*
Dog 43	17068	14684	6963	758	1760	Dog 43 - 91.7% ~ GU367865.1	MK434220	Unknown nematode
Dog 43	17068	14684	6963	758	2120	Dog 43 - 91.2% ~ LC197946.1	MK434221	Unknown nematode
Dog 43	17068	14684	6963	758	358	Dog 43 - 90.3% ~ KY081230.1	MK434222	Unknown nematode
Dog 41	157320	152594	74595	3404	65917	Dog 41 - 91.2% ~ KY640299.1	MK434223	*Ancylostoma* sp.
Dog 37	17796	15610	7146	1318	4764	Dog 37 - 86.2% ~ AB526306.1	MK434224	Unknown nematode
Dog 28	43854	38986	14430	10126	3567	Dog 28 - 93.1% ~ JX184003.1	MK434225	Unknown eukaryote
Dog 22	11928	11082	5385	312	1558	Dog 22 - 90.8% ~ AB526307.1	MK434226	Unknown nematode

Dog 22	11928	11082	5385	312	2373	Dog 22 - 89.4% ~ AB793563.1	MK434227	Unknown nematode
Dog 21	137434	132270	64024	4222	9394	Dog 21 - 100.0% ~ AP017673.1	MK434228	*Ancylostoma caninum*
Dog 21	137434	132270	64024	4222	34524	Dog 21 - 91.2% ~ KY640299.1	MK434229	*Ancylostoma* sp.
Dog 20	42410	38378	18753	872	956	Dog 20 - 100.0% ~ AP017673.1	MK434230	*Ancylostoma caninum*
Dog 20	42410	38378	18753	872	827	Dog 20 - 91.2% ~ KY640299.1	MK434231	*Ancylostoma* sp.
Dog 20	42410	38378	18753	872	536	Dog 20 - 91.2% ~ AP017673.1	MK434232	*Ancylostoma* sp.
Dog 19	44336	41544	19989	1566	824	Dog 19 - 100.0% ~ FJ483518.1	MK434233	*Ancylostoma caninum*
Dog 19	44336	41544	19989	1566	3335	Dog 19 - 100.0% ~ AP017673.1	MK434234	*Ancylostoma caninum*
Dog 19	44336	41544	19989	1566	1063	Dog 19 - 91.2% ~ KY640299.1	MK434235	*Ancylostoma* sp.
Dog 19	44336	41544	19989	1566	4381	Dog 19 91.2% ~ KY640299.1	MK434236	*Ancylostoma* sp.
Dog 18	8526	6658	3276	106	422	Dog 18 - 99.5% ~ LC085498.1	MK434237	*Strongyloides stercoralis*
Dog 16	29664	27054	13189	676	3812	Dog 16 - 97.7% ~ GQ888715.1	MK434238	*Metastrongylus* sp.
Dog 16	29664	27054	13189	676	2170	Dog 16 - 96.3% ~ GQ888715.1	MK434239	*Metastrongylus* sp.
Dog 15	14546	12574	6100	374	363	Dog 15 - 98.6% ~ AP017673.1	MK434240	*Ancylostoma caninum*
Dog 15	14546	12574	6100	374	855	Dog 15 - 97.7% ~ GQ888715.1	MK434241	*Metastrongylus* sp.
Dog 15	14546	12574	6100	374	1189	Dog 15 - 91.2% ~ KY640299.1	MK434242	*Ancylostoma* sp.
Dog 15	14546	12574	6100	374	554	Dog 15 - 91.2% ~ AP017673.1	MK434243	*Ancylostoma* sp.
Dog 14	5684	5388	2604	180	346	Dog 14 - 98.2% ~ GQ888715.1	MK434244	*Metastrongylus* sp.
Dog 14	5684	5388	2604	180	1021	Dog 14 - 96.3% ~ GQ888715.1	MK434245	*Metastrongylus* sp.
Dog 13	20416	18802	9012	778	1021	Dog 13 - 100.0% ~ FJ483518.1	MK434246	*Ancylostoma caninum*

Dog 13	20416	18802	9012	778	1216	Dog 13 - 100.0% ~ AP017673.1	MK434247	*Ancylostoma caninum*
Dog 13	20416	18802	9012	778	615	Dog 13 - 91.7% ~ KY640299.1	MK434248	*Ancylostoma* sp.
Dog 12	39494	34354	15449	3456	3506	Dog 12 - 100.0% ~ FJ483518.1	MK434249	*Ancylostoma caninum*
Dog 12	39494	34354	15449	3456	4265	Dog 12 - 100.0% ~ AP017673.1	MK434250	*Ancylostoma caninum*
Dog 10	23224	21386	10417	552	3822	Dog 10 - 100.0% ~ FJ483518.1	MK434251	*Ancylostoma caninum*
Dog 10	23224	21386	10417	552	2288	Dog 10 - 99.1% ~ AP017673.1	MK434252	*Ancylostoma caninum*
Dog 10	23224	21386	10417	552	457	Dog 10 - 97.7% ~ AP017673.1	MK434253	*Ancylostoma caninum*
Dog 7	52918	45836	22568	700	293 (551 reads if unmerged)**	Dog 7 - 100.0% ~ KX226377.1	MK434254	*Strongyloides stercoralis*
Dog 6	29822	25880	12544	792	2417	Dog 6 - 100.0% ~ KX226377.1	MK434255	*Strongyloides stercoralis*
Dog 6	29822	25880	12544	792	1742	Dog 6 - 99.1% ~ AJ558163.1	MK434256	*Strongyloides stercoralis*
Dog 6	29822	25880	12544	792	2786	Dog 6 - 91.2% ~ KY640299.1	MK434257	*Ancylostoma* sp.
Dog 4	50144	45480	22504	472	881	Dog 4 - 91.2% ~ KY640299.1	MK434258	*Ancylostoma* sp.

**Indicates a contig with coverage just below the threshold set in this study. When reads that were not merged were mapped to the original *de novo* assembled contig using the mapping parameters described (150 bases and 100% identity), 551 reads mapped with no misaligned reads or gaps and the contig generated was identical to that generated using merged reads. Consequently, this contig was considered valid and was retained for downstream analysis.

18S HVR-I sequencing metrics

Specimen Name	# Paired End Reads Generated	# Reads after adapter and quality trimming	# Reads merged	# Reads that could not be merged	# Merged and unmearged reads mapped back to contig (100% identity, overlap of 150 bases)	GenBank Accession	Haplotype	Organism
Dog 18	69378	62120	2881	56358	20962	MK468657	II	*Strongyloides stercoralis*
Dog 18	69378	62120	2881	56358	21526	MK468660	VI	*Strongyloides stercoralis*
Dog 22	10252	7004	1657	3690	2656	MK468661	VIII	*Strongyloides* sp.
Dog 45	1826	1524	308	908	251 (354 if unmerged)**	MK468662	X	*Strongyloides* sp.
Dog 6	54312	49260	5116	39028	7555	MK778085	II	*Strongyloides stercoralis*
Dog 6	54312	49260	5116	39028	26260	MK468659	IV	*Strongyloides stercoralis*
Dog 7	7638	4546	2081	384	379	MK468663	IV	*Strongyloides* sp.
Human 1	10522	6856	2385	2086	1433	MK468655	II	*Strongyloides stercoralis*
Human 3	2916	2142	318	1506	1183	MK468656	II	*Strongyloides stercoralis*
Human 4	5196	4108	771	2566	736	MK468658	II	*Strongyloides stercoralis*

**Indicates a contig with coverage just below the threshold set in this study. When reads that were not merged were mapped to the original *de novo* assembled contig using the mapping parameters described (150 bases and 100% identity), 354 reads mapped with no misaligned reads or gaps and the contig generated was identical to that generated using merged reads. Consequently, this contig was considered valid and was retained for downstream analysis.

18S HVR-IV sequencing metrics

Specimen Name	# Paired End Reads Generated	# Reads after adapter and quality trimming	# Reads merged	# Reads that could not be merged	# Merged and unmearged reads mapped back to contig (100% identity, overlap of 150 bases)	GenBank Accession	Haplotype	Organism
Dog 13	8648	7866	3684	498	484	MK468668	A	*Strongyloides stercoralis*
Dog 13	8648	7866	3684	498	516	MK468676	G	*Strongyloides* sp.
Dog 18	12498	11334	5299	736	2139	MK468665	A	*Strongyloides stercoralis*
Dog 18	12498	11334	5299	736	1643	MK468674	E	*Strongyloides* sp.
Dog 22	10318	9166	4363	440	3031	MK468675	F	*Strongyloides* sp.
Dog 32	7998	7284	3402	480	2393	MK468669	A	*Strongyloides stercoralis*
Dog 45	5008	4510	2110	290	1253	MK468677	I	*Strongyloides* sp.
Dog 6	10436	9350	4437	476	503	MK468667	A	*Strongyloides stercoralis*
Dog 6	10436	9350	4437	476	2217	MK468672	B	*Strongyloides stercoralis*
Dog 7	10976	9878	4625	628	3254	MK468673	B	*Strongyloides stercoralis*
Human 1	13968	12784	6132	520	4532	MK468666	A	*Strongyloides stercoralis*
Human 2	9686	8598	3999	600	2757	MK468670	A	*Strongyloides stercoralis*
Human 3	4918	4422	2039	344	1249	MK468664	A	*Strongyloides stercoralis*
Human 4	4068	3644	1683	278	1051	MK468671	A	*Strongyloides stercoralis*

Table 5.S2 Sequences included in *cox1* BLAST database

>AB526282.1|NCBI_Strongyloides fuelleborni mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: HumJPImported-1 TTTAATTTATCAACATTTGTTTTGATTTTTTGGTCATCCTGAGGTATATATTTTAATTCTTCCTGCTTTTGGTATTATTA GTCAAAGTACTTTATATTTAACTGGTAAAAAGGAGGTCTTTGGTACTTTAGGTATAATTTATGCTATTTTAAGAATTGG TTTAATTGGTTGTGTGGTTTGGGCTCATCATATGTATACTGTTGGTATGGATATTGAT
>AB526282|AB526282.1|DDBJ_Strongyloides fuelleborni mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: HumJPImported-1. TTTAATTTATCAACATTTGTTTTGATTTTTTGGTCATCCTGAGGTATATATTTTAATTCTTCCTGCTTTTGGTATTATTA GTCAAAGTACTTTATATTTAACTGGTAAAAAGGAGGTCTTTGGTACTTTAGGTATAATTTATGCTATTTTAAGAATTGG TTTAATTGGTTGTGTGGTTTGGGCTCATCATATGTATACTGTTGGTATGGATATTGAT
>AB526283.1|NCBI_Strongyloides fuelleborni mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: ChimpTanzXP-1 TTTAATTTATCAACATTTGTTTTGATTTTTTGGTCATCCTGAGGTATATATCTTAATTCTTCCTGCTTTTGGTATTATTA GTCAAAGTACTTTATATTTAACTGGTAAAAAGGAGGTCTTTGGTACTTTAGGTATAATTTATGCTATTTTAAGAATTGG TTTAATTGGTTGTGTGGTTTGGGCTCATCATATGTATACTGTTGGTATGGATATTGAT
>AB526284|AB526284.1|DDBJ_Strongyloides fuelleborni mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: ChimpTanzCE-8. TTTAATTTATCAACATTTGTTTTGATTTTTTGGTCATCCTGAGGTATATATCTTAATTCTTCCTGCTTTTGGTATTATTA GTCAAAGTACTTTATATTTAACTGGTAAAAAGGAGGTCTTTGGTACTTTAGGTATAATTTATGCTATTTTAAGAATTGG TTTAATTGGTTGTGTGGTTTGGGCTCATCATATGTATACTGTTGGTATGGATATTGAT
>AB526287|AB526287.1|DDBJ_Strongyloides fuelleborni mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: ChimpGabon-1. TTTAATTTATCAACATTTATTTTGATTCTTTGGACATCCTGAAGTATATATTTTAATTCTTCCTGCTTTTGGTATCATTA GGCAAAGTACTCTTTATTTAACTGGTAAAAAGGAAGTCTTTGGTACCTTAGGTATAATTTATGCTATTTTAAGAATTG GTTTAATTGGTTGTGTAGTATGGGCTCATCATATGTATACCGTTGGTATAGATATTGAT
>AB526288|AB526288.1|DDBJ_Strongyloides fuelleborni mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: ChimpGabon-2. TTTAATTTATCAACATTTATTTTGATTCTTTGGCCATCCTGAGGTATATATTTTAATTCTTCCTGCTTTTGGTATCATTA GGCAAAGTACTCTTTATTTAACTGGTAAAAAGGAAGTCTTTGGTACCTTAGGTATAATTTATGCTATTTTAAGAATTG GTTTAATTGGTTGTGTAGTATGGGCTCATCATATGTATACCGTTGGTATAGATATTGAT
>AB526290.1|NCBI_Strongyloides fuelleborni mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: MacaqueOita-1 TTTGATTTATCAGCATTTGTTTTGATTTTTTGGTCATCCTGAGGTTTATATTTTAATTCTTCCTGCTTTTGGAATTATTA GTCAAAGTACTCTTTATTTAACTGGTAAAAAGGAAGTTTTTGGTACTTTAGGTATGATTTATGCTATTTTAAGAATTGG TTTGATTGGTTGTGTTGTTTGGGCTCATCATATGTATACTGTTGGTATAGATATTGAT
>AB526290|AB526290.1|DDBJ_Strongyloides fuelleborni mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: MacaqueOita-1. TTTGATTTATCAGCATTTGTTTTGATTTTTTGGTCATCCTGAGGTTTATATTTTAATTCTTCCTGCTTTTGGAATTATTA GTCAAAGTACTCTTTATTTAACTGGTAAAAAGGAAGTTTTTGGTACTTTAGGTATGATTTATGCTATTTTAAGAATTGG TTTGATTGGTTGTGTTGTTTGGGCTCATCATATGTATACTGTTGGTATAGATATTGAT
>AB526291.1|NCBI_Strongyloides fuelleborni mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: MacaqueShodoshima-1 TTTGATTTATCAGCATTTGTTTTGATTTTTTGGTCATCCTGAGGTTTATATTTTAATTCTTCCTGCTTTTGGAATTATTA GTCAAAGTACTCTTTATTTAACTGGTAAAAAGGAGGTTTTTGGTACTTTAGGTATGATTTATGCTATTTTAAGAATTGG TTTGATTGGTTGTGTTGTTTGGGCTCATCATATGTATACTGTTGGTATAGATATTGAT
>AB526292|AB526292.1|DDBJ_Strongyloides fuelleborni mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: MacaqueShodoshima-2. TTTGATTTATCAGCATTTGTTTTGATTTTTTGGTCATCCTGAGGTTTATATTTTAATTCTTCCTGCTTTTGGAATTATTA GTCAAAGTACTCTTTATTTAACTGGTAAAAAGGAGGTTTTTGGTACTTTAGGTATGATTTATGCTATTTTAAGAATTGG TTTGATTGGTTGTGTTGTTTGGGCTCATCATATGTATACTGTTGGTATAGATATTGAT
>AB526293.1|NCBI_Strongyloides fuelleborni mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds,

isolate: MacaqueYamaguchi-1
TTTGATTTATCAGCATTTGTTTTGATTTTTTGGTCATCCTGAGGTTTATATTTTAATTCTTCCTGCTTTTGGAATTATTA
GTCAAAGTACTCTTTATTTAACTGGTAAAAAGGAAGTTTTTGGTACTTTAGGTATGATTTATGCTATTTTAAGAATTGG
TTTAATTGGTTGTGTTGTTTGGGCTCATCATATGTATACTGTTGGTATAGATATTGAT

>AB526293|AB526293.1|DDBJ_Strongyloides fuelleborni mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: MacaqueYamaguchi-1.
TTTGATTTATCAGCATTTGTTTTGATTTTTTGGTCATCCTGAGGTTTATATTTTAATTCTTCCTGCTTTTGGAATTATTA
GTCAAAGTACTCTTTATTTAACTGGTAAAAAGGAAGTTTTTGGTACTTTAGGTATGATTTATGCTATTTTAAGAATTGG
TTTAATTGGTTGTGTTGTTTGGGCTCATCATATGTATACTGTTGGTATAGATATTGAT

>AB526294.1|NCBI_Strongyloides fuelleborni mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: MacaqueYaku-1
TTTGATTTATCAGCATTTGTTTTGATTTTTTGGTCATCCTGAGGTTTATATTTTAATTCTTCCTGCTTTTGGAATTATTA
GTCAAAGTACTCTTTATTTGACTGGTAAAAAGGAAGTTTTTGGTACTTTAGGTATGATTTATGCTATTTTAAGAATTGG
TTTGATTGGTTGTGTTGTTTGGGCTCATCATATGTATACTGTTGGTATAGATATTGAT

>AB526294|AB526294.1|DDBJ_Strongyloides fuelleborni mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: MacaqueYaku-1.
TTTGATTTATCAGCATTTGTTTTGATTTTTTGGTCATCCTGAGGTTTATATTTTAATTCTTCCTGCTTTTGGAATTATTA
GTCAAAGTACTCTTTATTTGACTGGTAAAAAGGAAGTTTTTGGTACTTTAGGTATGATTTATGCTATTTTAAGAATTGG
TTTGATTGGTTGTGTTGTTTGGGCTCATCATATGTATACTGTTGGTATAGATATTGAT

>AB526295|AB526295.1|DDBJ_Strongyloides planiceps mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: RaccoondogWakayama-1.
TTTAATCTATCAACATCTTTTTTGATTTTTTGGTCATCCCGAAGTTTATATTTTAATTTTGCCTGCATTTGGGATTATTA
GTCAAAGTACTTTATATTTAACTGGTAAAAAAGAAGTATTTGGTACTTTAGGTATAATCTATGCTATCCTAAGTATTGG
TTTAATTGGTTGTGTTGTTTGGGCTCATCATATGTATACTGTTGGTATGGATTTAGAC

>AB526296|AB526296.1|DDBJ_Strongyloides planiceps mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: RaccoondogYaku-1.
TTTAATCTATCAACATCTTTTTTGATTTTTTGGTCATCCCGAAGTTTATATTTTAATTTTGCCTGCATTTGGGATTATTA
GTCAAAGTACTTTATATTTAACTGGTAAAAAAGAAGTATTTGGTACTTTAGGTATAATTTATGCTATTCTAAGTATTGG
TTTAATTGGTTGTGTTGTTTGGGCTCATCATATGTATACTGTTGGTATGGATCTAGAC

>AB526297.1|NCBI_Strongyloides stercoralis mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: HumTanz-1
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGTATAGATTTTGAT

>AB526298.1|NCBI_Strongyloides stercoralis mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: HumJPTokyo-1
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTTGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGAGCTCACCACATGTATACTGTTGGTATAGATTTTGAT

>AB526298|AB526298.1|DDBJ_Strongyloides stercoralis mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: HumJPTokyo-1.
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTTGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGAGCTCACCACATGTATACTGTTGGTATAGATTTTGAT

>AB526299.1|NCBI_Strongyloides stercoralis mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: HumJPOkinawa-1
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGAGCTCACCACATGTATACTGTTGGTATAGATTTTGAT

>AB526299|AB526299.1|DDBJ_Strongyloides stercoralis mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: HumJPOkinawa-1.
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGAGCTCACCACATGTATACTGTTGGTATAGATTTTGAT

>AB526300.1|NCBI_Strongyloides stercoralis mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: HumJPOkinawa-2
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGCACTTTGTATTTGACTGGTAAGAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTGAGTATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATGGATTTTGAT

>AB526300|AB526300.1|DDBJ_Strongyloides stercoralis mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: HumJPOkinawa-2.
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGCACTTTGTATTTGACTGGTAAGAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTGAGTATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATGGATTTTGAT

>AB526302.1|NCBI_Strongyloides stercoralis mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: DogJP-1
TTTAATTTATCAGCATTTGTTTTGGTTTTTTGGCCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTCTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATAGATTTTGAT

>AB526302|AB526302.1|DDBJ_Strongyloides stercoralis mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: DogJP-1.
TTTAATTTATCAGCATTTGTTTTGGTTTTTTGGCCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTCTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATAGATTTTGAT

>AB526304.1|NCBI_Strongyloides stercoralis mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: ChimpJPCaptive-1
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCCTTCGGTATTATTA
GTCAATGCACTTTGTATTTGACTGGTAAGAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTGAGTATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATGGATTTTGAT

>AB526304|AB526304.1|DDBJ_Strongyloides stercoralis mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: ChimpJPCaptive-1.
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCCTTCGGTATTATTA
GTCAATGCACTTTGTATTTGACTGGTAAGAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTGAGTATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATGGATTTTGAT

>AB526305.1|NCBI_Strongyloides stercoralis mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: ChimpTanzCE-2
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTCGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGAATAGATTTTGAT

>AB526305|AB526305.1|DDBJ_Strongyloides stercoralis mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: ChimpTanzCE-2.
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTCGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGAATAGATTTTGAT

>AB526306.1|NCBI_Strongyloides fuelleborni mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: YelBaboonTanz-1
TTTAATTTATCAACATTTGTTTTGATTTTTTGGTCATCCTGAGGTATATATTTTAATTCTTCCTGCTTTTGGTATTATTA
GTCAAAGTACTTTATATTTAACTGGTAAAAAGGAGGTCTTTGGTACTTTAGGTATAATTTATGCTATTTTAAGAATTGG
TTTAATTGGTTGTGTGGTTTGGGCTCATCATATGTATACTGTTGGTATAGATATTGAT

>AB526306|AB526306.1|DDBJ_Strongyloides fuelleborni mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: YelBaboonTanz-1.
TTTAATTTATCAACATTTGTTTTGATTTTTTGGTCATCCTGAGGTATATATTTTAATTCTTCCTGCTTTTGGTATTATTA
GTCAAAGTACTTTATATTTAACTGGTAAAAAGGAGGTCTTTGGTACTTTAGGTATAATTTATGCTATTTTAAGAATTGG
TTTAATTGGTTGTGTGGTTTGGGCTCATCATATGTATACTGTTGGTATAGATATTGAT

>AB526307.1|NCBI_Strongyloides mirzai mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: Habu-1
TTTAATTTATCAACATTTGTTTTGATTTTTTGGGCATCCTGAGGTTTATATTTTAATTTTACCTGCTTTTGGTATTATTA
GCCAAAGAACTATATATTTAACAGGTAAGAAAGAAGTATTTGGAACTCTTGGAATAGTTTATGCTATTTTAAGAATTG

GTTTGATTGGTTGTGTAGTTTGGGCTCATCATATATATACTGTTGGAATAGATTTAGAT

>AB526307|AB526307.1|DDBJ_Strongyloides mirzai mitochondrial Cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: Habu-1.
TTTAATTTATCAACATTTGTTTTGATTTTTTGGGCATCCTGAGGTTTATATTTTAATTTTACCTGCTTTTGGTATTATTA
GCCAAAGAACTATATATTTAACAGGTAAGAAAGAAGTATTTGGAACTCTTGGAATAGTTTATGCTATTTTAAGAATTG
GTTTGATTGGTTGTGTAGTTTGGGCTCATCATATATATACTGTTGGAATAGATTTAGAT

>AB793537.1|NCBI_Necator americanus mitochondrial Cox1 gene for cytochrome c oxidase subunit 1, partial cds, haplotype: Type A-1
GTTAATTTATCAGCATTTGTTTTGATTTTTTGGGCATCCTGAGGTTTATATTTTAATTTTACCAGCATTTGGTATTATTA
GGCAATCAACTTTATATTTAACTGGAAAAAAAGAAGTGTTTGGTTCTTTAGGTATAGTATATGCGATTTTAAGGATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGTATGGATTTGGAT

>AB793538.1|NCBI_Necator americanus mitochondrial Cox1 gene for cytochrome c oxidase subunit 1, partial cds, haplotype: Type A-2
GTTGATTTATCAGCATTTGTTTTGATTTTTTGGTCATCCTGAGGTTTATATTTTAATTTTGCCAGCATTTGGTATTATTA
GGCAATCAACTTTATATTTAACTGGAAAAAAAGAAGTGTTTGGTTCTTTAGGTATAGTATATGCGATTTTAAGAATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGTATGGATTTGGAT

>AB793540.1|NCBI_Necator americanus mitochondrial Cox1 gene for cytochrome c oxidase subunit 1, partial cds, haplotype: Type A-3
GTTAATTTATCAGCATTTGTTTTGATTTTTTGGTCATCCTGAGGTTTATATTTTAATTTTACCAGCATTTGGTATTATTA
GGCAATCAACTTTATATTTAACTGGAAAAAAAGAAGTGTTTGGTTCTTTAGGTATAGTGTATGCGATTTTAAGAATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTCGGTATGGATTTAGAT

>AB793542.1|NCBI_Necator americanus mitochondrial Cox1 gene for cytochrome c oxidase subunit 1, partial cds, haplotype: Type A-5
GTTGATTTATCAGCATTTGTTTTGATTTTTTGGTCATCCTGAGGTTTATATTTTAATTTTGCCAGCATTTGGTATTATTA
GGCAATCAACTTTATATTTAACTGGAAAAAAAGAAGTGTTTGGTTCTTTAGGTATAGTATATGCGATTTTAAGGATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGTATGGATTTGGAT

>AB793543.1|NCBI_Necator americanus mitochondrial Cox1 gene for cytochrome c oxidase subunit 1, partial cds, haplotype: Type A-6
GTTAATTTATCAGCATTTGTTTTGATTTTTTGGTCATCCTGAGGTTTATATTTTAATTTTGCCAGCATTTGGTATTATTA
GGCAATCAACTTTATATTTAACTGGAAAAAAAGAAGTGTTTGGTTCTTTAGGTATAGTATATGCGATTTTAAGAATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGTATGGATTTGGAT

>AB793548.1|NCBI_Necator sp. HH-2013d mitochondrial Cox1 gene for cytochrome c oxidase subunit 1, partial cds, haplotype: Type B-1
ATTAATTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTTTATATTTTAATTTTACCAGCTTTTGGTATTATTA
GTCAGTCTACATTGTATTTAACTGGAAAAAAGGAGGTTTTTGGTTCTTTAGGTATGGTGTATGCAATTTTAAGGATTG
GTTTAATTGGTTGTGTGGTTTGAGCTCATCATATGTATACTGTTGGAATAGATTTGGAT

>AB793549.1|NCBI_Necator sp. HH-2013d mitochondrial Cox1 gene for cytochrome c oxidase subunit 1, partial cds, haplotype: Type B-2
ATTAATTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTTTATATTTTAATTTTACCAGCTTTTGGTATTATTA
GTCAGTCAACATTGTATTTAACTGGAAAAAAGGAGGTTTTTGGTTCTTTAGGTATGGTGTATGCAATTTTAAGGATTG
GTTTAATTGGTTGTGTGGTTTGAGCTCACCATATGTATACTGTTGGAATAGATTTGGAT

>AB793550.1|NCBI_Necator sp. HH-2013e mitochondrial Cox1 gene for cytochrome c oxidase subunit 1, partial cds, haplotype: Type C-5
TTTAATTTATCAACATTTGTTTTGATTTTTTGGACATCCAGAGGTTTATATTTTGATTTTACCGGCTTTTGGTATTATTA
GTCAGTCAACGTTATATTTAACGGGTAAAAAAGAAGTATTTGGTTCTTTGGGTATGGTGTATGCAATTTTGAGGATTG
GTTTAATTGGTTGTGTAGTTTGAGCACATCATATGTATACTGTTGGGATGGATTTAGAT

>AB793551.1|NCBI_Necator sp. HH-2013d mitochondrial Cox1 gene for cytochrome c oxidase subunit 1, partial cds, haplotype: Type B-3
ATTAATTTATCAGCATTTATTTTGATTTTTTGGTCACCCTGAGGTTTATATTTTAATTTTACCAGCTTTTGGTATTATTA
GTCAGTCAACATTGTATTTAACTGGAAAAAAGGAGGTTTTTGGTTCTTTAGGTATGGTATATGCAATTTTAAGGATTG
GTTTAATTGGTTGTGTGGTTTGAGCTCATCATATGTATACTGTTGGAATAGATTTGGAT

>AB793555.1|NCBI_Necator sp. HH-2013d mitochondrial Cox1 gene for cytochrome c oxidase subunit 1, partial cds, haplotype: Type B-7

ATTAATTTATCAGCATTTATTTTGATTTTTTGGTCATCCAGAGGTTTATATTTTAATTTTACCAGCTTTTGGTATTATTA
GTCAGTCAACATTGTATTTGACTGGAAAAAAGGAGGTTTTTGGTTCTTTAGGTATGGTGTATGCAATTTTAAGGATTG
GTTTAATTGGTTGTGTGGTTTGAGCTCATCATATGTATACTGTTGGAATAGATTTGGAT

>AB793556.1|NCBI_Necator sp. HH-2013d mitochondrial Cox1 gene for cytochrome c oxidase subunit 1, partial cds, haplotype: Type B-8
ATTAATTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTTTATATTTTAATTTTACCAGCTTTTGGTATTATTA
GTCAGTCAACATTGTATTTAACTGGAAAAAAGGAGGTTTTTGGTTCTTTAGGTATGGTATATGCAATTTTAAGGATTG
GTTTAATTGGTTGTGTGGTTTGAGCTCATCATATGTATACTGTTGGAATAGATTTGGAT

>AB793557.1|NCBI_Necator sp. HH-2013d mitochondrial Cox1 gene for cytochrome c oxidase subunit 1, partial cds, haplotype: Type B-9
ATTAATTTATCAGCATTTATTTTGATTTTTTGGTCACCCTGAGGTTTATATTTTAATTTTACCAGCTTTTGGTATTATTA
GTCAGTCAACATTGTATTTAACTGGAAAAAAGGAGGTTTTTGGTTCTTTAGGTATGGTGTATGCAATTTTAAGGATTG
GTTTAATTGGTTGTGTGGTTTGAGCTCATCATATGTATACTGTTGGGATAGATTTGGAT

>AB793558.1|NCBI_Necator sp. HH-2013d mitochondrial Cox1 gene for cytochrome c oxidase subunit 1, partial cds, haplotype: Type B-10
ATTAATTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTTTATATTTTAATTTTACCGGCTTTTGGTATTATTA
GTCAGTCAACATTGTATTTAACTGGAAAAAAGGAGGTTTTTGGTTCTTTAGGTATGGTGTATGCAATTTTAAGGATTG
GTTTAATTGGTTGTGTGGTTTGAGCTCATCATATGTATACTGTTGGAATAGATTTGGAT

>AB793559.1|NCBI_Necator sp. HH-2013d mitochondrial Cox1 gene for cytochrome c oxidase subunit 1, partial cds, haplotype: Type B-11
ATTAATTTATCAGCATTTATTTTGATTTTTTGGTCATCCCGAGGTTTATATTTTAATTTTACCAGCTTTTGGTATTATTA
GTCAGTCAACATTGTATTTAACTGGAAAAAAGGAGGTTTTTGGTTCTTTAGGTATGGTATATGCAATTTTAAGGATTG
GTTTAATTGGTTGTGTGGTTTGAGCTCATCATATGTATACTGTTGGAATAGATTTGGAT

>AB793561.1|NCBI_Necator sp. HH-2013d mitochondrial Cox1 gene for cytochrome c oxidase subunit 1, partial cds, haplotype: Type B-13
ATTAATTTATCAGCATTTGTTTTGATTTTTTGGTCATCCTGAGGTTTATATTTTAATTTTACCAGCTTTTGGTATTATTA
GTCAGTCAACATTGTATTTAACTGGAAAAAAGGAGGTTTTTGGTTCTTTAGGTATGGTGTATGCAATTTTAAGGATTG
GTTTAATTGGTTGTGTGGTTTGAGCTCATCATATGTATACTGTTGGAATAGATTTGGAT

>AB793562.1|NCBI_Necator sp. HH-2013d mitochondrial Cox1 gene for cytochrome c oxidase subunit 1, partial cds, haplotype: Type B-14
ATTAATTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTTTATATTTTAATTTTACCAGCTTTTGGTATTATTA
GTCAGTCAACATTGTATTTAACTGGAAAAAAGGAGGTTTTTGGTTCTTTAGGTATGGTGTATGCAATTTTAAGGATTG
GTTTAATTGGTTGTGTGGTTTGAGCTCATCATATGTATACTGTTGGAATAGATTTGGAT

>AB793563.1|NCBI_Necator sp. HH-2013d mitochondrial Cox1 gene for cytochrome c oxidase subunit 1, partial cds, haplotype: Type B-15
ATTAATTTATCAGCATTTATTTTGATTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCAGCTTTTGGTATTATTA
GTCAGTCAACATTGTATTTAACTGGAAAAAAGGAGGTTTTTGGTTCTTTAGGTATGGTGTATGCAATTTTAAGGATTG
GTTTAATTGGTTGTGTGGTTTGAGCTCATCATATGTATACTGTTGGAATGGATTTGGAT

>AB793565.1|NCBI_Necator sp. HH-2013e mitochondrial Cox1 gene for cytochrome c oxidase subunit 1, partial cds, haplotype: Type C-1
TTTAATTTATCAACATTTGTTTTGATTTTTTGGGCATCCAGAGGTTTATATTTTGATTTTACCGGCTTTTGGTATTATTA
GTCAGTCAACATTATATTTAACGGGTAAAAAAGAAGTATTTGGTTCTTTAGGTATGGTGTATGCGATTTTGAGGATTG
GTTTAATTGGTTGTGTAGTTTGAGCACATCATATGTATACTGTTGGAATGGATTTAGAT

>AB793566.1|NCBI_Necator sp. HH-2013e mitochondrial Cox1 gene for cytochrome c oxidase subunit 1, partial cds, haplotype: Type C-2
TTTAATTTATCAACATTTGTTTTGATTTTTTGGACATCCAGAGGTTTATATTTTGATTTTACCGGCTTTTGGTATTATTA
GTCAGTCAACGTTATATTTAACGGGTAAAAAAGAAGTATTTGGTTCTTTGGGTATGGTGTATGCAATTTTGAGGATTG
GTTTAATTGGTTGTGTAGTTTGAGCACACCATATGTATACTGTTGGAATGGATTTAGAT

>AB793567.1|NCBI_Necator sp. HH-2013e mitochondrial Cox1 gene for cytochrome c oxidase subunit 1, partial cds, haplotype: Type C-3
TTTAATTTATCAACATTTGTTTTGATTTTTTGGGCATCCAGAGGTTTATATTTTGATTTTACCGGCTTTTGGTATTATTA
GTCAGTCAACGTTATATTTAACGGGTAAAAAAGAAGTATTTGGTTCTTTAGGTATGGTGTATGCGATTTTGAGGATTG
GTTTAATTGGTTGTGTAGTTTGAGCACATCATATGTATACTGTTGGAATGGATTTAGAT

>AB793568.1|NCBI_Necator sp. HH-2013e mitochondrial Cox1 gene for cytochrome c oxidase subunit 1, partial cds, haplotype: Type C-4
TTTAATTTATCAACATTTGTTTTGATTTTTTGGGCATCCAGAGGTTTATATTTTGATTTTACCGGCTTTCGGTATTATTA
GTCAGTCAACGTTATATTTAACGGGTAAAAAAGAAGTATTTGGTTCTTTAGGTATGGTGTATGCGATTTTGAGGATTG
GTTTAATTGGTTGTGTAGTTTGAGCACATCATATGTATACTGTTGGAATGGATTTAGAT

>AB793569.1|NCBI_Necator americanus mitochondrial Cox1 gene for cytochrome c oxidase subunit 1, partial cds, haplotype: Type A-11
GTTAATTTATCAGCATTTGTTTTGATTTTTTGGTCATCCTGAGGTTTATATTTTAATTTTACCAGCATTTGGTATTATTA
GGCAATCAACTTTATATTTAACTGGAAAAAAAGAAGTGTTTGGTTCTTTAGGTATAGTATATGCGATTTTAAGAATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGTATGGATTTGGAT

>AB793570.1|NCBI_Necator americanus mitochondrial Cox1 gene for cytochrome c oxidase subunit 1, partial cds, haplotype: Type A-12
GTTGATTTATCAGCATTTGTTTTGATTTTTTGGTCATCCTGAGGTTTATATTTTAATTTTGCCAGCATTTGGTATTATTA
GGCAATCAACTTTATATTTAACTGGAAAGAAAGAAGTGTTTGGTTCTTTAGGTATAGTATATGCGATTTTAAGAATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGTATGGATTTGGAT

>AB793571.1|NCBI_Necator americanus mitochondrial Cox1 gene for cytochrome c oxidase subunit 1, partial cds, haplotype: Type A-13
ATTAATTTATCAGCATTTGTTTTGATTTTTTGGTCATCCTGAGGTTTATATTTTAATTTTACCAGCATTTGGTATTATTA
GGCAATCAACTTTATATTTAACTGGAAAAAAAGAAGTGTTTGGTTCTTTAGGTATAGTATATGCGATTTTAAGAATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCACCATATGTATACTGTTGGTATGGATTTGGAT

>AJ417719.2|NCBI_Necator americanus complete mitochondrial genome
GTTGATTTATCAGCATTTGTTTTGATTTTTTGGTCATCCTGAGGTTTATATTTTAATTTTGCCAGCATTTGGTATTATTA
GGCAATCAACTTTATATTTAACTGGAAAAAAAGAAGTGTTTGGTTCTTTAGGTATAGTATATGCGATTCTAAGAATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGTATGGATTTGGAT

>AJ558163.1|NCBI_Strongyloides stercoralis complete mitochondrial genome
TTTAATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTCTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATAGATTTTGAT

>AJ558163|AJ558163.1|DDBJ_Strongyloides stercoralis complete mitochondrial genome.
TTTAATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTCTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATAGATTTTGAT

>AP017673.1|NCBI_Ancylostoma caninum mitochondrial DNA, complete sequence
TTTGATTTATCAACATTTATTTTGATTTTTTGGTCACCCTGAGGTTTATATTTTAATTTTACCTGCTTTTGGTATTGTTA
GACAGTCTACTTTATATTTAACTGGTAAAAAAGAAGTATTTGGTTCTTTAGGTATGGTATACGCTATTTTAAGAATTG
GTTTGATTGGTTGTGTGGTTTGAGCACATCATATGTATACGGTGGGTATGGATTTAGAT

>AP017674.1|NCBI_Ancylostoma ceylanicum mitochondrial DNA, complete sequence
TTTAATTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAGGTTTATATTTTAATTTTACCTGCTTTTGGTATTGTAA
GACAGTCTACTTTGTATTTAACAGGTAAAAAAGAGGTGTTTGGATCTTTGGGGATGGTTTATGCAATTTTAAGGATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCACCATATGTATACTGTAGGGATAGATTTAGAT

>AP017676.1|NCBI_Ancylostoma duodenale mitochondrial DNA, complete sequence
TTTAATTTATCAACATTTGTTTTGGTTTTTTGGTCACCCTGAAGTTTATATTTTGATTTTACCAGCTTTTGGTATTGTTA
GTCAATCTACTTTATATTTAACAGGTAAAAAAGAAGTATTTGGTTCTTTAGGTATGGTTTATGCAATTTTAAGTATTGG
TTTGATTGGTTGTGTTGTGTGAGCTCATCATATGTATACTGTGGGTATGGATTTGGAT

>BI324166|BI324166.1|DDBJ_kt54e11.y3 Strongyloides ratti L1 pAMP1 v3 Chiapelli McCarter Strongyloides ratti cDNA 5' similar to SW:COX1_ASCSU P24881 CYTOCHROME C OXIDASE POLYPEPTIDE I, mRNA sequence.
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGACATCCTGAGGTTTATATTTTAATTTTGCCTGCTTTTGGAATTATTA
GACAGAGGATTTTATATTTGACTGGTAAGAAGGAAGTTTTTGGAGTTTTGGGGATGGTTTATGCAATTTTGAGTATT
GGATTGATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGAATAGATTTGGAT

>BI741952|BI741952.1|DDBJ_kt83c11.y1 Strongyloides ratti L1 pAMP1 v3 Chiapelli McCarter Strongyloides ratti cDNA 5' similar to SW:COX1_ASCSU P24881 CYTOCHROME C OXIDASE POLYPEPTIDE I, mRNA sequence.
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGACATCCTGAGGTTTATATTTTAATTTTGCCTGCTTTTGGAATTATTA
GACAGAGGATTTTATATTTGACTGGTAAGAAGGAAGTTTTTGGAGTTTTGGGGGATGGTTTATGCAATTTTGAGTAT

TGGATTTGATTGGTTTGTGTAGTTTGAGCTCATCATATGCATACTGTTGGAATAGATTTGGAT

>FJ483518.1|NCBI_Ancylostoma caninum mitochondrion, complete genome
TTTAATTTATCAACATTTATTTTGATTTTTTGGTCACCCTGAGGTTTATATTTTAATTTTACCTGCTTTTGGTATTGTTA
GACAGTCTACTTTATATTTAACTGGTAAAAAAGAAGTATTTGGTTCTTTAGGTATGGTATACGCTATTTTAAGAATTG
GTTTGATTGGTTGTGTGGTTTGGGCACATCATATGTATACGGTGGGTATGGATTTGGAT

>GQ888715.1|NCBI_Metastrongylus salmi mitochondrion, complete genome
TTTGATTTATCAACATTTGTTTTGGTTTTTTGGTCATCCAGAGGTATATGTTTTAATTTTGCCGGCTTTTGGTATTATTA
GTCATTCGGCTTTATATTTAACTGGTAAAAAGGAAATTTTTGGTTATTTAGGGATGGTGTATGCTATTTTGAGAATTG
GTATGATTGGTTGTGTGGTTTGGGCCCATCATATGTATACGGTAGGGATGGATTTGGAT

>GU367861.1|NCBI_Aphelenchoides sp. US02 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCTGAAGTATATATTTTAATTCTTCCTGCTTTTGGTATTGTAA
GTCAAAGAACACTATATCTTACTGGAAAGAAAGAGGTGTTTGGTTCTTTGGGTATGGTTTATGCAATTTTGAGGATT
GGTTTAATTGGTTGTGTAGTTTGAGCTCACCATATATATACTGTTGGTATGGATTTAGAT

>GU367862.2|NCBI_Aphelenchoides sp. US01 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCTGAAGTATATATTTTAATTCTTCCTGCTTTTGGTATTGTAA
GTCAAAGAACACTATATCTTACTGGAAAGAAAGAGGTGTTTGGTTCTTTGGGTATGGTTTATGCAATTTTGAGGATT
GGTTTAATTGGTTGTGTAGTTTGAGCTCACCATATATATACTGTTGGTACGGATTTAGAT

>GU367863.1|NCBI_Aphelenchoides sp. KP cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial
TTTGATTTATCAGCATTTATTTTGATTTTTTGGTCATCCTGAAGTTTATATTTTAATTCTTCCTGCTTTTGGGATTATTA
GTCAGAGAACTTTGTTTCTGACTGGGAAAAAAGAAGTATTTGGTAATTTGGGAATAGTTTATGCTATTTTAAGAATTG
GTTTGATTGGTTGTGTAGTTTGGGCTCATCATATGTATACTGTTGGGATGGATTTGGAT

>GU367865.1|NCBI_Aphelenchoides paradalianensis isolate HR3 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial
TTTAATTTATCAACATTTATTTTGATTTTTTGGTCATCCTGAAGTTTATATTTTAATTTTACCTGCTTTTGGTATTATTAG
ACAAAGTACTTTATTCTTAACTGGTAAAAAAGAAGTTTTTGGTAATTTAGGTATAGTTTATGCTATTTTAAGGATTGGT
TTAATTGGTTGTGTAGTTTGAGCTCATCATATATACACAGTTGGTATAGATTTAGAT

>GU367866.1|NCBI_Aphelenchoides sp. US03 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial
TTTAATCTATCAACATTTATTTTGATTTTTTGGTCATCCTGAAGTTTATATTTTAATTCTTCCTGCTTTTGGAATTATTA
GTCAGAGAACTTTGTTTCTGACTGGGAAAAAAGAAGTATTTGGTAATTTGGGAATAGTTTATGCTATTTTAAGAATTG
GTTTGATTGGTTGTGTAGTTTGGGCTCATCATATATATACTGTCGGGATGGATTTGGAT

>GU367867.1|NCBI_Aphelenchoides sp. Be cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial
TTTAATTTATCAACATTTATTTTGGTTTTTTGGTCACCCAGAAGTTTATATTTTAATTTTACCAGCTTTTGGAATTATTA
GTCAAAGAACTCTTTTTTTAACTGGTAAAAAGGAGGTTTTTGGAAATTTAGGAATAGTTTATGCAATTTTAAGAATTG
GTTTGATTGGTTGTGTTGTATGGGCCCATCATATATACACAGTTGGTATAGACCTTGAT

>GU367868.1|NCBI_Aphelenchoides sp. DG cytochrome c oxidase subunit I-like (COI) gene, partial sequence; mitochondrial
TTTAATTTATCAGCATTTATTTTGATTTTTTGGGCATCCAGAGGTTTATATTTTGATTTTGCCAGCTTTTGGTATTGTCA
GACAAAGTACTTTGTTTTTGACAGGTAAAAAAGAGGTGTTTGGAAATTTAGGTATAGTTTACGCTATTTTAAGAATTG
GGTTAATTGGTTGTGTAGTTTGAGCACATCATATGTATACAGTAGGTATAGATTTGGAT

>JX184003.1_NCBI_Macrotrachela quadricornifera isolate MQ cytochrome c oxidase subunit 1 (COX1) gene, partial cds; mitochondrial
TATTTTATATCAACATTTATTTTGATTTTTTGGGCATCCTGAAGTTTATATTTTAATTGTTCCTGGTTTTGGAATAATTT
CTCAAATTGTAATTAGAATTTCTAAAAAAGGTGAGATTTTTGGTTATTTAGGAATAGTTTATGCTATAATTTCTATTGG
TTTATTAGGATTCATTGTTTGAGCTCATCATATATTTACTGTTGGAATAGATGTAGAT

>KU962139.1|NCBI_Strongyloides stercoralis isolate SsLC17 cytochrome oxidase subunit 1 (cox1) gene, partial cds; mitochondrial
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTGAGTATTG
GTTTAATTGGTTGTGTAGTTTGGGCTCATCACATGTATACTGTTGGTATAGATTTTGAT

>KU962139|KU962139.1|DDBJ_Strongyloides stercoralis isolate SsLC17 cytochrome oxidase subunit 1 (cox1) gene, partial cds; mitochondrial.
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA

GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTGAGTATTG
GTTTAATTGGTTGTGTAGTTTGGGCTCATCACATGTATACTGTTGGTATAGATTTTGAT

>KU962141.1|NCBI_Strongyloides stercoralis isolate SsLC35 cytochrome oxidase subunit 1 (cox1) gene, partial cds; mitochondrial
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCGGAAGTTTATATTTTAATTTTACCTGCTTTTGGTATTATTA
GTCAATGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATGGATTTTGAT

>KU962141|KU962141.1|DDBJ_Strongyloides stercoralis isolate SsLC35 cytochrome oxidase subunit 1 (cox1) gene, partial cds; mitochondrial.
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCGGAAGTTTATATTTTAATTTTACCTGCTTTTGGTATTATTA
GTCAATGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATGGATTTTGAT

>KU962143.1|NCBI_Strongyloides stercoralis isolate SsLC58 cytochrome oxidase subunit 1 (cox1) gene, partial cds; mitochondrial
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGGGCTCATCACATGTATACTGTTGGTATGGATTTTGAT

>KU962143|KU962143.1|DDBJ_Strongyloides stercoralis isolate SsLC58 cytochrome oxidase subunit 1 (cox1) gene, partial cds; mitochondrial.
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGGGCTCATCACATGTATACTGTTGGTATGGATTTTGAT

>KU962156|KU962156.1|DDBJ_Strongyloides stercoralis isolate SsLCP cytochrome oxidase subunit 1 (cox1) gene, partial cds; mitochondrial.
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTGG
TTTGATTGGTTGTGTAGTTTGGGCTCATCACATGTATACTGTTGGTATAGATTTTGAT

>KU962157.1|NCBI_Strongyloides stercoralis isolate SsLN1 cytochrome oxidase subunit 1 (cox1) gene, partial cds; mitochondrial
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTGAGTATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATATATTTTGAT

>KU962157|KU962157.1|DDBJ_Strongyloides stercoralis isolate SsLN1 cytochrome oxidase subunit 1 (cox1) gene, partial cds; mitochondrial.
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTGAGTATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATATATTTTGAT

>KU962158.1|NCBI_Strongyloides stercoralis isolate SsLN10 cytochrome oxidase subunit 1 (cox1) gene, partial cds; mitochondrial
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GCCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTGAGTATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATAGATTTTGAT

>KU962158|KU962158.1|DDBJ_Strongyloides stercoralis isolate SsLN10 cytochrome oxidase subunit 1 (cox1) gene, partial cds; mitochondrial.
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GCCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTGAGTATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATAGATTTTGAT

>KU962160.1|NCBI_Strongyloides stercoralis isolate SsLN21 cytochrome oxidase subunit 1 (cox1) gene, partial cds; mitochondrial
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTGAGTATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATAGATTTTGAT

>KU962161.1|NCBI_Strongyloides stercoralis isolate SsLN24 cytochrome oxidase subunit 1 (cox1) gene, partial cds;

mitochondrial
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTCTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTGAGTATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATAGATTTTGAT

>KU962161|KU962161.1|DDBJ_Strongyloides stercoralis isolate SsLN24 cytochrome oxidase subunit 1 (cox1) gene, partial cds; mitochondrial.
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTCTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTGAGTATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATAGATTTTGAT

>KU962163.1|NCBI_Strongyloides stercoralis isolate SsLN39 cytochrome oxidase subunit 1 (cox1) gene, partial cds; mitochondrial
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATAGATTTTGAT

>KU962171|KU962171.1|DDBJ_Strongyloides stercoralis isolate SsLS31 cytochrome oxidase subunit 1 (cox1) gene, partial cds; mitochondrial.
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATAGATTTTGAT

>KU962172.1|NCBI_Strongyloides stercoralis isolate SsLS62 cytochrome oxidase subunit 1 (cox1) gene, partial cds; mitochondrial
TTTAATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAGGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTAGGTATGGTTTATGCTATTCTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATAGATTTTGAT

>KU962172|KU962172.1|DDBJ_Strongyloides stercoralis isolate SsLS62 cytochrome oxidase subunit 1 (cox1) gene, partial cds; mitochondrial.
TTTAATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAGGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTAGGTATGGTTTATGCTATTCTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATAGATTTTGAT

>KU962175.1|NCBI_Strongyloides stercoralis isolate SsLS87 cytochrome oxidase subunit 1 (cox1) gene, partial cds; mitochondrial
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAGGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTGTATTTAACTGGTAAAAAAGAAGTTTTTGGGTATTTGGGTATGGTTTATGCTATTTTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGGGCTCATCACATGTATACTGTTGGTATAGATTTTGAT

>KU962175|KU962175.1|DDBJ_Strongyloides stercoralis isolate SsLS87 cytochrome oxidase subunit 1 (cox1) gene, partial cds; mitochondrial.
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAGGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTGTATTTAACTGGTAAAAAAGAAGTTTTTGGGTATTTGGGTATGGTTTATGCTATTTTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGGGCTCATCACATGTATACTGTTGGTATAGATTTTGAT

>KX226367.1|NCBI_Strongyloides stercoralis isolate HumKHRovieng-1 cytochrome oxidase subunit I (cox1) gene, partial cds; mitochondrial
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCGGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATGGATTTTGAT

>KX226371.1|NCBI_Strongyloides stercoralis isolate HumKHRovieng-5 cytochrome oxidase subunit I (cox1) gene, partial cds; mitochondrial
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTGG
TTTGATTGGTTGTGTAGTTTGGGCTCATCACATGTATACTGTTGGTATAGATTTTGAT

>KX226376|KX226376.1|DDBJ_Strongyloides stercoralis isolate DogKHRovieng-3 cytochrome oxidase subunit I (cox1) gene, partial cds; mitochondrial.
TTTAATTTATCAACATTTGTTTTGGTTTTTCGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTTGGTATTATTA
GTCAGTGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTAGGAATGGTTTACGCTATCTTAAGTATTG
GATTGATTGGTTGTGTGGTTTGGGCTCATCATATGTATACTGTTGGAATGGATTTTGAT

>KX226377|KX226377.1|DDBJ_Strongyloides stercoralis isolate DogKHRovieng-4 cytochrome oxidase subunit I (cox1) gene, partial cds; mitochondrial.
TTTAATTTATCAACATTTGTTTTGGTTTTTCGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTTGGTATTATTA
GTCAGTGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTAGGAATGGTTTACGCTATCTTAAGTATTG
GATTGATTGGTTGTGTAGTTTGGGCTCATCATATGTATACTGTTGGAATGGATTTTGAT

>KX226378|KX226378.1|DDBJ_Strongyloides stercoralis isolate DogKHRovieng-5 cytochrome oxidase subunit I (cox1) gene, partial cds; mitochondrial.
TTTAATTTATCAACATTTGTTTTGGTTTTTTGGTCATCCGGAAGTTTATATTTTAATTTTGCCTGCTTTTGGTATTATTA
GTCAGTGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTAGGAATGGTTTACGCTATTTTAAGTATTGG
ATTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGAATGGATTTCGAT

>KX226380|KX226380.1|DDBJ_Strongyloides stercoralis isolate DogKHRovieng-7 cytochrome oxidase subunit I (cox1) gene, partial cds; mitochondrial.
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCGGAAGTTTATATTTTAATTTTGCCTGCTTTTGGTATTATTA
GTCAGTGTACTTTATATTTGACTGGTAAGAAAGAGGTTTTTGGTTATTTAGGTATGGTTTATGCTATTTTAAGTATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGTATGGATTTCGAT

>KX226381|KX226381.1|DDBJ_Strongyloides stercoralis isolate DogKHRovieng-8 cytochrome oxidase subunit I (cox1) gene, partial cds; mitochondrial.
TCTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTTGGTATTATTA
GTCAGTGTACTTTATATTTGACTGGTAAGAAAGAGGTTTTTGGTTATTTAGGTATGGTTTATGCTATTTTAAGTATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGTATGGATTTCGAT

>KX226382|KX226382.1|DDBJ_Strongyloides stercoralis isolate DogKHRovieng-9 cytochrome oxidase subunit I (cox1) gene, partial cds; mitochondrial.
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTGCCTGCTTTTGGTATTATTA
GTCAGTGTACTTTATATTTAACTGGTAAGAAAGAGGTTTTTGGTTATTTAGGAATGGTTTATGCTATTTTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGTATGGATTTTGAT

>KX226384|KX226384.1|DDBJ_Strongyloides stercoralis isolate DogKHRovieng-11 cytochrome oxidase subunit I (cox1) gene, partial cds; mitochondrial.
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTGCCTGCTTTTGGTATTATTA
GTCAGTGTACTTTATATTTGACTGGTAAGAAAGAGGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGTATGGATTTCGAT

>KY070315.1|NCBI_Ancylostoma tubaeforme mitochondrion, complete genome
TTTGATTTATCAACATTTATTTTGATTTTTTGGTCACCCTGAGGTTTATATTTTAATTTTACCAGCTTTTGGTATTGTTA
GTCAGTCAACTTTATATTTAACAGGTAAAAAAGAAGTATTTGGTTCTTTAGGTATGGTTTATGCAATTTTAAGTATTGG
TTTAATTGGTTGTGTTGTGTGAGCCCATCATATGTATACTGTTGGTATAGATTTGGAT

>KY081224|KY081224.1|DDBJ_Strongyloides stercoralis isolate MA3 cytochrome oxidase subunit 1 (cox1) gene, partial cds; mitochondrial.
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCGGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATGGATTTTGAT

>KY081230.1|NCBI_Strongyloides stercoralis isolate MA77 cytochrome oxidase subunit 1 (cox1) gene, partial cds; mitochondrial
TTTAATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTAGGTATGGTTTATGCTATTCTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATAGATTTTGAT

>KY081230|KY081230.1|DDBJ_Strongyloides stercoralis isolate MA77 cytochrome oxidase subunit 1 (cox1) gene, partial cds; mitochondrial.
TTTAATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTAGGTATGGTTTATGCTATTCTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATAGATTTTGAT

>KY081233.1|NCBI_Strongyloides fuelleborni isolate UD33 cytochrome oxidase subunit 1 (cox1) gene, partial cds; mitochondrial
TTTAATTTATCAGCATTTATTTTGGTTTTTTGGTCATCCTGAGGTTTATATTTTAATTCTTCCTGCTTTTGGTATTATTA
GTCAAAGTACTCTCTATTTAACTGGTAAAAAGGAGGTGTTTGGTACTTTAGGTATGATTTATGCAATTTTAAGTATTG
GTTTAATTGGTTGTGTTGTTTGGGCTCATCATATGTATACTGTTGGTATAGATATTGAT

>KY081233|KY081233.1|DDBJ_Strongyloides fuelleborni isolate UD33 cytochrome oxidase subunit 1 (cox1) gene, partial cds; mitochondrial.
TTTAATTTATCAGCATTTATTTTGGTTTTTTGGTCATCCTGAGGTTTATATTTTAATTCTTCCTGCTTTTGGTATTATTA
GTCAAAGTACTCTCTATTTAACTGGTAAAAAGGAGGTGTTTGGTACTTTAGGTATGATTTATGCAATTTTAAGTATTG
GTTTAATTGGTTGTGTTGTTTGGGCTCATCATATGTATACTGTTGGTATAGATATTGAT

>KY081234|KY081234.1|DDBJ_Strongyloides stercoralis isolate UD38 cytochrome oxidase subunit 1 (cox1) gene, partial cds; mitochondrial.
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGTATAGATTTTGAT

>KY081242|KY081242.1|DDBJ_Strongyloides stercoralis isolate UD72 cytochrome oxidase subunit 1 (cox1) gene, partial cds; mitochondrial.
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTGAGTATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATAGATTTTGAT

>KY548505|KY548505.1|DDBJ_Strongyloides stercoralis isolate DogKHRovieng-12 cytochrome oxidase subunit I (cox1) gene, partial cds; mitochondrial.
TTTAATTTATCAACATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTTGGTATTATTA
GTCAGTGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTAGGAATGGTTTATGCTATCTTAAGTATTGG
ATTGATTGGTTGTGTAGTTTGGGCTCATCATATGTATACTGTTGGAATGGATTTTGAT

>KY640299.1|NCBI_Ancylostoma ceylanicum mitochondrion, complete genome
TTTAATTTATCAGCATTTGTTTTGATTTTTTGGTCACCCTGAGGTTTATATTTTGATTTTACCTGCTTTTGGTATTGTAA
GACAGTCTACTTTATATTTAACAGGTAAGAAAGAGGTGTTTGGATCTTTGGGAATGGTTTATGCAATTTTAAGGATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCACCATATGTATACTGTAGGGATAGATTTAGAT

>LC036566.1|NCBI_Necator americanus mitochondrial COI gene for cytochrome c oxidase subunit 1, partial cds, isolate: ThakhekLao1
GTTGATTTATCAGCATTTGTTTTGATTTTTTGGTCATCCTGAGGTTTATATTTTAATTTTGCCAGCATTTGGTATTATTA
GGCAATCAACTTTATATTTAACTGGAAAAAAAGAAGTGTTTGGTTCTTTAGGTATAGTATATGCGATTNTAAGAATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGTATGGATTTGGAT

>LC050210|LC050210.1|DDBJ_Strongyloides papillosus mitochondrial DNA, complete genome, isolate: LIN.
CAGCATTTATTTTGATTCTTTGGTCATCCTGAGGTATATATTTTAATTTTACCTGCTTTTGGTATTATTAGTCAATCTTG
TTTATACTTGACTGGTAAGAAGGAAGTATTTGGAACACTAGGAATGGTATATGCTATTCTTAGAATTGGTTTAATCGG
TTGTGTAGTATGGGCTCATCATATATATACTGTTGGTATGGATATTGATTCTCGTGCTTAT

>LC050213|LC050213.1|DDBJ_Strongyloides venezuelensis mitochondrial DNA, complete genome, isolate: HH1.
CAACATTTGTTTTGGTTTTTTGGACATCCTGAGGTTTATATTTTAATTTTACCTGCTTTTGGTATTATTTCTCAAAGAA
CTTTGTATTTAACTGGTAAAAAGGAGGTTTTTGGAACCCTTGGGATGATTTATGCAATTTTAAGAATTGGATTAATCG
GTTGTGTTGTTTGAGCACATCATATGTATACAGTTGGTATGGATTTAGACTCTCGTGCTTAT

>LC085498.1|NCBI_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: 4AStr4
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTTGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATGGATTTTGAT

>LC085500|LC085500.1|DDBJ_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: 3EStr6.
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTTGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATGGATTTTGAT

>LC085501|LC085501.1|DDBJ_Strongyloides fuelleborni mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: 4AStr3.
TTTAATTTATCAACATTTATTCTGATTCTTTGGTCATCCTGAAGTTTATATTTTAATTCTTCCTGCTTTTGGTATTATTA
GACAAAGTACTCTTTATTTAACTGGTAAAAAGGAAGTTTTTGGTACCTTAGGTATAATTTATGCTATTTTAAGAATTGG
TTTAATTGGTTGTGTAGTATGGGCTCATCATATGTATACTGTTGGTATGGATATTGAT

>LC085503|LC085503.1|DDBJ_Strongyloides fuelleborni mitochondrial COX1 gene for cytochrome c oxidase subunit 1,

partial cds, isolate: 2AStr2.
TTTAATTTATCAACATTTATTCTGATTCTTTGGTCATCCTGAAGTTTATATTTTAATTCTTCCTGCTTTTGGTATTATTA
GACAAAGTACTCTTTATTTAACTGGTAAAAAGGAAGTTTTTGGTACCTTAGGTATAATTTATGCTATTTTAAGAATTGG
TTTAATTGGTTGTGTAGTGTGGGCTCATCATATGTATACTGTTGGTATGGATATTGAT

>LC085509|LC085509.1|DDBJ_Strongyloides fuelleborni mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: Gor46Str2.
TTTAATTTATCAACATTTATTTTGATTCTTTGGTCATCCCGAAGTATATATTTTAATTCTTCCTGCTTTTGGTATTATTA
GGCAAAGTACTCTTTATTTAACTGGTAAAAAGGAAGTCTTTGGTACCTTAGGTATAATTTATGCTATTTTAAGAATTG
GTTTAATTGGTTGTGTAGTATGGGCTCATCATATGTATACCGTTGGTATAGATATTGAT

>LC088303.1|NCBI_Necator sp. HH-2015 mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: D425-H1
TTTGATTTATCAACATTTGTTTTGATTTTTTGGTCACCCTGAGGTTTATATTTTGATTTTACCAGCTTTTGGTATTATTA
GTCAGTCAACATTGTATTTAACTGGAAAAAAAGAGGTTTTTGGTTCTTTAGGTATAGTATATGCAATTTTAAGAATTG
GTTTAATTGGTTGTGTGGTTTGAGCTCATCATATATATACTGTTGGGATAGATTTGGAT

>LC088305.1|NCBI_Necator americanus mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: G5-H4
GTTAATTTATCAGCATTTGTTTTGATTTTTTGGTCATCCTGAGGTTTATATTTTAATTTTACCAGCATTTGGTATTATTA
GGCAATCAACTTTATATTTAACTGGAAAAAAAGAAGTGTTTGGTTCTTTAGGTATAGTGTATGCGATTTTAAGAATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGTATGGATTTGGAT

>LC088307.1|NCBI_Necator americanus mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: TSG3-H9
GTTAATTTATCAGCATTTGTTTTGATTTTTTGGTCATCCCGAGGTTTATATTTTAATTTTACCAGCATTTGGTATTATTA
GGCAATCAACTTTATATTTAACTGGAAAAAAAGAAGTGTTTGGTTCTTTAGGTATGGTATATGCGATTTTAAGAATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCACCATATGTATACTGTTGGTATGGATTTGGAT

>LC088308.1|NCBI_Necator americanus mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: SG5-H2
GTTAATTTATCAGCATTTGTTTTGATTTTTTGGTCATCCTGAGGTTTATATTTTAATTTTGCCAGCATTTGGTATTATTA
GGCAATCAACTTTATATTTAACTGGAAAAAAAGAAGTGTTTGGTTCTTTAGGTATAGTATATGCAATTTTAAGAATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGTATGGATTTGGAT

>LC088310.1|NCBI_Necator sp. HH-2015 mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: D3-H1
ATTAATTTATCAGCATTTATTTTGATTTTTTGGTCATCCAGAGGTTTATATTTTAATTTTACCAGCTTTTGGTATTATTA
GTCAGTCAACGTTGTATTTAACTGGAAAAAAAGGAGGTTTTTGGTTCTTTAGGTATGGTGTATGCAATTTTAAGGATTG
GTTTAATTGGTTGTGTGGTTTGAGCTCATCATATGTATACTGTTGGAATAGATTTGGAT

>LC088311.1|NCBI_Necator sp. HH-2015 mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: D3-H2
TTTAATTTATCAACATTTGTTTTGATTTTTTGGACATCCAGAGGTTTATATTTTGATTTTACCAGCTTTTGGTATTATTA
GTCAGTCAACGTTATATTTAACGGGTAAAAAAGAAGTATTTGGTTCTTTGGGTATGGTGTATGCAATTTTGAGGATTG
GTTTAATTGGTTGTGTAGTTTGAGCACATCATATGTATACTGTTGGAATGGATTTAGAT

>LC088313.1|NCBI_Necator sp. HH-2015 mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: SG5-8-24-HD
ATTAATTTATCAGCATTTATTTTGATTTTTTGGCCATCCAGAGGTTTATATTTTAATTTTACCAGCTTTTGGTATTATTA
GTCAGTCAACATTGTATTTAACTGGAAAAAAGGAGGTTTTTGGTTCTTTAGGTATGGTGTATGCAATTTTAAGGATTG
GTTTAATTGGTTGTGTGGTTTGAGCTCATCATATGTATACTGTTGGAATAGATTTGGAT

>LC088314.1|NCBI_Necator sp. HH-2015 mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: SG5-24-HA
ATTAATTTATCAGCATTTATTTTGATTTTTTGGTCATCCAGAGGTTTATATTTTAATTTTACCAGCTTTTGGTATTATTA
GTCAGTCAACATTGTATTTAACTGGAAAAAAGGAGGTTTTTGGTTCTTTAGGTATGGTGTATGCAATTTTAAGGATTG
GTTTAATTGGTTGTGTGGTTTGAGCTCATCATATGTATACTGTTGGAATAGATTTGGAT

>LC088317.1|NCBI_Necator sp. HH-2015 mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: SG5-24-H4
TTTAATTTATCAACATTTGTTTTGATTTTTTGGACATCCAGAGGTTTATATTTTGATTTTACCGGCTTTTGGTATTATTA
GTCAGTCAACGTTATATTTAACGGGTAAAAAAGAAGTATTTGGTTCTTTGGGTATGGTGTATGCAATTTTGAGGATTG
GTTTAATTGGTTGTGTAGTTTGAGCACATCATATGTATACTGTTGGAATGGATTTAGAT

>LC088320.1|NCBI_Necator sp. HH-2015 mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: TS-H7
TTTAATTTATCAACATTTGTTTTGATTTTTTGGACATCCAGAGGTTTATATTTTGATTTTACCGGCTTTTGGTATTATTA
GTCAGTCAACGTTGTATTTAACGGGTAAAAAAGAAGTATTTGGTTCTTTGGGTATGGTGTATGCAATTTTGAGGATT
GGTTTAATTGGTTGTGTAGTTTGAGCACATCATATGTATACTGTTGGAATGGATTTAGAT

>LC088321.1|NCBI_Necator sp. HH-2015 mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: TS-H8
ATTAATTTATCAGCATTTATTTTGATTTTTTGGTCACCCTGAGGTTTATATTTTAATTTTACCAGCTTTTGGTATTATTA
GTCAGTCAACATTGTATTTAACTGGAAAAAAGGAGGTTTTTGGTTCTTTAGGTATGGTGTATGCAATTTTAAGGATTG
GTTTAATTGGTTGTGTGGTTTGAGCTCACCATATGTATACTGTTGGAATAGATTTGGAT

>LC179093.1|NCBI_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: HTB080_5
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTTGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTGAGTATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATAGATTTTGAT

>LC179094|LC179094.1|DDBJ_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: HTB080_7.
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTTGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTGAGTATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATAGATTTTGAT

>LC179138.1|NCBI_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: HTB176_1
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTGAGTATTG
GTTTAATCGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATAGATTTTGAT

>LC179138|LC179138.1|DDBJ_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: HTB176_1.
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTGAGTATTG
GTTTAATCGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATAGATTTTGAT

>LC179209.1|NCBI_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: MGD167_1
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAGAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTGAGTATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATAGATTTTGAT

>LC179209|LC179209.1|DDBJ_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: MGD167_1.
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAGAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTGAGTATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATAGATTTTGAT

>LC179211.1|NCBI_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: MGD167_3
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTTGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATAGATTTTGAT

>LC179211|LC179211.1|DDBJ_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: MGD167_3.
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTTGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATAGATTTTGAT

>LC179218.1|NCBI_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: MGD191_2
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCGGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTGG

TTTAATTGGTTGTGTAGTTTGGGCTCATCACATGTATACTGTTGGTATGGATTTTGAT

>LC179223|LC179223.1|DDBJ_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: MGD221_8.
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCGGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGGGCTCATCACATGTATACTGTTGGTATGGATTTTGAT

>LC179226.1|NCBI_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: MIE001_3
TTTGATTTACCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTTGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATGGATTTTGAT

>LC179229|LC179229.1|DDBJ_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: MIE001_7.
TTTGATTTACCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTTGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATGGATTTTGAT

>LC179237|LC179237.1|DDBJ_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: NDA009_1.
TTTAATTTATCAACATTTGTTTTGGTTTTTTGGTCATCCGGAAGTTTATATTTTAATTTTGCCTGCTTTTGGTATTATTA
GTCAGTGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTAGGAATGGTTTACGCTATTCTAAGTATTG
GGTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGAATGGATTTCGAT

>LC179296.1|NCBI_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: PSU033_01
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAGTGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATAGATTTTGAT

>LC179301|LC179301.1|DDBJ_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: PSU033_10.
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAGTGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGTATAGATTTTGAT

>LC179304.1|NCBI_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: PSU034_3
TTTGATTTATCAGCATTTGTTTTGGTTTTTCGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGTATAGATTTTGAT

>LC179304|LC179304.1|DDBJ_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: PSU034_3.
TTTGATTTATCAGCATTTGTTTTGGTTTTTCGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGTATAGATTTTGAT

>LC179397|LC179397.1|DDBJ_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: SCT028_4.
TTTAATTTATCAACATTTGTTTTGGTTTTTTGGTCATCCGGAAGTTTATATTTTAATTCTACCTGCTTTTGGTATTATTA
GTCAGTGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTAGGAATGGTTTATGCTATTTTAAGTATTGG
GTTGATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGAATGGATTTTGAT

>LC179409|LC179409.1|DDBJ_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: SCT032_7.
TTTAATTTATCAACATTTGTTTTGGTTTTTTGGTCATCCGGAAGTTTATATTTTAATTTTGCCTGCTTTTGGTATTATTA
GTCAGTGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGAATGGTTTACGCTATTTTAAGTATTG
GGTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGAATGGATTTCGAT

>LC179418|LC179418.1|DDBJ_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: SCT041_4.

TTTAATTTATCAACATTTGTTTTGGTTTTTTGGTCATCCGGAAGTTTATATTTTAATTTTGCCTGCTTTTGGTATTATTA
GTCAGTGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTAGGAATGGTTTACGCTATTTTAAGTATTGG
GTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGAATGGATTTTGAT

>LC179420|LC179420.1|DDBJ_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: SCT041_8.
TTTAATTTATCAACATTTGTTTTGGTTTTTTGGTCATCCGGAAGTTTATATTTTAATTTTGCCTGCTTTTGGTATTATTA
GTCAGTGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTAGGAATGGTTTACGCTATTTTAAGTATTGG
GTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGAATGGATTTCGAC

>LC179421|LC179421.1|DDBJ_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: SCT043_2.
TTTAATTTATCAACATTTGTTTTGGTTTTTTGGTCATCCGGAAGTTTATATTTTAATTCTACCTGCTTTTGGTATTATTA
GTCAGTGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTAGGAATGGTTTATGCTATTTTAAGTATTGG
GTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGAATGGATTTTGAT

>LC179424|LC179424.1|DDBJ_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: SCT046_6.
TTTAATTTATCAACATTTGTTTTGGTTTTTTGGTCATCCGGAAGTTTATATTTTAATTTTACCTGCTTTTGGTATTATTA
GTCAGTGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGAATGGTTTATGCTATTTTAAGTATTGG
GTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGAATGGATTTTGAT

>LC179428|LC179428.1|DDBJ_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: SCT047_4.
TTTAATCTATCAACATTTGTTTTGGTTTTTTGGTCATCCGGAAGTTTATATTTTAATTTTGCCTGCTTTTGGTATTATTA
GTCAGTGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTAGGAATGGTTTACGCTATTTTAAGTATTGG
GTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGAATGGATTTCGAT

>LC179430|LC179430.1|DDBJ_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: SCT047_7.
TTTAATTTATCAACATTTGTTTTGGTTTTTTGGTCATCCGGAAGTTTATATTTTAATTTTGCCTGCTTTTGGTATTATTA
GTCAGTGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTACTTAGGAATGGTTTATGCTATTTTAAGTATTGG
GTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGAATGGATTTCGAT

>LC179433|LC179433.1|DDBJ_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: SCT054_3.
TTTAATTTATCAACATTTGTTTTGGTTTTTCGGTCATCCAGAAGTTTATATTTTAATTTTGCCTGCTTTTGGTATTATTA
GTCAGTGTACTTTATATTTGACTGGTAAGAAAGAAGTTTTTGGTTATTTAGGAATGGTTTACGCTATTTTAAGTATTG
GATTGATTGGTTGTGTAGTTTGGGCTCATCATATGTATACTGTTGGAATGGATTTTGAT

>LC179439|LC179439.1|DDBJ_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: SCT055_7.
TTTAATTTATCAACATTTGTTTTGGTTTTTTGGTCATCCGGAAGTTTATATTTTAATTTTGCCTGCTTTTGGTATTATTA
GTCAGTGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTACTTAGGAATGGTTTACGCTATTTTAAGTATTG
GGTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGAATGGATTTCGAT

>LC179455|LC179455.1|DDBJ_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: SCT085_6.
TTTAATTTATCAACATTTGTTTTGGTTTTTTGGTCATCCGGAAGTTTATATTTTAATTTTGCCTGCTTTTGGTATTATCA
GTCAGTGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTAGGGATGGTTTATGCTATTTTAAGTATTGG
GTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGAATGGATTTCGAT

>LC179460.1|NCBI_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: TBN026_6
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTTGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGGGCTCACCACATGTATACTGTTGGTATAGATTTTGAT

>LC179460|LC179460.1|DDBJ_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: TBN026_6.
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTTGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTGG
TTTAATTGGTTGTGTAGTTTGGGCTCACCACATGTATACTGTTGGTATAGATTTTGAT

>LC179493|LC179493.1|DDBJ_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: TLD166_4.
TTTAATTTATCAACATTTGTTTTGGTTTTTTGGTCATCCGGAAGTTTATATTTTAATTTTACCTGCTTTTGGTATTATTA
GTCAGTGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTAGGAATGGTTTATGCTATTTTAAGTATTGG
GTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGAATGGATTTTGAT

>LC179496|LC179496.1|DDBJ_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: TLD166_7.
TTTAATTTATCAACATTTGTTTTGGTTTTTTGGTCATCCGGAAGTTTATATTTTAATTTTGCCTGCTTTTGGTATTATTA
GTCAGTGTACTTTATATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTAGGAATGGTTTACGCTATTTTAAGTATTGG
GTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGAATGGATTTCGAT

>LC179527.1|NCBI_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: UGL001_09
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTCGGTTATTTGGGTATGGTTTATGCTATTTTGAGTATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGTATAGATTTTGAT

>LC179532|LC179532.1|DDBJ_Strongyloides stercoralis mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: UGL001_14.
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACTTTGTATTTGACTGGTAAAAAAGAAGTTTTCGGTTATTTGGGTATGGTTTATGCTATTTTGAGTATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCATATGTATACTGTTGGTATAGATTTTGAT

>LC197946.1|NCBI_Strongyloides sp. Str_loris mitochondrial cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: Nmenagensis75-L01
TCTTATTTATCAACATCTTTTTTGGTTTTTTGGACATCCTGAGGTTTATATTTTGATTTTACCTGCTTTTGGTATTATTA
GTCAGTGTACTCTTTATTTAACTGGTAAAAAGGAGGTTTTTGGTTATCTTGGTATGGTTTATGCTATTTTAAGTATTG
GTTTGATTGGTTGTGTTGTTTGGGCTCATCATATGTATACTGTTGGTATGGATTTTGAT

>LC197958.1|NCBI_Strongyloides sp. Str_loris mitochondrial cox1 gene for cytochrome oxidase subunit 1, partial cds, isolate: Nmenagensis75-L13
TCTTATTTATCAACATCTTTTTTGGTTTTTTGGGCATCCTGAGGTTTATATTTTGATTTTACCTGCTTTTGGTATTATTA
GTCAGTGTACTCTTTATTTAACTGGTAAAAAGGAGGTTTTTGGTTATCTTGGTATGGTTTATGCTATTTTAAGTATTG
GTTTGATTGGTTGTGTTGTTTGGGCTCATCATATGTATACTGTTGGTATGGATTTTGAT

>LM523328.1 2|NCBI_Parastrongyloides trichosuri genome assembly P_trichosuri_KNP ,scaffold PTRK_scaffold0000122
TTTAATTTATCAGCATTTGTTTTGATTTTTCGGTCATCCTGAGGTTTATATTTTGATTTTGCCTGCTTTTGGTATTATTA
GTCAGAGTACTCTTTATTTAACAGGAAAAAAAGAAGTATTTGGGGTTTTGGGTATGGTTTATGCAATTTTAAGTATTG
GTTTGATTGGTTGTGTAGTTTGGGCTCACCATATATACACTGTTGGTATGGATTTGGAT

>MG995852.1|NCBI_Strongyloides stercoralis cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACCTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGAATGGATTTTGAT

>MG995852|MG995852.1|DDBJ_Strongyloides stercoralis cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial.
TTTGATTTATCAGCATTTGTTTTGGTTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGCTTTCGGTATTATTA
GTCAATGTACCTTGTATTTGACTGGTAAAAAAGAAGTTTTTGGTTATTTGGGTATGGTTTATGCTATTTTAAGTATTG
GTTTAATTGGTTGTGTAGTTTGAGCTCATCACATGTATACTGTTGGAATGGATTTTGAT

>MH049699.1|NCBI_Strongyloides fuelleborni isolate K23 cytochrome oxidase subunit 1 gene, partial cds; mitochondrial
TTTAATTTATCAGCATTTATTTTGGTTTTTTGGTCATCCTGAGGTTTATATTTTAATTCTTCCTGCTTTTGGTATTATTA
GTCAAAGTACTCTTTATTTGACTGGTAAGAAGGAGGTGTTTGGTACTTTAGGTATGATTTATGCAATTTTAAGTATTG
GTTTAATTGGTTGTGTTGTTTGGGCTCATCATATGTATACTGTTGGTATGGATATTGAT

>MH049702.1|NCBI_Strongyloides fuelleborni isolate K58 cytochrome oxidase subunit 1 gene, partial cds; mitochondrial
TTTAATTTATCAGCATTTATTTTGGTTTTTTGGTCATCCTGAGGTTTATATTTTAATTCTTCCTGCTTTTGGTATTATTA
GTCAAAGTACTCTCTATTTAACTGGTAAAAAGGAGGTGTTTGGTACTTTAGGTATGATTTATGCAATTTTAAGTATTG
GTTTAATTGGTTGTGTTGTTTGGGCTCATCATATGTATACTGTTGGTATGGATATTGAT

>MH049703.1|NCBI_Strongyloides fuelleborni isolate K94 cytochrome oxidase subunit 1 gene, partial cds; mitochondrial

TTTAATTTATCAGCATTTATTTTGGTTTTTTGGTCATCCTGAGGTTTATATTTTAATTCTTCCTGCTTTTGGTATTATTA
GTCAAAGTACTCTTTATTTGACTGGTAAAAAGGAGGTGTTTGGTACTTTAGGTATGATTTATGCAATTTTAAGTATTG
GTTTAATTGGTTGTGTTGTTTGGGCTCATCATATGTATACTGTTGGTATGGATATTGAT

>MH049704.1|NCBI_Strongyloides fuelleborni isolate K95 cytochrome oxidase subunit 1 gene, partial cds; mitochondrial
TTTAATTTATCAGCATTTATTTTGGTTTTTTGGTCATCCTGAGGTTTATATTTTAATTCTTCCTGCTTTTGGTATTATTA
GTCAAAGTACTCTTTATTTAACTGGTAAAAAGGAGGTGTTTGGTACTTTAGGTATGATTTATGCAATTTTAAGTATTG
GTTTAATTGGTTGTGTTGTTTGGGCTCATCATATGTATACTGTTGGTATGGATATTGAT

>MH049710.1|NCBI_Strongyloides fuelleborni isolate S15 cytochrome oxidase subunit 1 gene, partial cds; mitochondrial
TTTGATTTATCAGCATTTATTTTGGTTTTTTGGTCATCCTGAGGTTTATATTTTAATTCTTCCTGCTTTTGGTATTATTA
GTCAAAGTACTCTTTACTTGACTGGTAAAAAGGAGGTGTTTGGTACTTTAGGTATGATTTATGCAATTTTAAGTATTG
GTTTAATTGGTTGTGTTGTTTGGGCTCATCATATGTATACTGTTGGTATGGATATTGAT

>MH049719.1|NCBI_Strongyloides fuelleborni isolate S58 cytochrome oxidase subunit 1 gene, partial cds; mitochondrial
TTTGATTTATCAGCATTTATTTTGGTTTTTTGGTCATCCTGAGGTTTATATTTTAATTCTTCCTGCTTTTGGTATTATTA
GTCAAAGTACTCTTTACTTAACTGGTAAAAAGGAGGTGTTTGGTACTTTAGGTATGATTTATGCAATTTTAAGTATTG
GTTTAATTGGTTGTGTTGTTTGGGCTCATCATATGTATACTGTTGGTATGGATATTGAT

>MH049720.1|NCBI_Strongyloides fuelleborni isolate S66 cytochrome oxidase subunit 1 gene, partial cds; mitochondrial
TTTAATTTATCAGCATTTATTTTGGTTTTTTGGTCATCCTGAGGTTTATATTTTAATTCTTCCTGCTTTTGGTATTATTA
GTCAAAGTACTCTTTACTTAACTGGTAAAAAGGAGGTGTTTGGTACTTTAGGTATGATTTATGCAATTTTAAGTATTG
GTTTAATTGGTTGTGTTGTTTGGGCTCATCATATGTATACTGTTGGTATGGATATTGAT

>MH049722.1|NCBI_Strongyloides fuelleborni isolate L2 cytochrome oxidase subunit 1 gene, partial cds; mitochondrial
TTTAATTTATCAGCATTTATTTTGGTTTTTTGGTCATCCTGAGGTTTATATTTTAATTCTTCCTGCTTTTGGTATTATTA
GTCAAAGTACTCTTTATTTGACTGGTAAGAAGGAGGTGTTTGGTACTTTAGGTATGATTTATGCAATTTTAAGTATTG
GTTTAATCGGTTGTGTTGTTTGGGCTCATCATATGTATACTGTTGGTATGGATATTGAT

>MH049723.1|NCBI_Strongyloides fuelleborni isolate L3 cytochrome oxidase subunit 1 gene, partial cds; mitochondrial
TTTAATTTATCAGCATTTATTTTGGTTTTTTGGTCATCCTGAGGTTTATATTTTAATTCTTCCTGCTTTTGGTATTATTA
GTCAAAGTACTCTTTATTTAACCGGTAAAAAAGAGGTATTTGGTACTTTAGGTATGATTTATGCGATTTTAAGTATTG
GTTTAATTGGTTGTGTTGTTTGGGCTCATCATATGTATACTGTTGGTATGGATATTGAT

>MH049725.1|NCBI_Strongyloides fuelleborni isolate L9 cytochrome oxidase subunit 1 gene, partial cds; mitochondrial
TTTAATTTATCAGCATTTATTTTGGTTTTTTGGTCATCCTGAGGTTTATATTTTAATTCTTCCTGCTTTTGGTATTATTA
GTCAAAGTACTCTTTATTTAACTGGTAAAAAGGAGGTATTTGGTACTTTAGGTATGATTTACGCGATTTTAAGTATTG
GTTTAATTGGTTGTGTTGTTTGGGCTCATCATATGTATACTGTTGGTATGGATATTGAT

>MH049726.1|NCBI_Strongyloides fuelleborni isolate L13 cytochrome oxidase subunit 1 gene, partial cds; mitochondrial
TTTAATTTATCAGCATTTATTTTGGTTTTTTGGTCATCCTGAGGTTTATATTTTGATTCTTCCTGCTTTTGGTATTATTA
GTCAAAGTACTCTTTATTTAACCGGTAAAAAGGAAGTATTTGGTACTTTAGGTATGATTTACGCGATTTTAAGTATTG
GTTTAATTGGTTGTGTTGTTTGGGCTCATCATATGTATACTGTTGGTATGGATATTGAT

>MH049727.1|NCBI_Strongyloides fuelleborni isolate L16 cytochrome oxidase subunit 1 gene, partial cds; mitochondrial
TTTAATTTATCAGCATTTATTTTGGTTTTTTGGTCATCCTGAGGTTTATATTTTAATTCTTCCTGCTTTTGGTATTATTA
GTCAAAGTACTCTTTATTTAACCGGTAAAAAGGAGGTATTTGGTACTTTAGGTATGATTTACGCGATTTTAAGTATTG
GTTTAATTGGTTGTGTTGTTTGGGCTCATCATATGTATACTGTTGGTATGGATATTGAT

>MH049729.1|NCBI_Strongyloides fuelleborni isolate L27 cytochrome oxidase subunit 1 gene, partial cds; mitochondrial
TTTGATTTATCAGCATTTATTTTGGTTTTTTGGTCATCCTGAGGTTTATATTTTAATTCTTCCTGCTTTTGGTATTATTA
GTCAAAGTACTCTTTACTTGACTGGTAAAAAGGAGGTGTTTGGTACTTTGGGTATGATTTATGCAATTTTAAGTATTG
GTTTAATTGGTTGTGTTGTTTGAGCTCATCATATGTATACTGTTGGTATGGATATTGAT

6. OPPORTUNISTIC MAPPING OF *STRONGYLOIDES STERCORALIS* AND HOOKWORM SPECIES IN DOGS IN REMOTE AUSTRALIAN COMMUNITIES

This chapter describes the prevalence of *S. stercoralis* and hookworm species in dogs living in remote Indigenous communities in Australia. This is the first large-scale study that assessed the prevalence of these zoonotic parasites in dogs and mapped the cases across remote communities in the north parts in Australia. The findings of this study emphasise the need for applying a One Health approach to protect animal and public health.

Opportunistic mapping of *Strongyloides stercoralis* and hookworm in dogs in remote Australian communities

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Working paper

This manuscript will be submitted to the *International Journal for Parasitology*

Keywords *S. stercoralis*, hookworm, soil-transmitted helminths, zoonotic, parasite, Australia, remote communities, zoonotic parasites, one health

6.1 Abstract

Both S*trongyloides stercoralis* and hookworms are common soil-transmitted helminths in remote Australian communities. As well as infecting humans, *S. stercoralis* and some species of hookworms infect canids and therefore both environmental and zoonotic sources of transmission to humans. Currently, there is limited information available on the prevalence of hookworms and *S. stercoralis* infecting dogs living in communities across the Northern Territory in Australia. In this study, 274 dog faecal samples and 11 faecal samples of unknown origin were collected from the environment and directly from animals across 27 remote communities located in northern and central Australia. Samples were examined using real-time PCR for the presence of *S. stercoralis* and four hookworm species; *Ancylostoma caninum, Ancylostoma ceylanicum, Ancylostoma braziliense* and *Uncinaria stenocephala*. The prevalence of *S. stercoralis* in dogs was found to be 21.9% (60/274). *A. caninum* was the only hookworm detected in the dog samples with a prevalence of 31.4% (86/274). This study provides an insight into the prevalence of *S. stercoralis* and hookworm in dogs and informs future intervention and prevention strategies aimed at controlling these parasites in both dogs and humans**.** A One Health approach is crucial for the prevention of these diseases in Australia.

6.2 Introduction

Soil-transmitted helminths (STHs) are estimated to infect up to 2 billion people worldwide with high prevalence levels recorded in Southeast Asia (Bethony et al., 2006, Pullan et al., 2014, Jex et al., 2011). Australia as a whole, has a relatively low prevalence of STHs due to access to adequate hygiene and sanitation as well as clean water (Gordon et al., 2017). *Strongyloides stercoralis,* distributed throughout the tropics, is estimated to infect up to 370 million people worldwide, predominantly in socioeconomically disadvantaged communities (Olsen et al., 2009, Beknazarova et al., 2016b). Strongyloidiasis is a major health concern in Australian remote communities with up to 60% of Indigenous populations found seropositive to infection (Adams et al., 2003, Gordon et al., 2017, Johnston et al., 2005). *S. stercoralis* can infect humans chronically and in immunocompromised individuals, develop into severe hyperinfective or disseminated strongyloidiasis, which has a mortality rate of up to 90% (Geri et al., 2015).

Genetic studies worldwide and in Australia have shown that there are at least two genetically different strains of *S. stercoralis*, one that is zoonotic, infecting both humans and dogs, and the other restricted to infecting dog only (Jaleta et al., 2017, Nagayasu et al., 2017, Beknazarova et al., 2019). There is sufficient evidence to suggest that dogs can act as potential reservoirs for human strongyloidiasis and that controlling the parasite in dogs may play a role in preventing the disease in humans.

Hookworms infect up to half a billion people worldwide (Forouzanfar et al., 2016). The most prevalent hookworms in humans in southeast Asia and the Pacific are *Necator americanus, Ancylostoma*

ceylanicum and *Ancylostoma duodenale* (Holt et al., 2010, Palmer et al., 2007). Hookworms in humans can contribute to iron deficiency anemia and impact on maternal and child health (Hotez and Whitham, 2014). Hookworm infection in humans used to be considered a widespread public health problem in parts of Australia until intervention campaigns successfully eradicated it from the mainstream population (Bearup, 1931, Bradbury and Traub, 2016, Prociv and Luke, 1995, Holt et al., 2017, Davies et al., 2013). Only a single autochthonous case of *A. ceylanicum* in humans was reported in Western Australia and an imported case reported in an Australian soldier returning from the Solomon Islands (Koehler et al., 2013, Speare et al., 2016). Recent studies found that hookworms, in particular *A. duodenale* (Smout et al., 2017) remain sporadically reported in the remote communities in far north Queensland, northern parts of New South Wales, Western Australia and Northern Territory. In the Northern Territory hookworm prevalence in humans is reported significantly lower than that of *S. stercoralis* (Koehler et al., 2013, Bradbury and Traub, 2016, Davies et al., 2013, Hopkins et al., 1997). Overall, there has been reduction seen in both *S. stercoralis* and hookworm infections in humans in the remote communities in the NT attributed to the deworming programs (Holt et al., 2017). However, neither strongyloidiasis nor hookworm has been eradicated completely from the remote communities despite various intervention programs.

In Australia, like other regions of the Asia Pacific, dogs are considered a potential zoonotic reservoir for STH infections including strongyloidiasis and hookworms. Within Indigenous Australian communities, the risk of transmission might be increased given that dogs tend to live in close contact with humans (Constable et al., 2010).

The most common hookworms in dogs in Australia are *Ancylostoma caninum*, *A. ceylanicum*, *Ancylostoma braziliense,* and *Uncinaria stenocephala* (Palmer et al., 2007). These hookworm species are zoonotic and all are capable of causing cutaneous larva migrans in humans (Traub et al., 2004a). *A. ceylanicum* and *A. caninum* are of particular interest, as *A. ceylanicum* larvae can develop into the adult stage in humans and is now recognised as the second most common species of hookworm infecting humans in the Asia Pacific (Traub, 2013, Bradbury et al., 2017, Inpankaew et al., 2014). While *A. caninum* infection in humans is non-patent and strongly associated with eosinophilic enteritis (Prociv and Croese, 1990, McCarthy and Moore, 2000). Recent data show high prevalence of both *A. caninum* and *A. ceylanicum* in dogs, dingoes and soil in the remote communities in Western Australia and north-east Queensland. (Rusdi et al., 2018, Smout et al., 2018a). Both *A. ceylanicum* and *A. caninum* are considered neglected zoonotic parasites and accurate data on their prevalence in dogs and humans residing in Indigenous communities of northern Australia is largely lacking (Palmer et al., 2007, McCarthy and Moore, 2000, Walker et al., 1995, Traub, 2013, Smout et al., 2017).

The aim of this study was to map the distribution of zoonotic *S. stercoralis* and hookworm species in dogs in remote communities in the northern Australia. To our knowledge this is the first large scale molecular study of dogs in the remote communities for the presence of *S. stercoralis* and hookworm.

6.3 Materials and Methods

Ethical Considerations

The project was registered with the Animal Welfare Committee Research Development and Support. The research was approved by the Social and Behavioural Research Ethics Committee (SBREC) (No 6852 dated 1st June 2015). For dog faeces collected from the residential or private land, consent from the owners of dogs or local managers of the communities was obtained.

Study area and population

Two hundred and eighty-five faecal samples presumed to be from dogs were collected from the remote communities across the Northern Territory, Central Australia, Western Australia, and north-west of South Australia during 2016 – 2019. The samples were collected from total 27 locations including 23 communities in the Northern Territory, two communities in the northern parts of Western Australia, one community in the north-west of South Australia and surroundings in Alice Springs.

Specimen collection and DNA extraction

Faeces were either collected by the Flinders University researchers, NT Health Environmental Health Officers (EHOs) or veterinarians primarily from the Animal Management in Rural and Remote Indigenous Communities (AMRRIC).

In the cases where samples were collected by EHOs or representatives of the AMRRIC they would do so during their routine inspection or dog treatment. A sampling package containing the project's information sheet, risk assessment and consent forms, sampling instructions and sampling equipment was provided to them in advance.

Permission from the community elders, Traditional Owners or community managers was obtained prior to collecting samples from private or residential land. Approximately 2-3 g faeces were collected and preserved immediately in 6 mL DESS (dimethyl sulfoxide, disodium EDTA, and saturated NaCl) and kept at room temperature (Beknazarova et al., 2017a). The samples were shipped to Environmental Health laboratory, Flinders University, within 30 days after collection for further sample processing. The genomic DNA was extracted using the PowerSoil DNA Isolation Kit (QIAGEN, Hilden, Germany) as described previously (Sultana et al., 2013, Beknazarova et al., 2019).

Real-time PCR assays

The real-time PCR assay was adopted from Verweij *et al*. (Verweij et al., 2009) using *S. stercoralis* specific primers, Stro18S-1530F and Stro18S-1630R, and a probe, Stro18S-1586T, targeting 101 bp region of the 18S rRNA, and conducted as described previously (Beknazarova et al., 2019). All qPCR reactions were performed in triplicates on the two channel Corbett Rotor-Gene 6000 machine (QIAGEN, Hilden, Germany). The primers and a probe and qPCR conditions are shown in the Table 6.1. It should be noted that while this this primer/probe set is considered specific for *S. stercoralis*, it can also amplify other species of *Strongyloides, including Stronglyoides ratti*.

Positive, non-template and negative control samples were included in each qPCR run. The Cq threshold value for *S. stercoralis* was 0.02-0.03. A sample was considered positive when the Ct value was lower than the mean negative Ct minus 2.6 standard deviations of a mean negative control Ct (Beknazarova et al., 2017a). Positive samples were amplified in every qPCR reaction.

Multiplex quantitative qPCR assays for detection of *A. ceylanicum, A. caninum, A. braziliense* and *U. stenocephala* using primers and probes targeting internal transcribed spacer 1 (ITS1) gene were adopted and performed as described by Massetti et al (unpublished).

Synthetic block gene fragments (IDT Technologies, Skokie, Illinois, USA) of ITS1 genes targeted by the PCRs primers and probes for *A. ceylanicum, A. caninum, A. braziliense* and *U. stenocephala* were used as positive controls in the PCR runs (Table 6.2). Nuclease-free water was used as the non-template or negative control, synthetic block gene fragment (IDT Technologies, Skokie, Illinois, USA) of a herpes virus (Equine herpesvirus type 4, accession number KT324745.1) was used as an internal control, and primers and a probe to amplify a region of the dog mtDNA (*Canis lupus familiaris or Canis lupus dingo*) was used as DNA extraction controls in all runs. Primers and probes and qPCR conditions are shown in Table 6.1. The GenBank Accession numbers and sequences of the synthetic block gene fragments used as controls in this study are presented in the Table 6.2. All hookworm qPCR reactions were performed in duplicates on the multiplex channel Corbett Rotor-Gene 6000 machine (QIAGEN, Hilden, Germany).

Table 6.1 Primers and probes and PCR conditions

Primer/Probe	Amplicon	Sequence	Reaction conditions
Stro18S-1530F Stro18S-1630R Stro18S-1586T FAM	rDNA 101 bp	5'-GAATTCCAAGTAAACGTAAGTCATTAGC-3' 5'-TGCCTCTGGATATTGCTCAGTTC-3' 5'-FAM-ACACACCGGCCGTCGCTGC-3'-BHQ1	**Step 1:** 95°C for 15 min, **Step 2:** 95°C for 15 s, **Step 3:** 60°C for 30 s. Repeat steps two and three 40 times.
A. cancey F A. cancey R Ahumanceylanicum probe Acantub probe	ITS1 region	5'- GGGAAGGTTGGGAGTATCG-3' 5'- CGAACTTCGCACAGCAATC-3' 5'- Cy5/ CCGTTC+CTGGGTGGC/3IABkRQSp/-3' 5'-HEX/ AG+T+CGT+T+A+C+TGG/3IABkRFQ/-3'	**Step 1:** 95°C for 2 min, **Step 2:** 95°C for 15 s, **Step 3:** 60°C for 60 s. Repeat steps two and three 40 times.
Uncbraz F Uncbraz R Unc Probe Abra probe	ITS1 region	5'- GAG CTT TAG ACT TGA TGA GCA TTG-3' 5'- GCA GAT CAT TAA GGT TTC CTG AC-3' 5'-/5HEX/CAT TAG GCG /ZEN/GCA ACG TCT GGT G/3IABkFQ/-3' 5'-/56FAM/TGA GCG CTA /ZEN/GGC TAA CGC CT/3IABkFQ/-3'	**Step 1:** 95°C for 2 min, **Step 2:** 95°C for 15 s, **Step 3:** 64°C for 60 s. Repeat steps two and three 40 times.
EMV F ENV R ENV probe	Equine herpesvirus type 4	5'-GATGACACTAGCG-ACTTCGA-3' 5'-CAGGGCAGAAACC-ATAGACA-3' 5'-TEX-TTTCGCGTGC-CTCCTCCAG-IBRQ-3'	**Step 1:** 95°C for 2 min, **Step 2:** 95°C for 15 s, **Step 3:** 60°C for 60 s. Repeat steps two and three 40 times.
Dog F Dog R Dog probe	mtDNA	5'-CGACCTCGATGTTGGATCAG-3' 5'-GAACTCAGATCACGTAGGACTTT-3' 5'-FAM/ CCTAATGGT/ ZEN/ GCAGCAGCTATTAA/ LABKFQ-3'	**Step 1:** 95°C for 2 min, **Step 2:** 95°C for 15 s, **Step 3:** 60°C for 60 s. Repeat steps two and three 40 times.

Table 6.2 Synthetic block gene fragments used for positive controls

Species	GenBank Accession number	Sequence
Ancylostoma ceylanicum	DQ780009.1	CGTGCTAGTCTTCAGGACTTTGTCGGGAAGGTTGGGAGTATCGCCCCCCGTTACA GCCCTACGTGAGGTGTCTATGTGCAGCAAGAGCCGTTCCTGGGTGGCGGCAGTGA TTGCTGTGCGAAGTTCGCGTTTCGCTGAGCTTTAGACTTGAG
Ancylostoma duodenale/ Ancylostoma caninum	EU344797.1	CGTGCTAGTCTTCACGACTTTGTCGGGAAGGTTGGGAGTATCGCCCCCCGTTATAG CCCTACGTAAGGTGTCTATGTGCAGCAAGAGTCGTTACTGGGTGACGGCAGTGATT GCTGTGCGAAGTTCGCGTTTCGCTGAGCTTTAGACTTGAT
Ancylostoma braziliense	JQ812692.1	TGTACGAAGCTCGCGGTTTCGTCAGAGCTTTAGACTTGATGAGCATTGCTAGAATG CCGCCTTACCTGCTTGTGTTGGTGGTTGAGCGCTAGGCTAACGCCTGGTGCGGCA CCTGTCTGTCAGGAAACCTTAATGATCTGCTAACGCGGACGCCAGCACAGCAAT
Uncinaria stenocephala	HQ262054.1	GCTGTGCGAAGTTCGCGTTTCGCTGAGCTTTAGACTTGATGAGCATTGCTGGAATG CCGCCTTACTGTTTGTGTTGGTGGTTGGGCATTAGGCGGCAACGTCTGGTGCGAC ACCTGTTTGTCAGGAAACCTTAATGATCTGCTCACGTGGACGCCAATACAGCACT
Equid herpesvirus	KT324745.1	ATGAAAGCTCTATACCCAATAACAACCAGGAGCCTTAAAAACAAAGCCAAAGCCTC ATACGGCCAAAACGACGATGATGACACTAGCGACTTCGATGAAGCCAAGCTGGAG GAGGCACGCGAAATGATCAAATATATGTCTATGGTTTCTGCCCTGGAAAAACAGGA AAAAAAGGCAATGAAGAAAAACAAGGGGGTTGGACTTATTGCC

The Cq threshold value for *A. ceylanicum* and *A. caninum* was 0.05 and a cut off level at Ct 32 was established. The Cq threshold values for *A. braziliense* and *U. stenocephala* were 0.08 and 0.1 accordingly a cut off level was at Ct 32.

Also, synthetic block gene fragments of hookworm were spiked with negative dog DNA and run by the qPCR to check for any inhibitors the might be contained in dog DNA. All spiked hookworm synthetic block gene fragments were amplified by the qPCR.

Statistical analysis

A chi-square independence test was performed to determine whether there was an association between hookworm and *S. stercoralis* infection. Data were analysed using SPSS (SPSS for Windows, Version 23, IBM) and Excel 2016 (Microsoft).

6.4 Results

Dog DNA origin

There were 285 fresh presumed dog faeces collected from communities across Northern Territory, Central Australia, northern parts in Western Australia and north-west of South Australia and screened for *Canis familiaris*. Two hundred and seventy-four (274) out of 285 DNA samples extracted from the faeces were confirmed to be of a dog origin (*Canis lupus familiaris* or *Canis lupus dingo*) by PCR based amplification of the partial mtDNA.

Prevalence of *S. stercoralis* and hookworm

The prevalence of *Strongyloides* spp. among 285 environmental faecal samples was 21.1% (60/285) as determined by PCR based on amplification of the partial 18S rRNA. The prevalence of *S. stercoralis* among 274 dog faecal samples was 21.9% (60/274) (Figure 6.1).

Out of four hookworm species tested, only *A. caninum* was detected. The prevalence of hookworm infection (*A. caninum*) among 285 environmental faecal samples was 30.2% (86/285) by PCR based on amplification of the partial ITS gene. The prevalence of hookworm infection (*A. caninum*) among 274 dog samples was 31.4% (86/274) (Figure 6.1).

Maps showing sample locations and *S. stercoralis* and hookworm prevalence in dogs are shown in Figures 6.2 and 6.3.

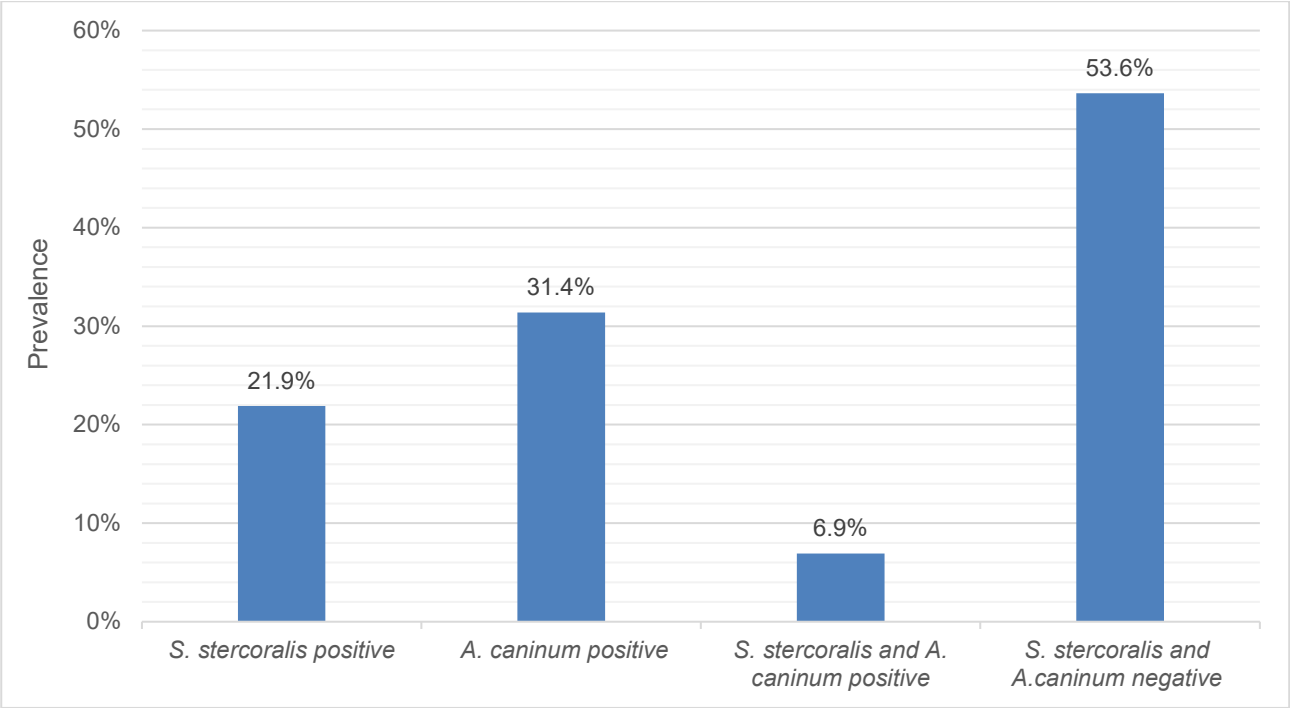

Figure 6.1 Dog faecal samples that were positive and negative for *Strongyloides stercoralis* and *Ancylostoma caninum*

Association of hookworm with strongyloidiasis

The chi-squared analysis did not identify any statistically significant association between *S. stercoralis* and *A. caninum* (x^2 (1) = 0.003, p – 0.958, n = 274, phi = 0.003). Of the 274 dog faecal samples, 6.9% (19/274) were positive for both *S. stercoralis* and *A. caninum,* and 53.6% were negative for either parasite (Figure 6.2).

There was no non-dog faecal sample that was infected with *S. stercoralis* or *A. caninum.*

6.5 Discussion and conclusion

This study used qPCR to detect zoonotic *S. stercoralis* and hookworms in dog faecal samples collected from the remote communities in northern and central Australia. The prevalence of *S. stercoralis* in dogs was 21.9% and the prevalence of *A. caninum* was 31.4%. All samples were negative for *A. ceylanicum*, *A. braziliense* and *U. stenocephala*, which support previous studies demonstrating that *A. caninum* is the most common hookworm in dogs living in the remote communities in Australia (Palmer et al., 2007, Rusdi et al., 2018, Smout et al., 2018b).

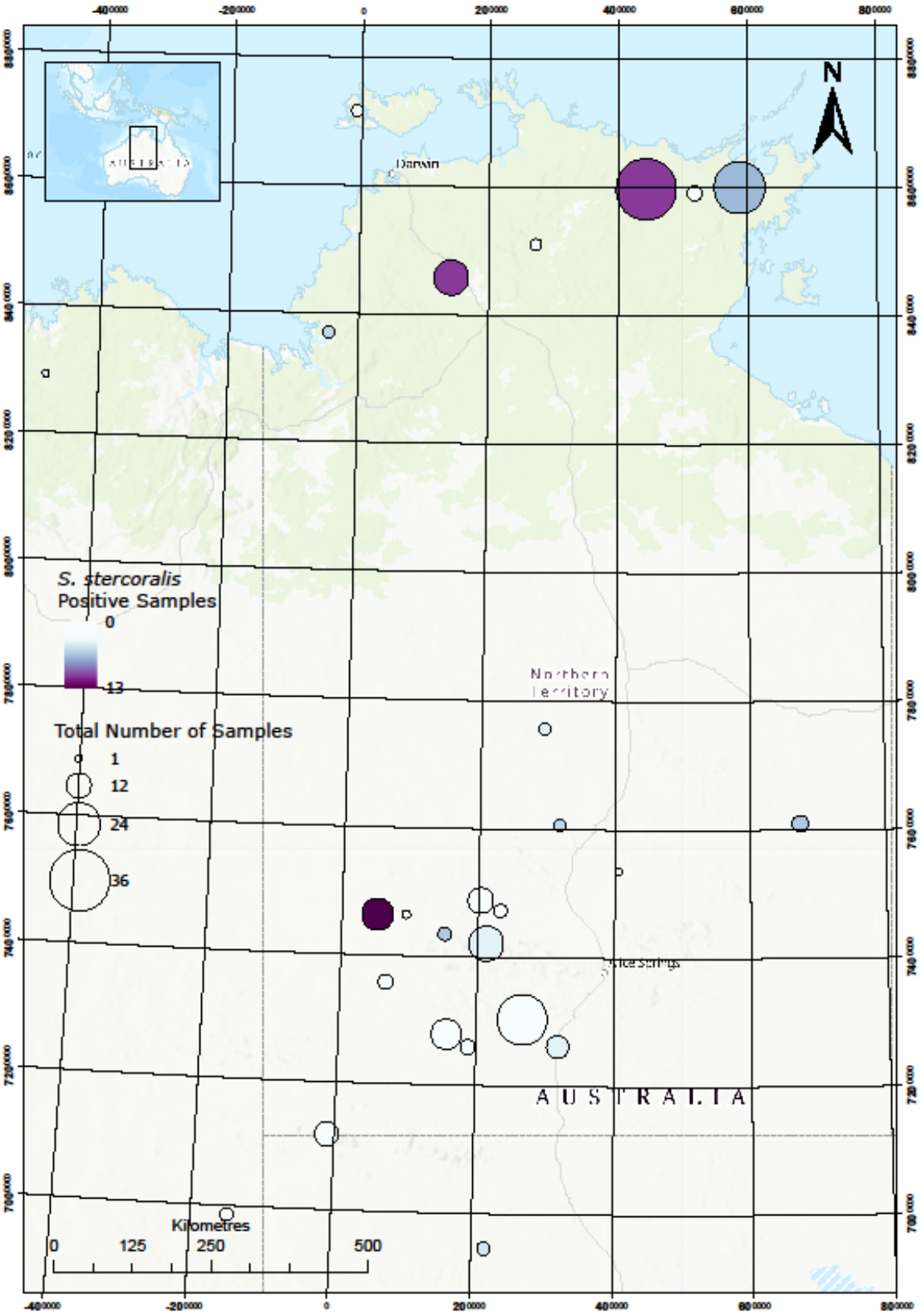

Figure 6.2 Opportunistic mapping of *Strongyloides stercoralis* in dogs in the remote communities

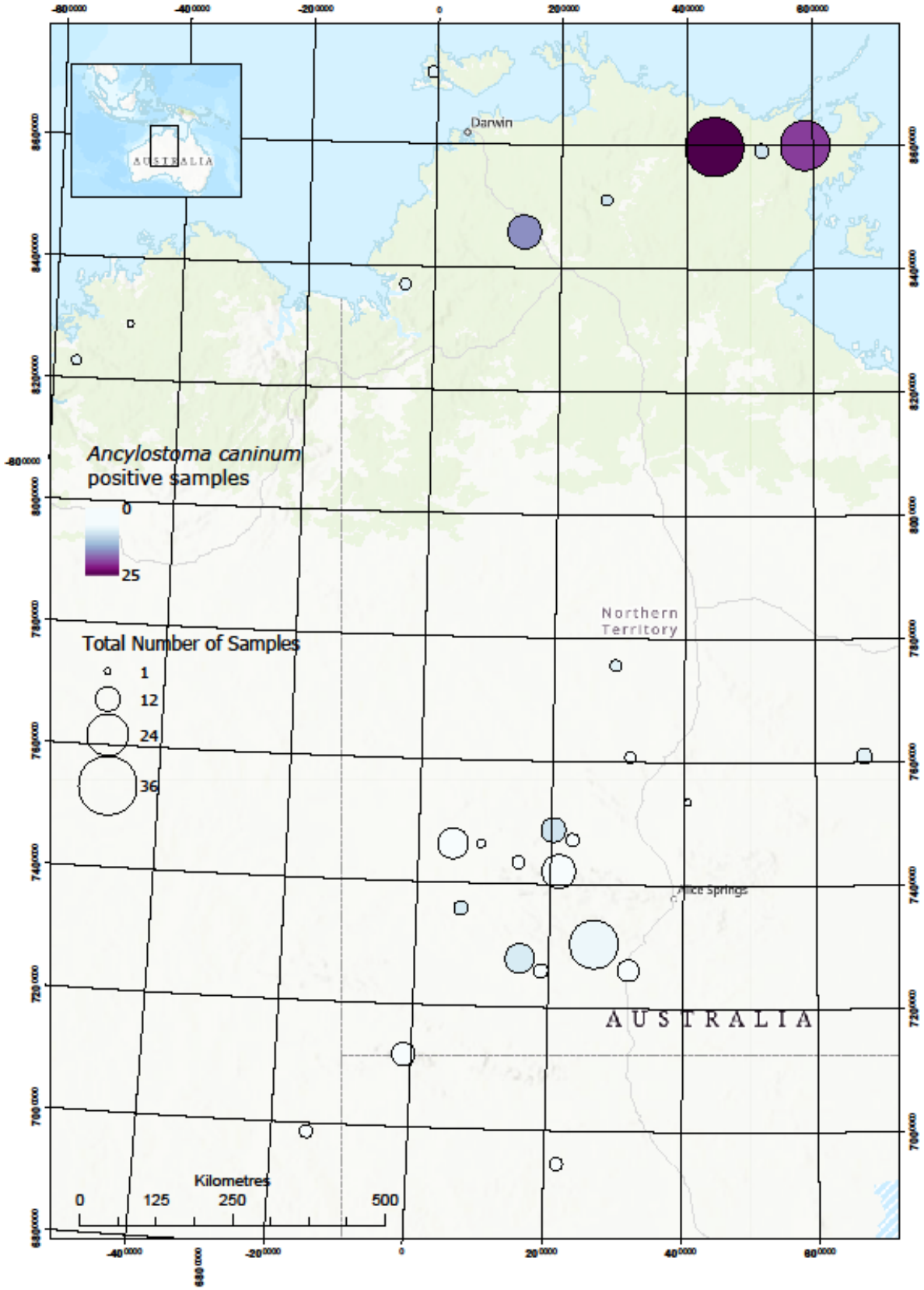

Figure 6.3 Opportunistic mapping of *Ancylostoma caninum* in dogs in the remote communities

A. caninum is known to cause eosinophilic enteritis in humans. Although infection is asympotmic in most cases, some individuals may suffer strong abdominal pain with or without peripheral eosinophilia, nausea, diarrhoea, anorexia, and/or allergic reactions (Prociv and Croese, 1996). In case of the infection being patent, the impact on nutritional status and immunocompetence caused by hookworm might be associated with other health problems, including greater susceptibility to other helminth infections (Fleming et al., 2006).

Although *A. ceylanicum* is the predominant hookworm of dogs and cats in Asia, it was only recently reported in dogs in Australia (Traub, 2013), although its presence in a cat from far north Queensland was retrospectively dated back to 1994 (Traub et al., 2008). *A. ceylanicum* was detected for the first time in Australia in 6.5% dogs from rural and urban areas in Broome, Brisbane, Sunshine Coast, Melbourne and Alice Springs (Palmer et al., 2007). More recently, *A. caninum* and *A. ceylanicum* infections reported for the first time at a prevalence of 98.4% (62/63) and 1.6% (1/64) respectively in domestic dogs in far north Queensland (Smout et al., 2017). The same study discovered 25% to 100% prevalence of *A. ceylanicum* in the soil in different communities in far north Queensland (Smout et al., 2017). Further, a study on dingoes and dogs in the north-east Queensland reported 100% (35/35) and 11% (4/35) prevalence of *A. caninum* and *A. ceylanicum* respectively in dingoes, and 92% (78/85) prevalence of *A. caninum* in dogs based on both necropsy and faecal examination (Smout et al., 2018a). A very recent study found 66% (93/141) camp dogs in remote communities in Western Australia infected with *A. caninum* based on molecular examination (Rusdi et al., 2018). The absence of *A. ceylanicum* in this study is likely owing to the climactic conditions of the study area such as dry weather at the time of sampling. *A. ceylanicum* doesn't have the biological advantage of undergoing "arrested development", in which larvae undergo a period of hypobiosis in host tissue only to re-develop to adults in the intestinal track when climatic conditions favour transmission (Schad and Page, 1982).

The absence of *U. stenocephala* in the samples is supported by its association with lower temperatures (Beveridge, 2002). Predominately, *U. stenocephala* is found in the southern regions of Australia, as the optimum temperature conditions for *U. stenocephala* larvae development to the infective stage is 7.5 ºC to 27 ºC and the ideal temperature for the free-living stages is 20 ºC (Gibbs and Gibbs, 1959). Likewise, previous studies have only detected *A. braziliense* from dogs located in north Queensland (Stewart, 1994, Palmer et al., 2007).

Molecular detection methods have been shown to be highly effective in detection of *S. stercoralis* and hookworm in faecal samples (Schär et al., 2013a, Verweij et al., 2009, Gasser et al., 2008, Hii et al., 2018) (Massetti, et al., unpublished). It has been however that the sensitivity of PCR for the detection of *S. stercoralis* is decreased when there they can amplify *S. ratti* as was shown previously (Sultana et al., 2013), meaning that for environmental samples we can only assume that positive samples contain *Strongyloides* spp. As for the dogs, we know from the previous genotyping study

on dogs living in remote communities in Australia that they are infected with *S. stercoralis* strains (Beknazarova et al., 2019). However, owing to the potential for hunting coprophagia, we cannot rule out the possibility of mechanical ingestion of other species of *Strongyloides*, including human sourced.

Normally, increased humidity and temperature are associated with the presence of *Ancylostoma* spp. and *S. stercoralis.* Tropical climates have been shown to be associated with multiple parasite infections in humans, (Brooker et al., 2000, Fleming et al., 2006). Hookworm infection intensity has been also associated with multiparasitism, as co-infection with hookworm weakens the immune system of the host. The intensity of strongyloidiasis infection is in turn highly dependent on the immune status of the host (Fleming et al., 2006, Brooker et al., 2000). This emphasises the importance of detecting and differentiating parasite infections. Moreover, indiscriminate use of anthelmintic drugs may cause development of anthelmintic resistance (Thompson and Roberts, 2001). A study conducted in Brazil showed a strong association between hookworm and other helminth infection (but not *S. stercoralis)* in humans (Fleming et al., 2006). In our study we did not find any significant association between *Strongyloides* spp. and *A. caninum* in dogs (Figure 6.S1). Non-infected dogs might be a result of dog health programs targeted at desexing and deworming dogs in the communities that are run by the AMRRIC.

Infection of both *S. stercoralis* spp. and hookworm occurs through exposure to soil contaminated with free-living infective stages of a parasite (Bethony et al., 2006). In the studied locations, dogs live in close proximity with their owners. Climate, sanitation and hygiene, environmental contamination with human or dog faeces and lack of STH disease knowledge are the main factors for the disease persistence and can influence transmission (Olsen et al., 2009, Traub et al., 2004b, McCarthy and Moore, 2000). The findings of this study emphasise the importance of the One Health initiative, which considers veterinary and public health interventions together. One Health approach should be central in developing methods to eliminating *S. stercoralis* and hookworms. In order to maintain health of both dogs and humans, veterinarians and pet owners are encouraged to coordinate and work in partnership (Willis and Ross, 2019).

The findings of this study need to be interpreted in light of its limitations. The faeces were collected from the ground rather than directly from the rectum of a dog. Therefore some of the samples that were collected were found to not be from dogs. Samples collected from the environment might have been contaminated with extraneous environmental organisms or their DNA, which could have caused further inhibition of the DNA of the target organisms (*Strongyloides* spp. or hookworm) (Alaeddini, 2012, Schrader et al., 2012), or researchers could have accidentally collected faeces that were old enough for parasites' DNA to degrade. Both limitations could have resulted in false negatives. Furthermore, the potential of fogs to hunting and coprophagia could lead to false positives results. The opportunistic sampling method did not let us take into account risk factors associated

with the parasite prevalence such as seasonal variation, climate conditions or anthelmintic use. Furthermore, there was significant variation in the number of samples from each geographical area.

The aim of this study was to map the prevalence of *S. stercoralis* and hookworm in dogs in remote communities in Australia based on molecular screening of dog faeces. The objective was to develop and optimise detection methods that can be applied in similar environmental settings without laboratory facilities and in respectful and non-intrusive manner. This study detected 21.9% and 31.4% of *S. stercoralis* and *A. caninum* in dog faeces respectively collected from the remote communities. Future research is needed to examine parasite prevalence in both dogs and humans from the same communities to determine whether there is an association to assess the zoonotic potential of dogs to transmit the diseases. Given the zoonotic nature of these parasitic species, the findings of this study can be used to develop control measures to maintain dog and human health.

6.6 Author contributions

MB: Designed study, obtained ethics approvals, collected specimens, extracted DNA, optimized and ran real-time PCR assays for S. stercoralis and hookworms, performed data analysis and interpretation, wrote and reviewed manuscript drafts; RT: helped with real-time PCR assays, reviewed manuscript drafts; HW: Provided PhD supervision of MB, designed study, obtained ethics approvals, obtained funding, reviewed manuscript drafts; KR: Provided PhD supervision of MB, designed study, obtained ethics approvals, obtained funding, reviewed manuscript drafts.

6.7 Acknowledgments

The authors would like to thank Dr Jan Allen and Dr. Madeleine Kelso from the Animal Management in Rural and Remote Indigenous Communities, Dr Ted Donelan from the West Arnhem Regional Council, Fiona Smith, Aaron Clifford, Kiri Gould, Russel Spargo and Ryan McLean from the Environmental Health Branch at the Department of Health, NT for helping us collecting dog faeces. We wish to thank Dr Rogan Lee, Dr Matthew Watts, John Clancy and Vishal Ahuja at the Westmead Hospital, NSW for sending us *S. ratti* infected rat faeces. The authors would also like to thank Ms Patsy Zanjedes from the Melbourne Veterinary School, University of Melbourne for her assistance in the lab.

The work has been supported by the Australian Government Research Training Program Scholarship and Flinders University Overseas Travelling Fellowship.

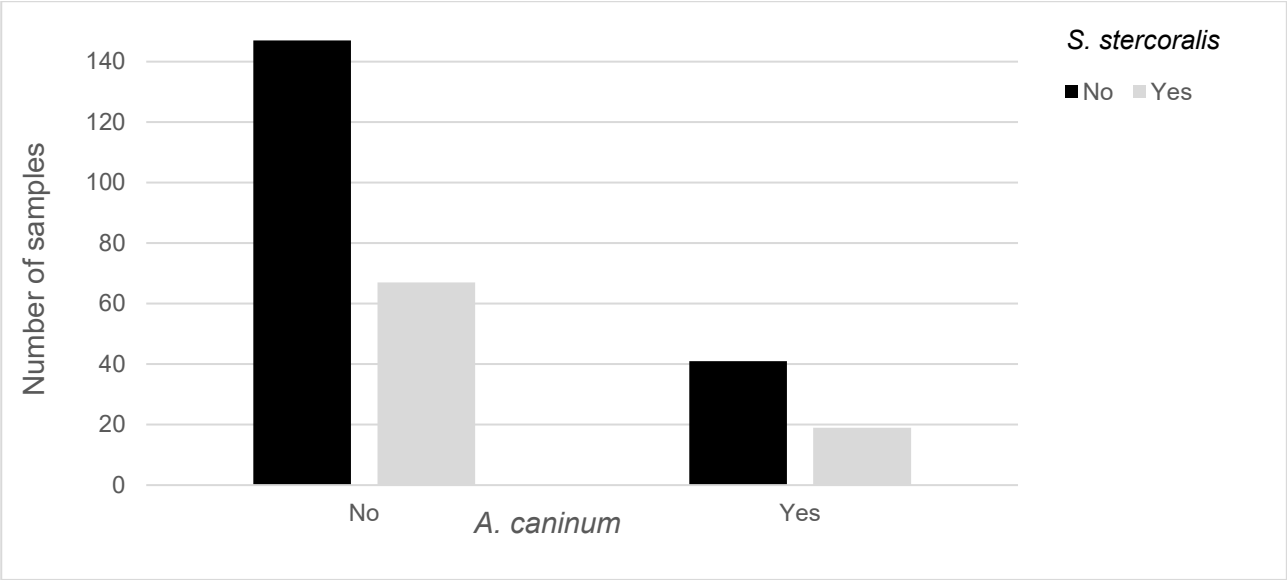

Figure 6.S1 Chi-square test association between *Strongyloides stercoralis* and *Ancylostoma caninum* infection in dogs

7. GENERAL DISCUSSION, RECOMMENDATIONS AND CONCLUSION

This research examined *Strongyloides stercoralis* in Australia and was conducted in four sections:

- The first phase of the research was to assess the global prevalence of *S. stercoralis* in relation to its presence in subtropical, tropical and temperate zones with mild and cold winters, and to explore socioeconomic and corresponding sanitary and hygiene conditions of these regions. Current treatment method of strongyloidiasis that includes administration of anthelminthic drugs was then explored. Additionally, the potential of nematicide application as an alternative or additional way of controlling *S. stercoralis* in the environment was also reviewed.
- The second part was research to determine whether there was a case for making strongyloidiasis a notifiable disease in Australia. The 12 criteria required for a disease to be included in the Australian National Notifiable Disease List were addressed.
- In the third part the dogs' role in human strongyloidiasis was examined. Firstly, a DNA preservation method was developed and validated that would prevent *Strongyloides* DNA in the dog faeces from degrading during transportation. Then, the literature was reviewed on the association of dog and human strongyloidiasis, and lastly, Australian dog and human *S. stercoralis* were genotyped and compared.
- Finally, the last part was an opportunistic mapping study of the zoonotic parasites *S. stercoralis* and hookworm species detected in faeces from dogs living in the remote communities in Australia.

7.1 Sanitary and hygiene factors associated with strongyloidiasis and control of *S. stercoralis* in the environment

In the first part of the research, the existing data on strongyloidiasis cases around the world, published from 1990 to 2016, were collected, reviewed and summarized. A review assessing the existing treatment options of strongyloidiasis was then conducted.

Socioeconomic factors and associated sanitary and hygiene conditions in the areas prevalent with strongyloidiasis.

In this review strongyloidiasis was shown to be predominantly found in tropical and subtropical regions such as Southeast Asia, Africa, Central and South America (Genta, 1989a, Ahmad et al., 2013, Repetto et al., 2013, Paula and Costa-Cruz, 2011, Babatunde et al., 2010). However, it was further shown that reported endemic areas are countries with developing economies and low socioeconomic status resulting in inadequate living and poor sanitary and hygiene conditions. Given

the life cycle of *S. stercoralis* and the route of exposure to the parasite, improper toileting systems, lack of an access to clean water, and overcrowding are the main factors relating to disease transmission (Steinmann et al., 2007, Paula and Costa-Cruz, 2011). Therefore, it was concluded that in tropical and subtropical regions, the disease is most likely to occur in areas with poor hygiene and living conditions.

Next, it was demonstrated that while strongyloidiasis is predominantly found in the tropics and subtropics, it had also been reported in continental climate zones with temperatures reaching below zero such as Appalachia regions in the USA and North Caucasus in Russia (Safdar et al., 2004, Prokhorov and Golovan, 1983). The studies demonstrated that larvae can survive in lower temperatures and infect humans. Further analysis showed that these reported regions belonged to populations with low socioeconomic status. This again demonstrated that strongyloidiasis is not strictly linked to climatic conditions but is instead influenced by poor sanitation hardware.

This work aimed to better understand the factors influencing the strongyloidiasis occurrence. Currently, strongyloidiasis is being classified as a neglected tropical disease, and this research showed that this is not completely true. While subtropical and tropical conditions favour strongyloidiasis transmission, sanitation and hygiene of the areas are the main factors causing the disease. Classifying strongyloidiasis based on the climatic conditions is misleading and prevents strongyloidiasis from being recognized as a disease of poverty.

The main conclusion of this study is that strongyloidiasis is a problem of all the countries with low socioeconomic status. Improving living conditions, infrastructure, sewerage and wastewater systems, and providing access to clean water should be primary approaches in controlling strongyloidiasis.

Control of *S. stercoralis* in the environment

In the second review, current treatment methods of strongyloidiasis were explored. It was found that they are restricted to administration of anthelminthic drugs. There are two classes of anthelminthic drugs that are recommended by the World Health Organisation (WHO, 2006). Benzimidazole class that includes albendazole and mebendazole, and macrocyclic lactone class drugs, that include ivermectin with the last considered as the drug of choice (Toma et al., 2000).

In this review, animal studies were examined and it was shown that there has been a high resistance reported to ivermectin and benzimidazole class drugs in the *Strongyloides* species of sheep and horse (Kaplan et al., 2004, Maroto et al., 2011, Mohamed and Al-Farwachi, 2008, Molento, 2009). It was also found that the same receptors are involved in ivermectin and albendazole resistance. These findings, coupled with the small number of drugs that are successful against *Strongyloides*,

leads to the conclusion that there is enough evidence to suggest there is potential for human resistance.

Furthermore, the unique autoinfection feature of *S. stercoralis* was identified as another issue associated with anthelminthic drug treatment. A single remaining larvae can reproduce through parthenogenesis, leading to the recrudescence of disease.

In this review there were two main issues identified in the current treatment of strongyloidiasis that relies purely on anthelminthic drug administration. Potential resistance and reinfection make drug treatment alone unreliable in the long term, and other options of controlling the disease should be considered. As an example the potential of nematicide application was reviewed. There are around 20 types of commercially available nematicides in Australia (Authority, 2016). The main chemical groups of nematicides, such as organophosphorus, carbamate-methylcarbamate and thiazole, have been tested on *C. elegans* and showed a potential for use on animal and human parasites.

Based on this research, it was proposed that a combined approach to control *S. stercoralis* that includes drug treatment and environmental control aimed at the prevention of *S. stercoralis* infection is needed. However it cannot be claimed that this is a new idea, indeed, it was more than twenty years ago that Grove said the most effective way of fighting *Strongyloides* is controlling it in the environment (Grove, 1990).

7.2 Making strongyloidiasis a notifiable disease in Australia

Strongyloidiasis has been endemic in remote communities in Australia since the early 1900s (Johnston, 1916, Nicoll, 1917, Willis, 1920). Nowadays, it remains endemic in the remote communities predominantly in the Northern Territory (Page et al., 2016). Despite high mortality and morbidity, it remains a highly neglected disease. Accurate incidence and prevalence data and an understanding of the geographical distribution, and the rate of transmission of the disease are lacking worldwide and in Australia. This is partially because of under-diagnosis due to the lack of a gold diagnostic standard, and also due to the absence of a mechanism to maintain surveillance data.

To address these issues strongyloidiasis should be made notifiable in Australia. Notification of the disease would allow cases around Australia be registered. Having the national surveillance system in place will help identifying susceptible populations, determine hotspots, understand the transmission of the disease and implement and assess intervention programs.

A systematic assessment of strongyloidiasis in Australia was performed to address the 12 criteria required for a disease to be included in the Australian National Notifiable Disease List (NNDL) issued by the Communicable Disease Network Australia operating under the Australian Health Protection Committee (Australia, 2017). Based on this assessment strongyloidiasis scored 28 to 30 fulfilling the

requirements for national notification to be recommended. There were six main key arguments identified:

- The disease is important to Indigenous health, and closing the health inequity gap between Indigenous and non-Indigenous Australians is a priority for the following reasons;

- A public health response is required to detect cases of strongyloidiasis and to establish the true incidence and prevalence of the disease;

- There is no alternative national surveillance system to gather data on the disease;

- There are preventive measures with high efficacy and low side effects;

- Data collection is feasible as cases are definable by microscopy, polymerase chain reaction, or serological diagnostics;

- Achievement of the Sustainable Development Goal # 6 on clean water and sanitation

After publishing this work collaboratively with the National Strongyloidiasis Working Group in Australia, there was a media release issued based on this case that caught wide media attention. As a result we were asked to give interviews through a number of media outlets, including the national television channel, on national radio and print media. This showed that there is great public concern around this disease as well as lack of general knowledge among general population. Following the process of making a disease notifiable, we wrote a letter to the Chief Health Officer of South Australia asking for the disease to be nominated. After receiving a negative answer from him, we submitted the case to the Chief Health Officer of Western Australia. It is a long process in which this systematic review and published manuscript serves as a solid start.

7.3 Dogs' role in human strongyloidiasis

Understanding the role of dogs in human strongyloidiasis was the main part of this research. As mentioned earlier in the discussion chapter, strongyloidiasis remains endemic in the remote communities predominantly in the Northern Territory in Australia. A study in the remote communities in the Northern Territory assessing strongyloidiasis and scabies prevalence and the efficacy of the ivermectin mass drug administration (MDA) showed reduction in both parasite prevalence after the MDA. However, it failed to eliminate the diseases from the communities. Reappearance of strongyloidiasis could potentially be due to exposure and reinfection from the environmental reservoirs such as dog faeces or soil. In rural and remote Indigenous communities in Australia dogs are generally not treated as pets but humans and dogs have very close and intimate cultural bond (Willis and Ross, 2019).

This part of the research consisted of three parts.

Strongyloides spp. DNA preservation method

In the first study, the capacity of the DESS (dimethyl sulfoxide, disodium EDTA, and saturated NaCl) solution to preserve *Strongyloides* spp. DNA in the faeces was investigated. Collecting dog faecal samples from the remote communities for molecular screening was the main part of this research. Collected samples had to be shipped interstate to be processed. Therefore the aim was to find a method to preserve DNA at room temperature and using a non-ethanol based/non-refrigeration preservation method to avoid any complexities with air transportation.

Firstly, 1:1 and 1:3 sample to DESS ratios of non-infected dog faeces spiked with rat faeces infected with *Strongyloides ratti* at a 1:9 rat to dog faeces ratio were tested. DESS was shown to preserve the DNA for 56 days at room temperature with the 1:3 sample to DESS ratio performing better. This method was then validated on the field collected dog faeces.

Although in these experiments DESS showed a slight inhibitory effect on the DNA, it was not significant enough to not be amplified by the PCR. The results demonstrated that DNA starts dramatically degrading after around two weeks if not preserved, and 1:3 sample to DESS ratio still preserved DNA better. In this study, the manufacturer's protocol for DNA extraction was also modified by introducing an incubation step at 56ºC overnight after the cell lysis step, and reducing a ten minute shaking step instructed in the protocol to three minutes.

The DESS DNA preservation method has been subsequently used throughout the research and shown to be the most applicable and feasible for the field-collected faeces.

Australian dog and human *S. stercoralis* genotypes

Next, available literature that described the role of dogs in the spread of human strongyloidiasis was reviewed. In 2016, when this study commenced, there was no genetic evidence confirming the zoonotic potential of dogs to transmit strongyloidiasis to humans in Australia or worldwide. However, this review assessed studies that showed strongyloidiasis association between dogs and dog keepers (Goncalves et al., 2007, Georgi and Sprinkle, 1974), or laboratory studies showing that *Strongyloides* spp. can be detected in dogs using *S. stercoralis* primers and probes commonly used in humans (Buonfrate et al., 2017). The findings of this review showed that there was enough evidence to suggest that further research is needed to explore this potential route of infection.

Successful examples of MDA programs targeting animals to eliminate human diseases, such as treatment of dogs for rabies elimination or treating water buffaloes to control human schistosomiasis, were also reviewed as a part of this study. Furthermore, it was addressed in this review that animal

MDA programs were estimated to be cost effective presenting a low-cost and low-risk mechanism for controlling human disease by reducing the risk of reinfection. Based on this research, it was argued that dogs should be concomitantly treated for the prevention human strongyloidiasis.

Later in 2017, two independent studies conducted in Cambodia and Myanmar assessed *S. stercoralis* isolated from human and dog samples. The researchers studied the *SSU* markers in the 18S rDNA (Hypervariable-I (HVR-I) and hypervariable-IV (HVR-IV) region) and *cox*1 gene in the mtDNA. They found that there are at least two *S. stercoralis* populations, one is dog specific, and the other is human and dog infective (Jaleta et al., 2017, Nagayasu et al., 2017).

So, in the final part of this study, the regions of mitochondrial *cox*1 gene and nuclear *SSU* markers (HVR-I and HVR-IV regions) of the DNA extracted from Australian human faeces and sputum and dog faeces were amplified and sequenced. It was found for the first time that Australian dogs are infected with the zoonotic *S. stercoralis* genotype that also infects Australian humans. The same haplotype was also found in Cambodian dogs and humans. There was also a dog specific genotype discovered in Australian dogs, which was also reported previously in Cambodian and Myanmar dogs. Additionally, it was found that Australian dogs are infected with the genotype that was previously shown in a dog in Europe and a dog and chimpanzee in Japan. This genotype has not yet been found in Australian humans in this study nor in any other humans in other studies. Whether this genotype is zoonotic or not needs to be further confirmed. While the current study did not show the direct transmission of *S. stercoralis* from dogs to humans or vice versa, it confirmed the hypothesis of zoonotic transmission of strongyloidiasis. The findings of this work showed that dogs can be a potential reservoir for human strongyloidiasis. Mindful of the One Health approach (Rock et al., 2009) it is proposed again that dogs should be treated as well as humans to control strongyloidiasis in remote Australian communities.

Furthermore, in this genotyping study few unique *S. stercoralis* haplotypes were discovered in Australian dogs. These were two new *SSU* HVR-I haplotypes, four new HVR-IV haplotypes and several new *cox*1 gene haplotypes. Given that Australia has been isolated for a long geological period, and dogs were at least introduced 12,000 years ago (Clutton-Brock, 1995) it is quite possible that some unique genotypes or sub-species could have been harbored in Australian dogs.

Interestingly, a novel haplotype in the *cox*1 gene found in Australian dogs in this study was also shown to cluster closely to the *Strongyloides* sp. found in Southeast Asian loris. Another theory for unique haplotypes and potentially genotypes and subspecies of *Strongyloides* sp. in Australia might be their divergence from Southeast Asia as a result of a vicariance. However a larger sample size and additional sequencing and morphological examination is needed before these haplotypes can be assigned to a new genotypes or subspecies.

Description of *S. stercoralis* genotype present in Australian dogs and humans will help our understanding of the genetic diversity of the parasite and assessing its zoonotic potential.

7.4 Prevalence of zoonotic parasites in Australian dogs living in the remote communities

This part of the study was continuous throughout the whole project. During the three years faecal samples were collected during the field trips to the Indigenous communities in the Northern Territory in Australia. In other cases the samples were collected by others as per instructions and shipped to our lab.

Indigenous communities in the northern parts of Australia have been shown to have endemic strongyloidiasis infections for many decades (Adams et al., 2003, Gordon et al., 2017). Hookworm infection in humans is used to be a major public health problem in the mainstream population in Australia (Prociv and Luke, 1995, Holt et al., 2017, Bradbury and Traub, 2016), and now remains sporadically reported in the remote communities in north Queensland, New South Wales, Western Australia and Northern Territory (Davies et al., 2013, Hopkins et al., 1997, Koehler et al., 2013). These areas are known as low socioeconomic status areas and are associated with inadequate sanitary, hygiene and living conditions. The climate in the sampling areas ranges from dry and hot, to wet and warm. However, climate as a factor of strongyloidiasis and hookworm prevalence was not a focus of this study mainly due to the opportunistic method of sampling areas as a limiting factor.

Dogs present a potential zoonotic transmission of STHs including *S. stercoralis* and hookworms, and in remote communities in Australia there is a higher risk of such transmission due to the dogs living in close contact with humans (Constable et al., 2010).

The aim of this study was to firstly identify the presence and prevalence of *S. stercoralis* and determine the prevalence of hookworm in dogs living in the Indigenous communities, and secondly to map the distribution of these parasite cases. There were no data on the presence and prevalence of strongyloidiasis in dogs in remote communities in Australia. The latest data on the hookworm prevalence in dogs living in Indigenous communities date back to ten years ago and only covered a small number of dogs.

There were 274 dog faecal samples collected from the 27 remote Indigenous communities mostly in the Northern Territory that were screened for the presence of *S. stercoralis* and four zoonotic hookworm species including *Ancylostoma caninum*, *Ancylostoma ceylanicum*, *Ancylostoma braziliense* and *Uncinaria stenocephala*. Of the four hookworm species tested for, there was only *A. caninum* detected, and was found in 31.4% of dogs faeces. This support previous findings showing that *A. caninum* is the most common hookworm in dogs in Australia (Palmer et al., 2007, Smout et al., 2018a). *S. stercoralis* was found in 21.9% of faecal samples. The hookworm prevalence was

higher than that of *S. stercoralis* which is consistent with a human study suggesting that usually hookworm is likely to be higher than *S. stercoralis*, and hookworm infection can work as a proxy for *S. stercoralis* infection (Bisoffi et al., 2013). In this study hookworm infections did not correlate with *S. stercoralis* infection.

Not detecting *A. ceylanicum* was not surprising as it is not a very common hookworm in humans or dogs in Australia, unlike South East Asia. So, *A. ceylanicum* was only reported few times in dogs (Palmer et al., 2007, Smout et al., 2017) and dingoes (Smout et al., 2018a) in far north Queensland. Likewise, *A. braziliense* was only once detected in north Queensland, and *U. stenocephala* is associated with lower temperatures and is therefore more common in the southern parts of Australia (Beveridge, 2002, Palmer et al., 2007). Not discovering these hookworms in our dog faecal samples is consistent with what we know about hookworms.

S. stercoralis and *A. caninum* are zoonotic parasites and endemic to Indigenous populations. It is therefore important to understand their presence and prevalence in dogs living in these communities. The findings of this study can help us understand and control sources of human infection and develop appropriate intervention strategies that protect not only animal but also public health.

7.5 Limitations

The findings of this research need to be viewed in light of limitations. The main limitation of this study was inability to work with *S. stercoralis* DNA isolated directly from individual worms. Source of DNA plays an important factor in producing high quality readable sequencing data. Adaptation of next generation sequencing technique in this study was able to perform deep sequencing producing high quality reads, however, the quantity of readable sequence data was affected, resulting in a smaller number of haplotypes found in our samples. Additionally, whole genome sequencing could not be performed due to the absence of the whole organism DNA. Whole genome sequencing of *S. stercoralis* would strengthen our genotyping results.

Another major limitation was the opportunistic method of sampling. This was the most ethical and least intrusive method of collecting dog faecal samples in the remote communities. However, with this approach to sampling, seasonal and climate factors that might have an effect on the parasites' prevalence were not able to be considered. Furthermore, for many samples it was not possible to know whether and when the dogs had received the anthelminthic treatment. These are important factors that would influence *S. stercoralis* and hookworm prevalence in dogs in these communities. Additionally, sampling off the ground introduced a risk of collecting non dog faeces. Indeed, out of 285 collected faecal samples, 11 were confirmed to be not of a dog origin by the PCR amplification.

Additionally, other conventional tests such as serology or microscopy were not performed on our samples to confirm the molecular test results.

Working with Indigenous communities was another limiting factor. Despite the ethical approvals, there are multiple informal permissions required to enter the community and sample from the ground. Furthermore, a researcher had to be accompanied by people that were known and trusted by the community such as a local environmental health officer or veterinarian. This coupled with impaired communication with local people significantly slowed down the sampling process. However, showing respect and engaging with the communities over the longer-term was the main priority of this research.

The neglected status of the disease around the world and in Australia is illustrated by a limited number of studies addressing strongyloidiasis. In Australia, there are not many research groups that are interested in studying strongyloidiasis. This has impacted current research as there are fewer opportunities for collaboration or exchanging the knowledge.

7.6 Practical implications

In this study strongyloidiasis was demonstrated to be a disease of disadvantage. A focus on improving sanitary and hygiene conditions of the areas should be a priority to decrease the risk for the infection transmission. Furthermore, the limitations of the anthelminthic treatment examined in this study suggest the need of combined control of strongyloidiasis that should include drug treatment as well as controlling the parasite in the environment. The results of this study contribute significantly to the general understanding of the parasite and should be used in developing accurate control measures of the disease.

Next, a detailed assessment of strongyloidiasis in Australia was conducted and addressed the 12 criteria required for recommending the disease to be included in the Australian National Notifiable Disease List. This work demonstrated that strongyloidiasis fulfils the requirements to be recommended for its notification. The results of this study provide the foundation for further steps towards making strongyloidiasis notifiable in Australia as well as other endemic countries.

The genotyping research was the first of its kind and resulted in the discovery of the *S. stercoralis* genotypes in Australian humans and dogs. It was demonstrated that dogs living in the Indigenous communities in Australia are infected with zoonotic genotypes and present a potential risk for human strongyloidiasis. Furthermore, there were few unique *S. stercoralis* haplotypes discovered in Australian dogs. These findings add to our understanding of the genetic diversity of *S. stercoralis* in humans and dogs in Australia and also provide a starting point for future genetic studies. The data obtained in this study confirms the zoonotic potential of dogs in Australia and contributes significantly to the development of effective control measures targeting both veterinary and public health disciplines. It is however recommended to undertake a field intervention study combined with a mathematical modelling approach to get a better understanding of dogs' role in transmitting strongyloidiasis.

Further, the prevalence of zoonotic *S. stercoralis* and *A. caninum* based on the molecular examination of the faecal samples in the remote Indigenous communities in the Northern Territory were identified and cases were mapped. These data provide a foundation for future research into zoonotic diseases in dogs in these endemic communities.

Finally, this work was able to test and validate the applicability and accuracy of the molecular based tests (polymerase chain reaction) for the detection of *S. stercoralis* and hookworm species in dog faecal samples collected in the field.

7.7 Future research in Australia

This project investigated *S. stercoralis* with regard to the socioeconomic status and associated sanitary and hygiene hardware in endemic areas, looked at current treatment options, explored the status of strongyloidiasis in Australia and assessed the role of dogs in human strongyloidiasis in endemic remote Indigenous communities. This work also looked at the zoonotic hookworm prevalence in these dogs. The data presented within this thesis call for the following research needs:

1. The inaccurate association of *S. stercoralis* with the climatic conditions rather than socioeconomic status of the areas calls for studies to describe it more accurately. Studies should associate the disease with inadequate sanitary and hygiene conditions of the areas, focusing on targeting these primary causes of the spread of strongyloidiasis.
2. Current anthelminthic treatment of *S. stercoralis* is not sufficient and research should be conducted to look at options to control the parasite in the environment. This should focus on environmental health and One Health approaches to prevent the disease. Studies should investigate the free-living stage of the *S. stercoralis* larvae to identify what and how environmental factors influence its survival and determine the development cycle of larvae. Research looking at different ways of controlling the parasite in the environment would contribute significantly in reducing the prevalence of disease.
3. Strongyloidiasis in Australia should be included in the Australian National Notifiable Disease List. Researchers, the general population and government representatives of the country should work collaboratively towards making a disease notifiable.
4. Further molecular studies should be undertaken on a larger number of Australian dogs and humans to confirm and investigate further zoonotic and unique *S. stercoralis* haplotypes and genotypes in Australian dogs discovered in this study. Additionally, whole genome sequencing and morphological analyses need to be performed along to identify zoonotic genotypes and potentially new unique Australian subspecies.
5. A large scale study should look at the prevalence of zoonotic parasites in dogs and humans in endemic areas and perform quantitative analysis of zoonotic transmission in comparison to the anthroponotic transmission of the disease.

7.8 Conclusion

Strongyloides stercoralis in Australia is highly neglected and most prevalent in the Indigenous communities predominantly in the Northern Territory. Strongyloidiasis is mostly associated with inadequate sanitary and hygiene conditions that need to be improved to reduce the prevalence of the disease. Current treatment method relying purely on anthelminthic drug treatment is not reliable and should be combined with environmental control of *S. stercoralis*. There are gaps in the current knowledge on disease true prevalence, geographical distribution, rate and mode of transmission worldwide and in Australia. Making a strongyloidiasis a national notifiable disease will create a surveillance system that will help identifying susceptible populations, determine hotspots, understand the transmission of the disease and implement and assess intervention programs. It was found that dogs living in the Indigenous communities present a potential reservoir for zoonotic transmission of strongyloidiasis and shall be treated concurrently with humans to control strongyloidiasis and protect public health. Finally, it was shown that dogs in the remote communities are infected with another zoonotic hookworm, *A. caninum*. The results of this thesis stress the significance of applying environmental health and One Health approach as well as policy change in controlling zoonotic diseases caused by *S. stercoralis* and *A caninum* in the remote communities in endemic areas in Australia.

APPENDIX A. MATERIALS AND METHODS

This appendix describes general methods and equipment used throughout the research that have not been described elsewhere in the thesis, including publications. The chapter outlines optimisation of the real-time PCR assays for the detection of *Strongyloides* spp. and different DNA extraction methods used to extract DNA from faeces, wastewater, and a worm (*Strongyloides* spp.). It also describes microscopy performed during the research including Baermann technique and isolation of *Strongyloides* spp. from faeces.

1.1 DNA extraction

Different DNA extraction methods were used depending on the source of DNA. The PowerSoil DNA extraction kit was used to extract DNA from faeces, wastewater and human sputum. Freeze and thaw technique or lysis buffer solution were used to lyse the cell to extract DNA directly from the worm.

1.1.1 DNA extraction from faecal samples

DNA extraction from the faeces was performed using the PowerSoil DNA extraction kit (QIAGEN, Hilden, Germany).This kit was shown to be the most sensitive in extracting DNA fromfaecal matter when compared with other commercially available DNA extraction kits (Sultana et al., 2013). Throughout the research project, DNA extraction from the faecal samples and wastewater was performed at the Environmental Health Laboratory at Flinders University in Adelaide, SA, Australia (Method 1). A DNA extraction from the human sputum was performed at the Parasitic Disease Branch Laboratory at the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, US (Method 2). Although the same commercial DNA extraction kit was used, the methods were slightly different and are described below. The DNA extraction protocol used at the CDC laboratory was developed by the CDC researchers and adopted for this project.

Method 1: - Flinders

Slight additions and modifications to the standard PowerSoil DNA extraction kit protocol were introduced to reduce inhibition of the PCR reaction and facilitate breakdown of the helminth's cell membrane. In this method, an incubation step of incubation at 56ºC overnight after addition of a cell lysis solution was added to facilitate breakdown of fatty acids and lipids in a cell membrane (Sitta et al., 2014, Alonso et al., 2011). Additionally, a ten minute shaking step instructed in the protocol was reduced to three minutes to avoid excessive DNA shearing, which can lead to false negative results.

Washing step – removal of preservative/fixative solutions

Prior to DNA extraction, faecal samples preserved in DESS (dimethyl sulfoxide, disodium EDTA, and saturated NaCL) were centrifuged for three minutes at 3000 x g at the Orbital 400 Clements

(Phoenix, Lidcombe, Australia). The supernatant consisting of the preservative solution was removed.

DNA extraction

Approximately 250 mg of the remaining pellet was placed into a PowerBead tube containing lysis buffer (included in the PowerSoil DNA extraction kit). The PowerBead tube was then placed into the vortex homogeniser and run gently for 20 s to mix the content. Sixty µL of solution C1 (included in the Power Soil DNA extraction kit) containing SDS to aid in cell lysis was added to the PowerBead tube prior to incubation at 56ºC overnight (Sitta et al., 2014, Alonso et al., 2011). After incubation, the tube was vortexed for three minutes (this has been changed from the protocol) followed by centrifugation for 30 s at 10,000 x g. Up to 400-500 µL of the supernatant was then transferred to a clean 2 mL collection tube (included in the PowerSoil DNA extraction kit). Two hundred and fifty µL of solution C2 was added to precipitate humic acids and the tube was then vortexed for 5 s followed by incubation at 4 ºC for 5 min and centrifugation for one min at 10, 000 x g. Avoiding the pellet, around 600 µL of the supernatant was transferred into another 2 mL tube. Two hundred and fifty µL of solution C3 was added to the supernatant to precipitate proteins that might affect DNA, followed by brief vortex and another incubation at 4 ºC for 5 min. After incubation the tube was centrifuged for one min at 10,000 x g, after which up to 750 µL of the aliquot was transferred into a clean 2 mL tube. Twelve hundred µL of the DNA binding salt solution C4 was added to the supernatant to bind DNA to the silica. The tube was then vortexed for 5 s, and 675 µL aliquot was transferred onto an MB Spin Column tube (included in the PowerSoil DNA extraction kit). The tube was centrifuged for one min at 10,000 x g, after which the flow was discarded and a new portion of the sample was transferred to the MB Spin Column followed by centrifugation and removal of flow. This was repeated three times to process all of the sample. When the sample was processed, 500 µL of the ethanol based solution C5 was added to the supernatant to wash DNA from humic acids, salts, and other contaminants, and the sample was then centrifuged for 30 s at 10,000 x g. The flaw was discarded, and the tube was centrifuged for one min at 10,000 x g. The MB Spin Column was then carefully placed into a clean 2 mL tube without splashing of the solution C5. One hundred µL of the solution 6 containing sterile elution buffer was then added to the center of the white filter membrane to release DNA from the silica, and the tube was centrifuged for 30 s at 10,000 x g. The filter membrane was then discarded and the tube containing 100 µL of extracted DNA was stored at -20ºC.

Method 2: - at the Centers for Disease Control and Prevention

DNA extraction from the faeces was performed using the PowerSoil DNA isolation kit (QIAGEN, Hilden, Germany). Slight additions and modifications to the protocol were introduced to reduce inhibition of the PCR reaction and facilitate breakdown of the helminth's cell membrane. In this method, the standard kit's bead beating tubes were replaced with zirconia-silica beads due to the

superior performance of the latter in grinding parasites. An incubation step of a sample at 65ºC for ten minutes after addition of a cell lysis solution was added to facilitate breakdown of fatty acids and lipids in a cell membrane. As in the Method 1, a ten minute shaking step recommended in the protocol was reduced to three minutes to avoid excessive DNA shearing, which can lead to false negative results.

Washing step – removal of preservative/fixative solutions

Samples that were preserved in ethanol or ethanol containing solution were washed with sterile saline solution. Approximately one and a half microliter of an ethanol preserved faecal sample was added to a 2 mL tube followed by centrifugation for three minutes at 3,000 x g. All supernatant was removed and 1 mL of sterile saline solution was added to the tube. A tube was vortexed with the vortex homogeniser for five seconds and left overnight at -4ºC to equilibrate. The next day, a tube containing faeces and saline solution was centrifuged for three minutes at 3,000 x g. All supernatant was removed and a faecal sample was stored at -4ºC for DNA to be extracted within 12 h.

DNA extraction

Approximately 200 mg of the faecal samples were placed into a 2 mL collection tube and resuspended with distilled water to 1.7 mL. The tube was vortexed for 30 sec followed by the centrifugation for three minutes at 3, 000 x g. The supernatant was discarded and approximately 200 mg of the remaining pellet was placed into a 2 mL tube with silica beads and a 370 µL of lysis solution (lysis solution is taken from the PowerBead tubes included in the PowerSoil DNA extraction kit). Sixty µL of solution C1 (included in the Power Soil DNA extraction kit) containing SDS to aid in cell lysis was added to the silica bead tube followed by vortexing for 10 sec to mix the contents. Each faecal sample containing solution C1 was then incubated at 65ºC for 10 min. After incubation, the tube was vortexed for three min (this differs from the manufacturer'sprotocol) followed by centrifugation for one min at 10,000 x g. Up to 500 µL of the supernatant was then transferred to a clean 2 mL collection tube (included in the PowerSoil DNA extraction kit). Two hundred and fifty µL of solution C2 was added to precipitate humic acids and the tube was then vortexed for 5 sec followed by incubation at 4 ºC for 5 min and centrifugation for one min at 10,000 x g. Avoiding the pellet, around 600 µL of the supernatant was transferred into another 2 mL tube. Two hundred µL of solution C3 was added to the supernatant to precipitate proteins that might affect DNA, followed by brief vortex and another incubation at 4 ºC for 5 min. After incubation the tube was centrifuged for one min at 10, 000 x g, after which up to 750 µL of the aliquot was transferred into a clean 2 mL tube. Twelve hundred µL of the DNA binding salt solution C4 was added to the supernatant to bind DNA to the silica. The tube was then vortexed for 5 s, and 675 µL aliquot was transferred onto an MB Spin Column tube (included in the Power Soil DNA extraction kit). The tube was centrifuged for one min at 10,000 x g, after which the flow was discarded and a new portion of the sample was

transferred to the MB Spin Column followed by centrifugation and removal of flow. This was repeated three times to process all of the sample. When the sample was processed, 500 µL of the ethanol based solution C5 was added to the supernatant to wash DNA from humic acids, salts, and other contaminants, and the sample was then centrifuged for 30 s at 10,000 x g. The flaw was discarded, and the tube was centrifuged for one min at 10, 000 x g. The MB Spin Column was then carefully placed into a clean 2 mL tube without splashing of the solution C5. One hundred µL of the solution C6 containing sterile elution buffer was then added to the centre of the white filter membrane to release DNA from the silica, and the tube was centrifuged for 30 s at 10,000 x g. The filter membrane was then discarded and the tube containing 100 µL of extracted DNA was stored at -20ºC.

1.1.2 DNA extraction from wastewater

Briefly, about 700 mL wastewater was poured into a plastic funnel with a tubing and clamp supported on a single stand (Figure A.1). Wastewater was left for three to four hours to settle. About 200 mL of sediment wastewater was collected into 50 mL tubes and centrifuged for 5 min at 3000 x g at the Orbital 400 Clements (Phoenix, Lidcombe, Australia). Two hundred and fifty milligrams of precipitated pellet was then collected into the PowerBead tube for further DNA extraction using the PowerSoil DNA extraction kit (QIAGEN, Hilden, Germany), as described in the 1.1.1 (Method 1).

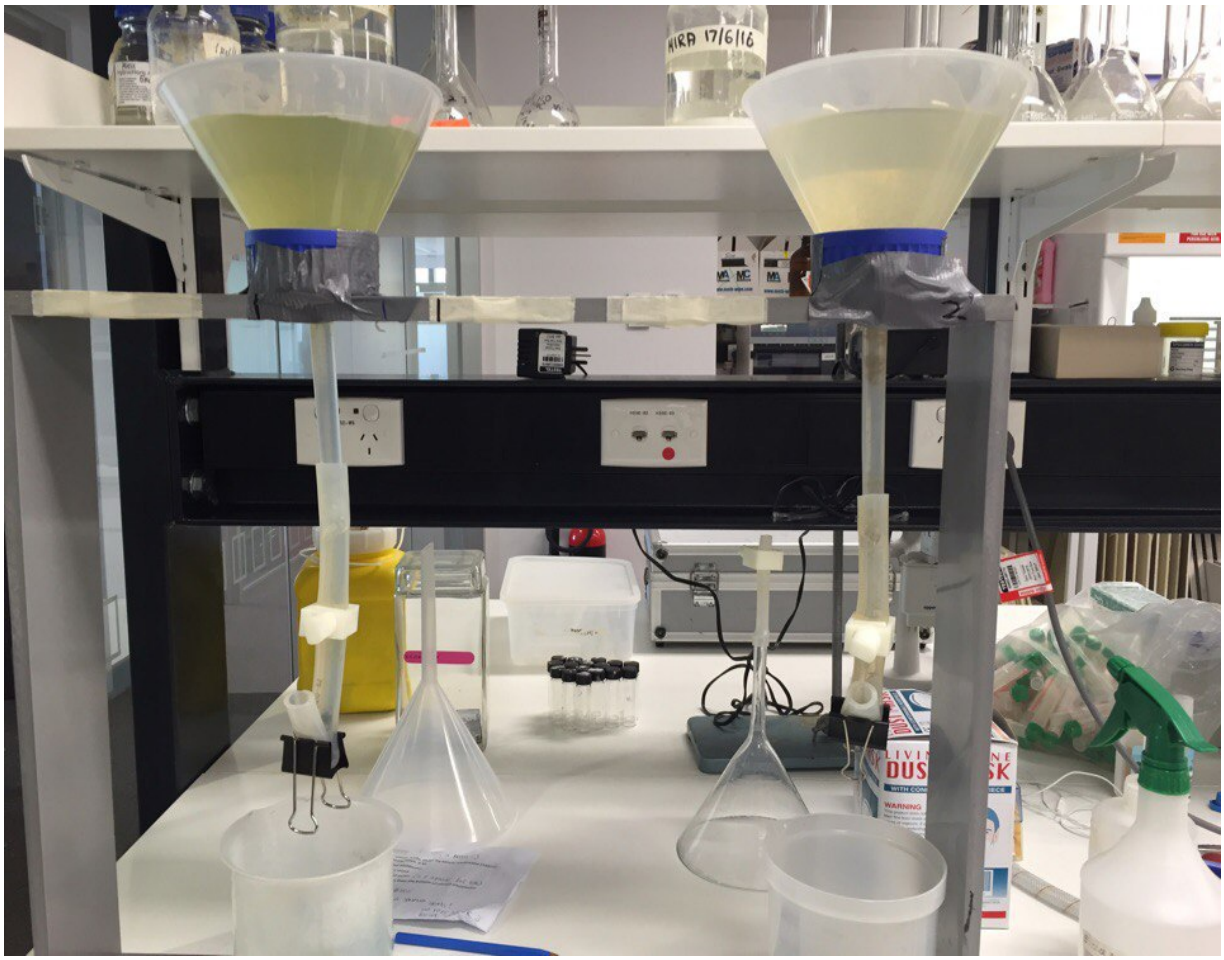

Figure A.1 Wastewater settling apparatus

1.1.3 DNA extraction from individual larvae

DNA extraction from individually isolated *S. ratti* and *S. stercoralis* was performed using different cell lysis techniques. Both the freeze and thaw method or a lysis buffer to break the cell's membrane and extract DNA from isolated worms were tried. There were two lysis buffers prepared and used with slightly different incubation conditions, with only one found to succeed in DNA extraction.

1.1.3.1 Freeze and thaw technique

The freeze and thaw technique was adapted from the Al-Warid, 2014 (Al-Warid, 2014). *Strongyloides* spp. larvae were individually isolated from the faeces and collected into separate tubes containing 20 µL deionised water. The tubes with a worm were then incubated at -80ºC for 15 min followed by thawing at 56ºC for 5 min. This was repeated three times. The tubes were then vortexed for five minutes and stored at -20 ºC for further applications.

1.1.3.2 Lysis buffer

There were two lysis buffer solutions used with slightly different incubation conditions to extract DNA from individual larvae as described below.

Method 1

Single larvae were isolated from faeces and collected into tubes containing 10 µL deionised water and 10 µL lysis buffer. Lysis buffer was prepared using 20 mM Tris-HCL, pH 8, 100 mM EDTA, and 1% SDS solution. The tube with an individual larvae was then incubated at 65 ºC for two hours followed by incubation at 80 ºC for 20 min (Gasser et al., 1993). However, this solution was not able to lyse cell membrane and extract DNA as was shown by qPCR.

Method 2

Single larvae were isolated from faeces and collected into tubes containing 10 µL deionised water and 10 µL lysis buffer. The lysis buffer recipe was modified and a new one was prepared containing 20 mM Tris-HCL, pH 8.3, 100 mM KCL, 5 mM $MgCL_2$, 0.9% NP-40 (Tergitol-type NP-40, nonyl phenoxypolyethoxylethanol), 0.9% Tween 20 (Polysorbate 20), 0.02% Gelatine, and 240 µg/mL Proteinase K. The tube with an individual larvae was then incubated at 65 ºC for two hours followed by incubation at 95 ºC for 15 min (Jaleta et al., 2017). These conditions were able to break a cell membrane and extract DNA from individual larvae as was shown by qPCR. Extracted DNA was stored at -20 ºC for further applications.

1.2 Real-time polymerase chain reaction for the detection of *Strongyloides* spp.

Molecular methods including real-time polymerase chain reaction (qPCR) are being widely used and have shown to be highly sensitive and specific in the detection of *Strongyloides* spp. in faeces.

(Sultana et al., 2012, Verweij et al., 2009). Its advantage of being able to amplify a target DNA region from small amount of a starting material is particularly useful in environmental sources of the DNA (Sultana et al., 2013).

1.2.1 Optimisation of *Strongyloides* spp. real-time PCR assays

There were two qPCR assays for the detection of *Strongyloides* spp. optimised and tested in this study. The first utilised primers and a second primers and a probe. The first assay using the primers did not show consistently repeatable results as it was found to be cross reacting with negative controls, and was therefore not used further in the research.

The probe method had been reported as species specific and had higher sensitivity and was chosen for further optimisation. This included trialling different annealing temperatures, number of cycles and methods to reduce inhibition (BSA and dilution). The optimised probe method was then used for the research described in this thesis (chapters 4, 5 and 6)

1.2.2 Real-time PCR quality control

For quality control, each qPCR run included a positive control (containing *Strongyloides ratti* DNA), a non-template control (containing double autoclaved sterile water), and a negative control (containing *Strongyloides* negative DNA). Fresh rat faeces were collected from rats subcutaneously infected with *S. ratti*. Rat faeces were collected 7-10 days post infection from the Centre for Infectious Diseases and Microbiology, Westmead Hospital, NSW, Australia. Infected rat faeces were used to spike non-infected dog faeces at a 1:9 ratio. DNA from the spiked faeces was extracted using PowerSoil DNA extraction kit as described previously and used as a qPCR positive control. DNA from non-infected dewormed canine faeces was extracted using PowerSoil DNA extraction kit as described previously and used as a qPCR negative control. For the results of a run to be included correct melt curves (assay 1) or Ct values (assay 2) of the positive control had to be positive, the negative and non-template controls had to be negative.

1.2.3 *Strongyloides* spp. real-time PCR assay 1 - with primers

S. stercoralis specific primers listed in Table A.1 were adopted from Verweij et al. (2009) and optimised for the detection of *Strongyloides* spp. (Verweij et al., 2009). The reagents used in a qPCR reaction are listed in Table A.2. Different qPCR cycling conditions were used to optimise assay as shown in Table A.3. For each reaction the melt curve was analysed and a positive *S. stercoralis* was confirmed with a T_m of 84.3 - 84.5 ºC. These primers were not used further throughout the PhD research.

Table A.1 *Strongyloides* spp. primer sequences

Primer	Amplicon	Sequence	Reference

Stro F	rRNA, 96bp	5'-TCCAGAAAAGTCTTCACTCTCCAG-3'	**(Verweij et al., 2009)**
Stro R	rRNA, 96bp	5'-TGCGTTAGAATTTAGATATTATTGTTGCT-3'	

Table A.2 Concentrations of reagents used for the *Strongyloides* spp. qPCR assay and amount of working stock added to each 25 µL reaction in order to achieve this concentration

Reagent	Concentration of working stock	Final concentration	Volume added to 25 µL reaction (µL)
Water			8.05
BSA	-	0.1 µg/µL	1
$MgCl_2$ (Invitrogen)	50 mM	2.5 mM	1.25
PCR Buffer (Invitrogen)	10 X	1 X	2.5
dNTP's (Invitrogen)	10 mM	0.2 mM	0.5
SYTO9 (BioRad)	25 µM	2.5 µM	2.5
Platinum Taq DNA polymerase (Invitrogen)	5 U/µL	1 U	0.2
F Primer	10 µM	0.5 µM	2.5
R primer	10 µM	0.5 µM	2.5
DNA sample	-	-	5

Table A.3 Optimisation of the qPCR cycling conditions for *Strongyloides* spp. primers

No	Initial denaturation		Step 1 Denaturation		Step 2 Annealing-		Step 3 Extension		No of cycles	Melt t	Result
1	temp	time	temp	time	temp	time	temp	time	45	83.7-84ºC	Did not work
	95ºC	5 min	94ºC	10 s	60ºC	20 s	73ºC	20 s			
2	temp	time	temp	time	temp	time	temp	time	45	75-95 ºC	Did not work
	95ºC	5 min	95ºC	15 s	50ºC	30 s	72 ºC	20 s			
3*	temp	time	temp	time	temp	time	temp	time	40	84.3-84.5 ºC	Worked
	95ºC	5 min	94ºC	10 s	60ºC	20 s	73ºC	20 s			

1.2.4 *Strongyloides* spp. real-time PCR assay 2

S. stercoralis species-specific primers and a probe were adopted from Verweij et al (2009) (Table A.4), and PCR conditions were developed and optimised for this study (Verweij et al., 2009). The forward primer is 100% homologous to other *Strongyloides* species targeting a 101 base pair region of 18s rRNA gene (GenBank accession no. **AF279916**) (Verweij et al., 2009). Real-time PCR reagents and conditions used in the assay are shown in Tables A.5 and A.6. To optimise the assay, a qPCR reaction was run with and without bovine serum albumin (BSA), at different cycling conditions, and with different concentrations of a template DNA.

Bovine serum albumin

BSA was previously shown to improve the amplification of the *S. stercoralis* DNA using the same *S. stercoralis* primers (Repetto et al., 2013). BSA was tested on different dilutions of a template DNA. Briefly, 20 µL reaction contained 10 µL Supermix (SSoAdvanced, Universal Probes Supermix, Bio-Rad Laboratories, CA, USA), 1 µL primers and probe mixture (Stro18S-1530F, Stro18S-1630R and Stro18S-1586T FAM) (Sto 18S PrimePCR probe assay Bio-Rad Laboratories, CA, USA), 1 µL BSA (or no BSA), 3 µL deionised H_2O (or 4 µL deionised H_2O) and 5 µL DNA template. The reagents used in qPCR reaction are listed in Table A.5. PCR positive controls containing undiluted DNA extract, 1 in 10, 1 in 100 and 1 in 1000 dilution of the DNA extract into nuclease free water were tested. The results showed that BSA exhibited an inhibition effect on the DNA amplification, and was excluded from the assay. Undiluted and 1 to 10 DNA concertation of a PCR positive control were shown to amplify the best. Subsequently, qPCR reactions were performed without BSA and in undiluted and 1 to 10 DNA concentrations.

For each reaction the Ct values were analysed. A sample was considered positive when the Ct value was lower the mean negative Ct minus 2.6 standard deviations of a mean negative control Ct. Positive samples were amplified in every PCR reaction.

PCR cycling conditions

To optimise an assay different cycling conditions were tested as shown in Table A.6. The reported cycling conditions included an initial hold at 95°C for 15 min, followed by 40 cycles consisting of 95 °C for 15 s and 60 °C for 30 s (Sultana et al., 2013). In our assay annealing temperatures of 50°C and 60°C were also tested and compared at 45 cycles.

The results showed that annealing temperatures of 50°C and 60°C at the 45 cycles caused slight amplification of the NTCs. Decreasing the number of cycles to 40, and maintaining the annealing temperature at 60°C was demonstrated to be the most optimised cycling conditions for the detection

of *Strongyloides* spp. These PCR cycling conditions were used further throughout the PhD research for the detection of *Strongyloides* spp.

Table A.4 *Strongyloides* spp. primer and a probe sequences

Primer/Probe	Amplicon	Sequence	Reference
Stro18S-1530F	rRNA, 101bp	5'-GAATTCCAAGTAAACGTAAGTCATTAGC-3'	**(Verweij et al., 2009)**
Stro18S-1630R	rRNA, 101bp	5'-TGCCTCTGGATATTGCTCAGTTC-3'	
Stro18S-1586T	rRNA, 101bp	FAM-5'-ACACACCGGCCGTCGCTGC03'-BHQ1	

Table A.5 Concentrations of reagents used for the *Strongyloides* spp. qPCR assay and amount of working stock added to each 20 µL reaction in order to achieve this concentration

Reagent	Final concentration	Volume added to 20 µL reaction (µL)
Water	-	4 (3)
Supermix (SSoAdvanced, Universal Probes Supermix, USA	1x	10
Mixture of primers and probes	1x	1
BSA	0.1µg/1µL	0 (1)
DNA	-	5

Table A.6 Optimisation of the qPCR cycling conditions for *Strongyloides* spp. primers and a probe

No	Initial denaturation		Step 1 Denaturation		Step 2 Annealing-Extension		Number of cycles	Result
1	temp	time	temp	time	temp	time	45	Did not work NTCs amplified
	95ºC	15 min	95ºC	15 s	60ºC	30 s		
2	temp	time	temp	time	temp	time	45	Did not amplify NTCs amplified
	95ºC	15 min	95ºC	15 s	50ºC	30 s		
3*	temp	time	temp	time	temp	time	40	Amplified
	95ºC	15 min	95ºC	15 s	60ºC	30 s		

1.2.5 Environmental inhibitors

To determine the presence of environmental inhibitors all samples were run undilutedand 1 in 10 dilution of the DNA extract into nuclease free water. If the cycle threshold (Ct) value for the pure DNA extract was 3.3 higher the Ct value of the 1 to 10 dilution of DNA extract, then the pure DNA was assumed to be inhibited by the environmental inhibitors and the diluted DNA extracts were used (Livak and Schmittgen, 2001). Figure A.2 demonstrates amplification curves of the neat and 1 to 10 DNA extracts of *Strongyloides* positive samples with neat DNA extracts amplifying more efficiently.

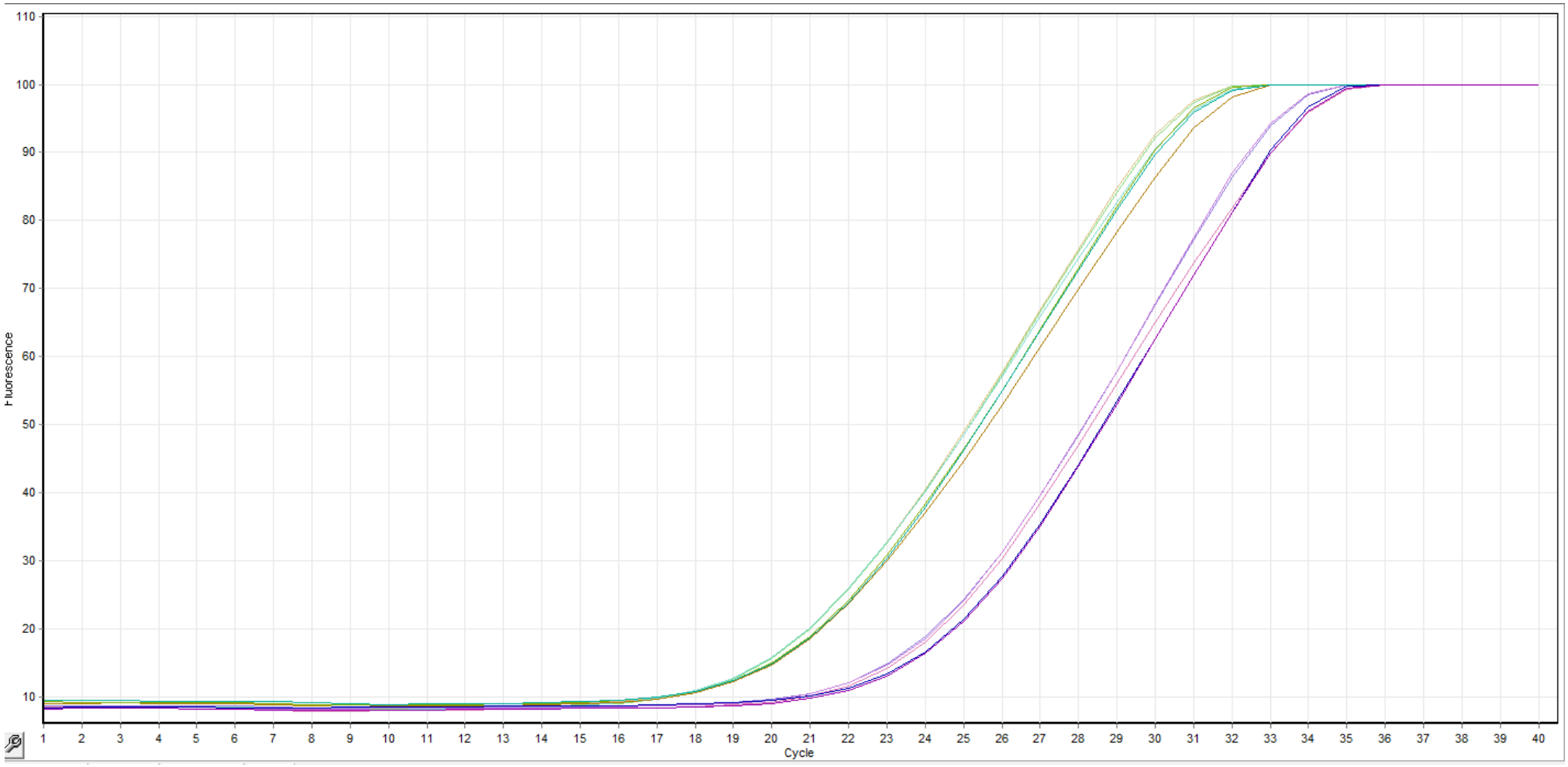

Figure A.2 Cycling curve of undiluted and 1 to 10 diluted DNA extracts

1.3 Microscopy tests for the detection of *Strongyloides*. spp.

1.3.1 Baermann technique

The Baermann method is based on active movement of larvae and performed in order to isolate worms from faeces or separate live worms from the dead ones. The Baermann technique was adopted and performed as described previously (Steinmann et al., 2007). Briefly, approximately 5 – 10 g of faeces were placed in a centre of a double layer disposable paper. A paper pouch containing faecal material was then placed into a funnel fit with tubing and closed with a clamp. The funnel was filled with water to cover the faeces and apparatus was left for about three hours to let larvae to crawl down the bottom of the funnel (Figure A.3). After three hours, a few millilitres of fluid from the stem of the funnel was collected into a test tube for further use.

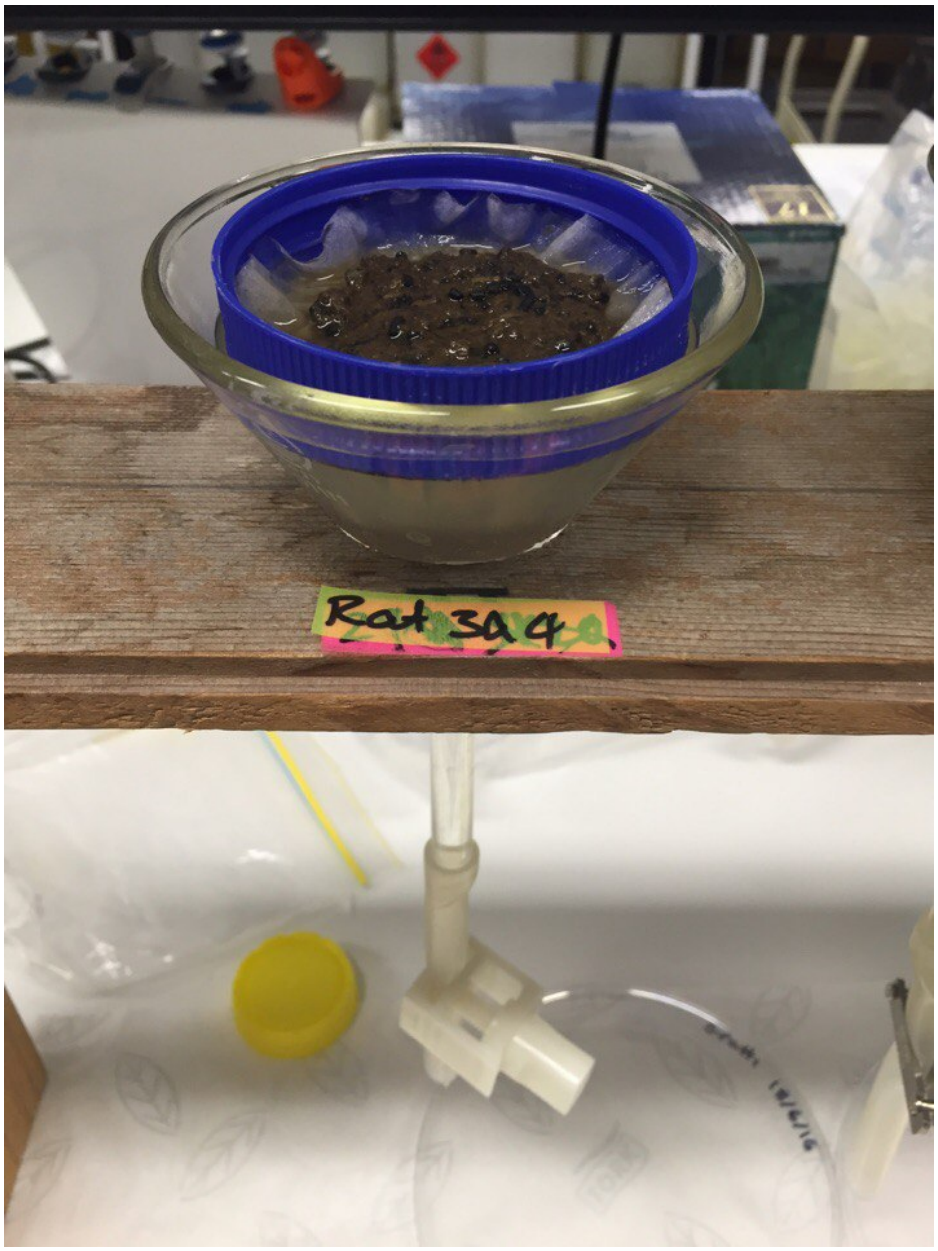

Figure A.3 Baermann technique

1.3.2 Isolation of the *Strongyloides ratti* from rat faeces

Individual *S. ratti* larvae were isolated from the rat faeces followed the Baermann technique (Figure A.4). Isolation of the worms was conducted by the researcher, with appropriate biosafety training, in a biosafety cabinet in a PC2 room. Closed shoes, lab coat, gloves and eye protection were worn at all times. Isolation was performed under the compound microscope using tweezers. This was conducted as a training for isolation of individual *Strongyloides* spp. larvae from collected dog faeces potentially infected with *S. stercoralis* for DNA sequencing purposes. However, the Baermann technique on collected dog faeces was never successful, since isolation of individual live worms was not performed on dog faeces.

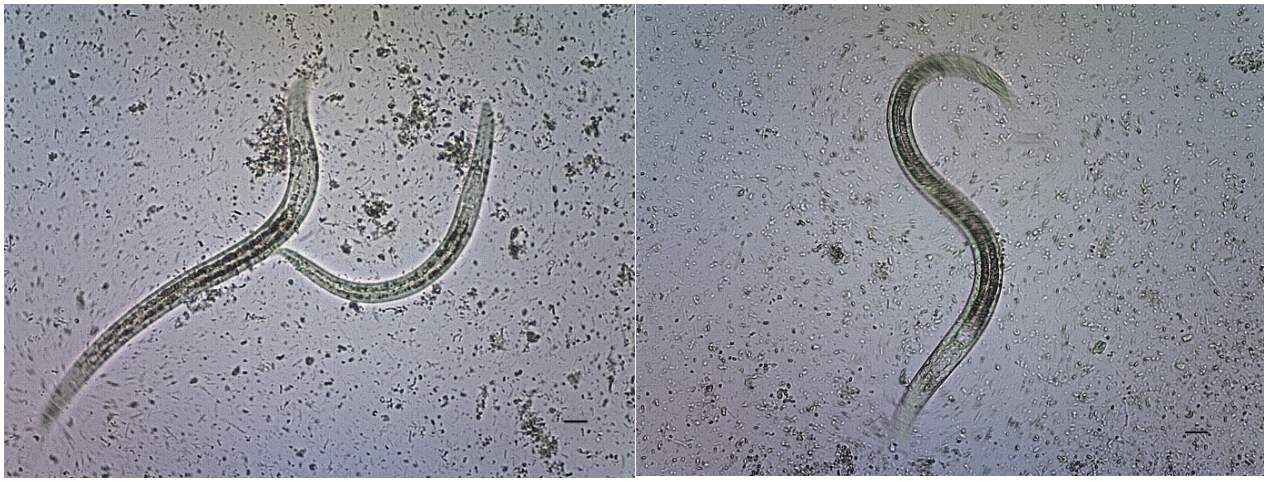

Figure A.4 Isolated *Strongyloides ratti,* rhabditiform larvae (scale bar – 50 µm)

APPENDIX B. STRONGYLOIDIASIS IS A DEADLY WORM INFECTING MANY AUSTRALIANS, YET HARDLY ANYBODY HAS HEARD OF IT

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September 5, 2017 12.08pm AEST

We all know about parasites, like tapeworms that can get into our intestines if we eat infected undercooked meat. There are many types of parasitic worms, including flatworms and roundworms, and they can all make humans sick.

But there's one infection by a parasitic worm that, worldwide, makes more people ill than malaria, and yet hardly anybody has ever heard of it. It's called strongyloidiasis, and estimates suggest up to 60% of Indigenous Australians in some communities carry the infection.

What is it?

Strongyloidiasis is an infection caused by a parasitic worm called *Strongyloides stercoralis* (*S. stercoralis*), which infects the gastrointestinal system (stomach, intestines and other digestive organs). Worldwide, it infects an estimated 370 million people, making it more common than malaria. But so few people are aware of it that it has been described as the most neglected of all neglected diseases.

Strongyloidiasis is generally considered a disease of developing countries, but we also see it in economically disadvantaged areas in the US, Asia and Europe. In Australia, the worm can infect anyone, but is most common in Indigenous Australian communities, refugees, returning overseas travellers and Vietnam veterans.

Estimates suggest the prevalence of infection in Indigenous Australiancommunities is 35-60%. Yet the true incidence in Australia could be much higher as the infection is difficult to detect, is often not tested for and is not a nationally notifiable disease. This means there is no centralised record of cases.

How can you get infected?

You can be infected with the worm by coming into contact with contaminated soil or faeces. The way it gets into your intestine is quite a process. First it enters your body through skin, normally the feet. From there it makes its way into the bloodstream and then the lungs. It is then coughed up and swallowed, which is how it ends up in the small intestine. It can live there for decades.

Infection can be undetected for many years. Patients can show no symptoms or suffer vague ones like weight loss, indigestion, abdominal cramps, diarrhoea, coughing, wheezing or a rash like hives on the buttocks or waist. Diagnosis can be confirmed by detecting the

worm in a stool sample or looking for antibodies (which means the immune system has developed proteins to fight the infection) in a blood sample.

However, the detection methods are not foolproof. Stool sample analysiscan result in false negatives because the worms can move into the faeces at different times. And blood tests can result in false positives due to the detection method reacting to other proteins and mistaking them for *Strongyloides*.
If infection goes undiagnosed and your immune system becomes compromised, or you are given steroids which suppress the immune response, the parasite can enter new phases of infection. These are hyperinfection or dissemination.

Hyperinfection is when the worm reproduces rapidly. The result is an enormous number of worms, which is often deadly.

Dissemination is when the worms spread throughout the body. This is almost always fatal as the gut bacteria carried by the worm are moved into other organs, causing massive infection.

How is it treated?

If diagnosed correctly, the infection can be treated by a drug called ivermectin, which is typically used against parasitic worms and other parasites including scabies. However, if one worm remains, it can reproduce (asexually), causing reinfection. Also, you do not develop immunity to the worm, so you can be immediately reinfected once the drug has left your system.

The biggest issue with drug treatment is that we have already started to see ivermectin-resistant *Strongyloides* in sheep and horses. Another drug family, called benzimidazoles, is sometimes used against human *Strongyloides*, but we've also seen resistance to this in infected animals.
Mass drug administration of ivermectin has been successful in significantly reducing the number of infected people in some communities in Australia. However, its roll-out has been patchy. Also reinfection can occur, suggesting the environmental part of the *Strongyloides* lifecycle plays an important role.

Controlling the environment

Very little is known about how the worm survives outside a host and little has been done to try to control it in the environment. The worm can reproduce once outside a host, but we don't know how long it can survive in the environment, although it is thought to be quite a while.

The worm's survival in the environment explains the prevalence of strongyloidiasis in low socioeconomic areas. Poor infrastructure, living conditions and sanitation result in contaminated soil and water where the worm can live and cause infection.

We must start targeting *Strongyloides* control in the environment and reduce our reliance on drugs. If human *Strongyloides* becomes resistant to ivermectin, the consequences could be devastating. However, the biggest challenge in fighting this disease is that many people have not even heard of the *Strongyloides* worm.

APPENDIX C. FLINDERS UNIVERSITY BIOSAFETY APPROVAL

APPENDIX D. FLINDERS UNIVERSITY SOCIAL AND BEHAVIOURAL RESEARCH ETHICS COMMITTEE APPROVAL

APPENDIX E. SOUTHERN ADELAIDE CLINICAL HUMAN RESEARCH ETHICS COMMITTEE APPROVAL

APPENDIX F. FLINDERS UNIVERSITY ANIMAL WELFARE COMMITTEE REGISTRY

REFERENCE LIST

ADAMS, M., PAGE, W. & SPEARE, R. 2003. Strongyloidiasis: an issue in Aboriginal communities. *Rural and Remote Health,* 3**,** 1-6.

ADLER, N. E., BOYCE, T., CHESNEY, M. A., COHEN, S., FOLKMAN, S., KAHN, R. L. & SYME, S. L. 1994. Socioeconomic status and health: the challenge of the gradient. *American Psychologist,* 49**,** 15.

ADLER, N. E. & OSTROVE, J. M. 1999. Socioeconomic status and health: what we know and what we don't. *Annals of the New York Academy of Sciences,* 896**,** 3-15.

AHMAD, A. F., HADIP, F., NGUI, R., LIM, Y. A. & MAHMUD, R. 2013. Serological and molecular detection of *Strongyloides stercoralis* infection among an Orang Asli community in Malaysia. *Parasitology Research,* 112**,** 2811-2816.

AL-WARID, H. S. 2014. The effect of freezing-thawing on DNA extraction from *Cryptosporidium* oocysts in fecal samples *International Journal of Pharmacy and Biological Sciences,* 4**,** 14-18.

ALAEDDINI, R. 2012. Forensic implications of PCR inhibition—a review. *Forensic Science International: Genetics,* 6**,** 297-305.

ALBONICO, M., ENGELS, D. & SAVIOLI, L. 2004. Monitoring drug efficacy and early detection of drug resistance in human soil-transmitted nematodes: a pressing public health agenda for helminth control. *International Journal for Parasitology,* 34**,** 1205-1210.

ALONSO, J. L., AMORÓS, I. & CAÑIGRAL, I. 2011. Development and evaluation of a real-time PCR assay for quantification of Giardia and *Cryptosporidium* in sewage samples. *Applied Microbiology and Biotechnology,* 89**,** 1203-1211.

ALZAIN, B. F. 2006. Study on the status of prevalence of *Strongyloides stercoralis* infection among children in agricultural areas in Beit Lahia, Gaza strip. *The Islamic University Journal (Series of Natural Studies and Engineering),* 14**,** 67-73.

ASHFORD, R., BARNISH, G. & VINEY, M. 1992. *Strongyloides fuelleborni kellyi*: Infection and disease in Papua New Guinea. *Parasitology Today,* 8**,** 314-318.

ASKARY, T. H. 2015. 19 limitations, research needs and future prospects in the biological control of Phytonematodes. *Biocontrol Agents of Phytonematodes***,** 446.

AUDY, J. R. & DUNN, F. L. 1974. Community health. *In:* SARGENT, F. (ed.) *Human Ecology.* Amsterdam: North Holland Publ. Co.

AUSTRALIA, C. D. N. 2017. Australian National Notifiable Diseases and Case Definitions. *In:* HEALTH, D. O. (ed.). Australia: Australian Government.

AUTHORITY, A. P. A. V. M. 2016. Public Chemical Registration Information System Search. Australia: Australian Government

BABATUNDE, S., SALAMI, A., FABIYI, J., AGBEDE, O. & DESALU, O. 2010. Prevalence of intestinal parasitic infestation in HIV seropositive and seronegative patients in Ilorin, Nigeria. *Annals of African Medicine,* 9.

BASSO, W., GRANDT, L.-M., MAGNENAT, A.-L., GOTTSTEIN, B. & CAMPOS, M. 2018. *Strongyloides stercoralis* infection in imported and local dogs in Switzerland: from clinics to molecular genetics. *Parasitology Research,* 118**,** 255-266.

BEARUP, A. 1931. The intensity and type of hookworm infestation in the Ingham district of North Queensland. *Medical Journal of Australia,* 2.

BEKNAZAROVA, M., BARRATT, J. L., BRADBURY, R. S., LANE, M., WHILEY, H. & ROSS, K. 2019. Detection of classic and cryptic *Strongyloides* genotypes by deep amplicon sequencing: A preliminary survey of dog and human specimens collected from remote Australian communities. *BioRxiv***,** 549535.

BEKNAZAROVA, M., MILLSTEED, S., ROBERTSON, G., WHILEY, H. & ROSS, K. 2017a. Validation of DESS as a DNA preservation method for the detection of *Strongyloides* spp. in canine feces. *International Journal of Environmental Research and Public Health,* 14**,** 624.

BEKNAZAROVA, M., WHILEY, H. & ROSS, K. 2016a. Advocating for both environmental and clinical approaches to control human strongyloidiasis. *Pathogens,* 5**,** 59.

BEKNAZAROVA, M., WHILEY, H. & ROSS, K. 2016b. Strongyloidiasis: A disease of socioeconomic disadvantage. *International Journal of Environmental Research and Public Health,* 13**,** 517.

BEKNAZAROVA, M., WHILEY, H. & ROSS, K. 2017b. Mass drug administration for the prevention human strongyloidiasis should consider concomitant treatment of dogs. *PLoS Neglected Tropical Diseases,* 11**,** e0005735.

BELOW, S., KÄMPFE, L. & MUELLER, A. 1987. Reaction of *Rhabditis oxycerca* after long-term exposure to aldicarb and oxamyl. II: Enzyme changes in nematicide resistance. *Nematologica,* 33**,** 298-309.

BESIER, R. & LOVE, S. 2004. Anthelmintic resistance in sheep nematodes in Australia: the need for new approaches. *Animal Production Science,* 43**,** 1383-1391.

BETHONY, J., BROOKER, S., ALBONICO, M., GEIGER, S. M., LOUKAS, A., DIEMERT, D. & HOTEZ, P. J. 2006. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *The Lancet,* 367**,** 1521-1532.

BEVERIDGE, I. 2002. Australian hookworms (*Ancylostomatoidea*): a review of the species present, their distributions and biogeographical origins. *Parassitologia,* 44**,** 83-88.

BILKERT, J. & RAO, P. 1985. Sorption and leaching of three nonfumigant nematicides in soils. *Journal of Environmental Science & Health Part B,* 20**,** 1-26.

BISOFFI, Z., BUONFRATE, D., MONTRESOR, A., REQUENA-MÉNDEZ, A., MUÑOZ, J., KROLEWIECKI, A. J., GOTUZZO, E., MENA, M. A., CHIODINI, P. L. & ANSELMI, M. 2013. *Strongyloides stercoralis*: a plea for action. *PLoS Neglected Tropical Diseases,* 7**,** e2214.

BOWMAN, D. D., MONTGOMERY, S. P., ZAJAC, A. M., EBERHARD, M. L. & KAZACOS, K. R. 2010. Hookworms of dogs and cats as agents of cutaneous larva migrans. *Trends in Parasitology,* 26**,** 162-167.

BRADBURY, R. & TRAUB, R. J. 2016. Hookworm infection in Oceania. *Neglected Tropical Diseases-Oceania.* Springer.

BRADBURY, R. S., HII, S. F., HARRINGTON, H., SPEARE, R. & TRAUB, R. 2017. *Ancylostoma ceylanicum* hookworm in the Solomon Islands. *Emerging Infectious Diseases,* 23**,** 252.

BROOKER, S., MIGUEL, E. A., MOULIN, S., LOUBA, A. I., BUNDY, D. A. & KREMER, M. 2000. Epidemiology of single and multiple species of helminth infections among school children in Busia District, Kenya. *East African Medical Journal,* 77.

BUONFRATE, D., ANGHEBEN, A., GOBBI, F., MUÑOZ, J., REQUENA-MENDEZ, A., GOTUZZO, E., MENA, M. A. & BISOFFI, Z. 2012. Imported strongyloidiasis: epidemiology, presentations, and treatment. *Current Infectious Disease Reports,* 14**,** 256-262.

BUONFRATE, D., FORMENTI, F., PERANDIN, F. & BISOFFI, Z. 2015a. Novel approaches to the diagnosis of *Strongyloides stercoralis* infection. *Clinical Microbiology and Infection,* 21**,** 543-552.

BUONFRATE, D., PARADIES, P., IARUSSI, F., FORMENTI, F., PERANDIN, F., OTRANTO, D. & BISOFFI, Z. 2017. Serological and molecular tests for the diagnosis of *Strongyloides stercoralis* infection in dogs. *Parasitology Research***,** 1-3.

BUONFRATE, D., SEQUI, M., MEJIA, R., CIMINO, R. O., KROLEWIECKI, A. J., ALBONICO, M., DEGANI, M., TAIS, S., ANGHEBEN, A. & REQUENA-MENDEZ, A. 2015b. Accuracy of five serologic tests for the follow up of *Strongyloides stercoralis* infection. *PLoS Neglected Tropical Diseases,* 9**,** e0003491.

BYARD, R., BOURNE, A., MATTHEWS, N., HENNING, P., ROBERTON, D. & GOLDWATER, P. 1993. Pulmonary strongyloidiasis in a child diagnosed on open lung biopsy. *Surgical Pathology,* 5**,** 55-62.

CABEZAS-FERNÁNDEZ, M. T., SALAS-CORONAS, J., LOZANO-SERRANO, A. B., VAZQUEZ-VILLEGAS, J., CABEZA-BARRERA, M. I. & COBO, F. 2015. Strongyloidiasis in immigrants in Southern Spain. *Enfermedades Infecciosas y Microbiología Clínica,* 33**,** 37-39.

CALVOPINA, M., CABALLERO, H., MORITA, T. & KORENAGA, M. 2016. Human pulmonary infection by the zoonotic *Metastrongylus salmi* Nematode. The first reported case in the Americas. *The American Journal of Tropical Medicine and Hygiene,* 95**,** 871-873.

CAMERON, S., FALLOWFIELD, H. & CROMAR, N. 2004. *Environmental Health in Australia & New Zealand*, Oxford University Press.

CARUANA, S. R., KELLY, H. A., NGEOW, J. Y., RYAN, N. J., BENNETT, C. M., CHEA, L., NUON, S., BAK, N., SKULL, S. A. & BIGGS, B. A. 2006. Undiagnosed and potentially lethal parasite infections among immigrants and refugees in Australia. *Journal of Travel Medicine,* 13**,** 233-239.

CARVALHO, E. & DA FONSECA PORTO, A. 2004. Epidemiological and clinical interaction between HTLV-1 and *Strongyloides stercoralis*. *Parasite Immunology,* 26**,** 487-497.

CASSEL, J. 1976. The contribution of the social environment to host resistance. *American Journal of Epidemiology,* 104**,** 107-123.

CAWOOD, P. A. & KORSCH, R. 2008. Assembling Australia: Proterozoic building of a continent. *Precambrian Research,* 166**,** 1-35.

CHOMEL, B., CHAPPUIS, G., BULLON, F., CARDENAS, E., DE BEUBLAIN, T. D., LOMBARD, M. & GIAMBRUNO, E. 1988. Mass vaccination campaign against rabies: are dogs correctly protected? The Peruvian experience. *Review of Infectious Diseases,* 10**,** S697-S702.

CHU, E., WHITLOCK, W. L. & DIETRICH, R. A. 1990. Pulmonary hyperinfection syndrome with *Strongyloides stercoralis*. *CHEST Journal,* 97**,** 1475-1477.

CLEAVELAND, S., KAARE, M., KNOBEL, D. & LAURENSON, M. K. 2006. Canine vaccination—providing broader benefits for disease control. *Veterinary Microbiology,* 117**,** 43-50.

CLUTTON-BROCK, J. 1995. Origins of the dog: domestication and early history. *In:* SERPELL, J. (ed.) *The domestic dog: Its evolution, behaviour and interactions with people.* Cambridge University Press.

COLES, G., JACKSON, F., POMROY, W., PRICHARD, R., VON SAMSON-HIMMELSTJERNA, G., SILVESTRE, A., TAYLOR, M. & VERCRUYSSE, J. 2006. The detection of anthelmintic resistance in nematodes of veterinary importance. *Veterinary Parasitology,* 136**,** 167-185.

CONSTABLE, S., DIXON, R. & DIXON, R. 2010. For the love of dog: the human–dog bond in rural and remote Australian indigenous communities. *Anthrozoös,* 23**,** 337-349.

CROKER, C., REPORTER, R., REDELINGS, M. & MASCOLA, L. 2010. Strongyloidiasis-related deaths in the United States, 1991–2006. *The American Journal of Tropical Medicine and Hygiene,* 83**,** 422-426.

CULLY, D. F., VASSILATIS, D. K., LIU, K. K., PARESS, P. S., VAN DER PLOEG, L., SCHAEFFER, J. M. & ARENA, J. P. 1994. Cloning of an avermectin-sensitive glutamate-gated chloride channel from *Caenorhabditis elegans*. *Nature,* 371**,** 707-711.

DAVIES, J., MAJUMDAR, S. S., FORBES, R., SMITH, P., CURRIE, B. J. & BAIRD, R. W. 2013. Hookworm in the Northern Territory: down but not out. *Medical Journal of Australia,* 198**,** 278-281.

DE CLERCQ, D., SACKO, M., BEHNKE, J., GILBERT, F., DORNY, P. & VERCRUYSSE, J. 1997. Failure of mebendazole in treatment of human hookworm infections in the southern region of Mali. *American Journal of Tropical Medicine and Hygiene,* 57**,** 25-30.

DE SILVA, N., GUYATT, H. & BUNDY, D. 1997. Anthelmintics. *Drugs,* 53**,** 769-788.

DE SILVA, S., SAYKAO, P., KELLY, H., MACINTYRE, C., RYAN, N., LEYDON, J. & BIGGS, B. 2002. Chronic *Strongyloides stercoralis* infection in Laotian immigrants and refugees 7–20 years after resettlement in Australia. *Epidemiology and Infection,* 128**,** 439-444.

DEL GIUDICE, P., DESALVADOR, F., BERNARD, E., CAUMES, E., . VANDENBOS, F., MARTY, P., LE FICHOUX, Y. & DELLAMONICA, P. 2002. Loeffler's syndrome and cutaneous larva migrans: a rare association. *British Journal of Dermatology,* 147**,** 386-387.

DEVI, U., BORKAKOTY, B. & MAHANTA, J. 2011. Strongyloidiasis in Assam, India: A community-based study. *Tropical Parasitology,* 1**,** 30.

DHAMMIKA, S. 1993. The Edicts of King Asoka: An English Rendering. *The Wheel Publication***,** 1-17.

DOBSON, R. J., LEJAMBRE, L. & GILL, J. H. 1996. Management of anthelmintic resistance: inheritance of resistance and selection with persistent drugs. *International Journal for Parasitology,* 26**,** 993-1000.

DORRIS, M., VINEY, M. E. & BLAXTER, M. L. 2002. Molecular phylogenetic analysis of the genus *Strongyloides* and related nematodes. *International Journal for Parasitology,* 32**,** 1507-1517.

EAGER, T. Strongyloides in Kuranda - an overview since 2006. 6th National Workshop on Strongyloides, Pullman Reef Hotel, Cairns, Australia 14 July 2011.

EINSIEDEL, L. & FERNANDES, L. 2008. *Strongyloides stercoralis:* a cause of morbidity and mortality for indigenous people in Central Australia. *Internal Medicine Journal,* 38**,** 697-703.

EINSIEDEL, L. & SPELMAN, D. 2006. *Strongyloides stercoralis*: risks posed to immigrant patients in an Australian tertiary referral centre. *Internal Medicine Journal,* 36**,** 632-637.

EINSIEDEL, L., SPELMAN, T., GOEMAN, E., CASSAR, O., ARUNDELL, M. & GESSAIN, A. 2014. Clinical associations of Human T-lymphotropic Virus type 1 infection in an indigenous Australian population. *PLoS Neglected Tropical Diseases,* 8**,** e2643.

EINSIEDEL, L. J., FERNANDES, L. A. & WOODMAN, R. J. 2008. Racial disparities in infection-related mortality at Alice Springs Hospital, Central Australia, 2000-2005. *Medical Journal of Australia,* 188**,** 568-571.

ERICSSON, C. D., STEFFEN, R., SIDDIQUI, A. A. & BERK, S. L. 2001. Diagnosis of *Strongyloides stercoralis* infection. *Clinical Infectious Diseases,* 33**,** 1040-1047.

FARDET, L., GÉNÉREAU, T., POIROT, J.-L., GUIDET, B., KETTANEH, A. & CABANE, J. 2007. Severe strongyloidiasis in corticosteroid-treated patients: case series and literature review. *Journal of Infection,* 54**,** 18-27.

FARRAR, J., HOTEZ, P., JUNGHANSS, T., KANG, G., LALLOO, D. & WHITE, N. J. 2013. *Manson's tropical diseases,* London, UK, Elsevier Saunders.

FEARON, D. & WILSON, A. Developing a protocol for the diagnosis and management of *Strongyloides* infections in paediatric patients in Central Australia. 12th National Workshop on Strongyloides, Charles Darwin University, Darwin, Australia, 23 September 2017.

FEINSTEIN, J. S. 1993. The relationship between socioeconomic status and health: a review of the literature. *The Milbank Quarterly*, 279-322.

FEITOSA, G., BANDEIRA, A. C., SAMPAIO, D. P., BADARÓ, R. & BRITES, C. 2001. High prevalence of giardiasis and strongyloidiasis among HIV-infected patients in Bahia, Brazil. *Brazilian Journal of Infectious Diseases,* 5, 339-344.

FENTON, L. 2004. Preventing HIV/AIDS through poverty reduction: the only sustainable solution? *The Lancet,* 364, 1186-1187.

FISHBEIN, D. B., MIRANDA, N. J., MERRILL, P., CAMBA, R. A., MELTZER, M., CARLOS, E. T., BAUTISTA, C. F., SOPUNGCO, P., MANGAHAS, L. C. & HERNANDEZ, L. M. 1991. Rabies control in the Republic of the Philippines: benefits and costs of elimination. *Vaccine,* 9, 581-587.

FISHER, D., MCCARRY, F. & CURRIE, B. 1993. Strongyloidiasis in the Northern Territory. Under-recognised and under-treated? *The Medical Journal of Australia,* 159, 88-90.

FLANNERY, G., WHITE, N., FLANNERY, G. & WHITE, N. 1993. Immunological parameters in northeast Arnhem Land Aborigines: consequences of changing settlement patterns and lifestyles. *Urban ecology and health in the third world. Cambridge University Press, Cambridge, United Kingdom*, 202-220.

FLEMING, F. M., BROOKER, S., GEIGER, S. M., CALDAS, I. R., CORREA-OLIVEIRA, R., HOTEZ, P. J. & BETHONY, J. M. 2006. Synergistic associations between hookworm and other helminth species in a rural community in Brazil. *Tropical Medicine & International Health,* 11, 56-64.

FOROUZANFAR, M. H., AFSHIN, A., ALEXANDER, L. T., ANDERSON, H. R., BHUTTA, Z. A., BIRYUKOV, S., BRAUER, M., BURNETT, R., CERCY, K. & CHARLSON, F. J. 2016. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *The Lancet,* 388, 1659-1724.

FRANTZEN, M., SILK, J., FERGUSON, J., WAYNE, R. & KOHN, M. 1998. Empirical evaluation of preservation methods for faecal DNA. *Molecular Ecology,* 7, 1423-1428.

GARRARD, T. A., ROSS, K. E. & TAYLOR, M. J. 2016. Biocontrol for environmental control of the human parasite, *Strongyloides stercoralis*: A necessary next step. *Biological Control,* 100, 25-28.

GASSER, R., CANTACESSI, C. & LOUKAS, A. 2008. DNA technological progress toward advanced diagnostic tools to support human hookworm control. *Biotechnology Advances,* 26**,** 35-45.

GASSER, R. B., CHILTON, N. B., HOSTE, H. & BEVERIDGE, I. 1993. Rapid sequencing of rDNA from single worms and eggs of parasitic helminths. *Nucleic Acids Research,* 21**,** 2525.

GEARY, T. G., WOO, K., MCCARTHY, J. S., MACKENZIE, C. D., HORTON, J., PRICHARD, R. K., DE SILVA, N. R., OLLIARO, P. L., LAZDINS-HELDS, J. K. & ENGELS, D. A. 2010. Unresolved issues in anthelmintic pharmacology for helminthiases of humans. *International Journal for Parasitology,* 40**,** 1-13.

GEERTS, S. & GRYSEELS, B. 2000. Drug resistance in human helminths: current situation and lessons from livestock. *Clinical microbiology reviews,* 13**,** 207-222.

GENTA, R. M. 1989a. Global prevalence of strongyloidiasis: critical review with epidemiologic insights into the prevention of disseminated disease. *Review of Infectious Diseases,* 11**,** 755-767.

GENTA, R. M. 1989b. Strongyloides stercoralis: loss of ability to disseminate after repeated passage in laboratory beagles. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 83**,** 539-541.

GENTA, R. M. 1992. Dysregulation of strongyloidiasis: a new hypothesis. *Clinical Microbiology Reviews,* 5**,** 345-355.

GENTA, R. M., SCHAD, G. A. & HELLMAN, M. E. 1986. *Strongyloides stercoralis:* parasitological, immunological and pathological observations in immunosuppressed dogs. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 80**,** 34-41.

GEORGI, J. R. & SPRINKLE, C. 1974. A case of human strongyloidosis apparently contracted from asymptomatic colony dogs. *The American Journal of Tropical Medicine and Hygiene,* 23**,** 899-901.

GERI, G., RABBAT, A., MAYAUX, J., ZAFRANI, L., CHALUMEAU-LEMOINE, L., GUIDET, B., AZOULAY, E. & PÈNE, F. 2015. *Strongyloides stercoralis* hyperinfection syndrome: a case series and a review of the literature. *Infection,* 43**,** 691-698.

GIANNAKOU, I. O. & KARPOUZAS, D. G. 2003. Evaluation of chemical and integrated strategies as alternatives to methyl bromide for the control of root-knot nematodes in Greece. *Pest Management Science,* 59**,** 883-892.

GIBBS, E. P. J. 2014. The evolution of One Health: a decade of progress and challenges for the future. *Veterinary Record,* 174**,** 85-91.

GIBBS, H. & GIBBS, K. 1959. The effects of temperature on the development of the free-living stages of *Dochmoides stenocephala* (Railliet, 1884)(Ancylostomidae: Nematoda). *Canadian Journal of Zoology,* 37**,** 247-257.

GIBNEY, K. B., MIHRSHAHI, S., TORRESI, J., MARSHALL, C., LEDER, K. & BIGGS, B.-A. 2009. The profile of health problems in African immigrants attending an infectious disease unit in Melbourne, Australia. *The American Journal of Tropical Medicine and Hygiene,* 80**,** 805-811.

GILL, G., WELCH, E., BAILEY, J., BELL, D. & BEECHING, N. 2004. Chronic *Strongyloides stercoralis* infection in former British Far East prisoners of war. *An International Journal of Medicine,* 97**,** 789-795.

GILLEARD, J. & BEECH, R. 2011. Anthelmintic resistance: markers for resistance, or susceptibility. *Parasitology,* 138**,** 160-174.

GLAZER, I., SALAME, L. & SEGAL, D. 1997. Genetic enhancement of nematicide resistance in entomopathogenic nematodes. *Biocontrol Science and Technology,* 7**,** 499-512.

GONCALVES, A., MACHADO, G., GONCALVES-PIRES, M., FERREIRA-JUNIOR, A., SILVA, D. & COSTA-CRUZ, J. 2007. Evaluation of strongyloidiasis in kennel dogs and keepers by parasitological and serological assays. *Veterinary Parasitology,* 147**,** 132-139.

GORDON, C. A., KURSCHEID, J., JONES, M. K., GRAY, D. J. & MCMANUS, D. P. 2017. Soil-transmitted helminths in Tropical Australia and Asia. *Tropical Medicine and Infectious Disease,* 2**,** 56.

GRAY, M. A., PRATTE, Z. A. & KELLOGG, C. A. 2013. Comparison of DNA preservation methods for environmental bacterial community samples. *FEMS Microbiology Ecology,* 83**,** 468-477.

GREINER, K., BETTENCOURT, J. & SEMOLIC, C. 2008. Strongyloidiasis: a review and update by case example. *Clinical Laboratory Science,* 21**,** 82-89.

GROVE, D. & NORTHERN, C. 1982. Infection and immunity in dogs infected with a human strain of Strongyloides stercoralis. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 76**,** 833-838.

GROVE, D. I. 1982. Strongyloidiasis: is it transmitted from husband to wife? *British Journal of Venereal Diseases,* 58**,** 271.

GROVE, D. I. 1989. *Strongyloidiasis: a major roundworm infection of man,* London, UK, Taylor and Francis Ltd.

GROVE, D. I. 1990. *A history of human helminthology*, CAB International Wallingford, Oxford, UK.

GROVE, D. I. 1995. Human strongyloidiasis. *Advances in Parasitology,* 38**,** 251-309.

GROVE, D. I. 1996. Human strongyloidiasis. *Advances in Parasitology.* Elsevier.

GUO, J., LI, Y., GRAY, D., NING, A., HU, G., CHEN, H., DAVIS, G. M., SLEIGH, A. C., FENG, Z. & MCMANUS, D. P. 2006. A drug-based intervention study on the importance of buffaloes for human *Schistosoma japonicum* infection around Poyang Lake, People's Republic of China. *The American Journal of Tropical Medicine and Hygiene,* 74**,** 335-341.

GUPTA, R. C. 2011. *Toxicology of organophosphate & carbamate compounds,* USA, Academic Press.

GYORKOS, T. W., GENTA, R. M., VIENS, P. & MACLEAN, J. D. 1990. Seroepidemiology of *Strongyloides* infection in the Southeast Asian refugee population in Canada. *American Journal of Epidemiology,* 132**,** 257-264.

HASEGAWA, H., HAYASHIDA, S., IKEDA, Y. & SATO, H. 2009. Hyper-variable regions in 18S rDNA of *Strongyloides* spp. as markers for species-specific diagnosis. *Parasitology Research,* 104**,** 869-874.

HASEGAWA, H., KALOUSOVA, B., MCLENNAN, M. R., MODRY, D., PROFOUSOVA-PSENKOVA, I., SHUTT-PHILLIPS, K. A., TODD, A., HUFFMAN, M. A. & PETRZELKOVA, K. J. 2016. *Strongyloides infections* of humans and great apes in Dzanga-Sangha Protected Areas, Central African Republic and in degraded forest fragments in Bulindi, Uganda. *Parasitology International,* 65**,** 367-370.

HASEGAWA, H., SATO, H., FUJITA, S., NGUEMA, P. P. M., NOBUSUE, K., MIYAGI, K., KOORIYAMA, T., TAKENOSHITA, Y., NODA, S. & SATO, A. 2010. Molecular identification of the causative agent of human strongyloidiasis acquired in Tanzania: dispersal and diversity of *Strongyloides* spp. and their hosts. *Parasitology International,* 59**,** 407-413.

HAUBER, H., GALLE, J., CHIODINI, P., RUPP, J., BIRKE, R., VOLLMER, E., ZABEL, P. & LANGE, C. 2005. Fatal outcome of a hyperinfection syndrome despite successful eradication of *Strongyloides* with subcutaneous ivermectin. *Infection,* 33**,** 383-386.

HENRIQUEZ-CAMACHO, C., GOTUZZO, E., ECHEVARRIA, J., WHITE JR, A. C., TERASHIMA, A., SAMALVIDES, F., PÉREZ-MOLINA, J. A. & PLANA, M. N. 2016. Ivermectin versus albendazole or thiabendazole for *Strongyloides stercoralis* infection. *Cochrane Database of Systematic Reviews,* 1.

HEYDON, G. & GREEN, A. 1931. Some Worm Infestations of Man. in Australia. *Medical Journal of Australia,* 1.

HII, S. F., SENEVIRATHNA, D., LLEWELLYN, S., INPANKAEW, T., ODERMATT, P., KHIEU, V., MUTH, S., MCCARTHY, J. & TRAUB, R. J. 2018. Development and evaluation of a multiplex quantitative real-time Polymerase Chain Reaction for hookworm species in human stool. *The American Journal of Tropical Medicine and Hygiene,* 99**,** 1186-1193.

HIRATA, T., NAKAMURA, H., KINJO, N., HOKAMA, A., KINJO, F., YAMANE, N. & FUJITA, J. 2007. Prevalence of *Blastocystis hominis* and *Strongyloides stercoralis* infection in Okinawa, Japan. *Parasitology Research,* 101**,** 1717-1719.

HOCHBERG, N. S., MORO, R. N., SHETH, A. N., MONTGOMERY, S. P., STEURER, F., MCAULIFFE, I. T., WANG, Y. F., ARMSTRONG, W., RIVERA, H. N. & LENNOX, J. L. 2011. High prevalence of persistent parasitic infections in foreign-born, HIV-infected persons in the United States. *PLoS Neglected Tropical Diseases,* 5**,** e1034.

HOLDEN-DYE, L. & WALKER, R. J. 2005. Anthelmintic drugs and nematicides: studies in *Caenorhabditis elegans*.

HOLT, D. C., MCCARTHY, J. S. & CARAPETIS, J. R. 2010. Parasitic diseases of remote Indigenous communities in Australia. *International Journal for Parasitology,* 40**,** 1119-1126.

HOLT, D. C., SHIELD, J., HARRIS, T. M., MOUNSEY, K. E., ALAND, K., MCCARTHY, J. S., CURRIE, B. J. & KEARNS, T. M. 2017. Soil-transmitted helminths in children in a remote Aboriginal community in the Northern Territory: hookworm is rare but *Strongyloides stercoralis* and *Trichuris trichiura* persist. *Tropical Medicine and Infectious Disease,* 2**,** 51.

HOPKINS, R. M., HOBBS, R. P., THOMPSON, R. A., GRACEY, M. S., SPARGO, R. M. & YATES, M. 1997. The prevalence of hookworm infection, iron deficiency and anaemia in an Aboriginal community in north-west Australia. *Medical Journal of Australia,* 166**,** 241-244.

HORTON, J. 2000. Albendazole: a review of anthelmintic efficacy and safety in humans. *Parasitology,* 121**,** S113-S132.

HOTEZ, P. 2009. Mass drug administration and integrated control for the world's high-prevalence neglected tropical diseases. *Clinical Pharmacology & Therapeutics,* 85**,** 659-664.

HOTEZ, P. & WHITHAM, M. 2014. Helminth infections: a new global women's health agenda. *Obstetrics & Gynecology,* 123**,** 155-160.

HOTEZ, P. J., ALVARADO, M., BASÁÑEZ, M.-G., BOLLIGER, I., BOURNE, R., BOUSSINESQ, M., BROOKER, S. J., BROWN, A. S., BUCKLE, G. & BUDKE, C. M. 2014. The global burden of disease study 2010: interpretation and implications for the neglected tropical diseases. *PLoS Neglected Tropical Diseases,* 8**,** e2865.

HOY, W. E. 2009. Closing the gap" by 2030: aspiration versus reality in Indigenous health. *Medical Journal of Australia,* 190**,** 542-4.

HUNT, V. L., TSAI, I. J., COGHLAN, A., REID, A. J., HOLROYD, N., FOTH, B. J., TRACEY, A., COTTON, J. A., STANLEY, E. J. & BEASLEY, H. 2016. The genomic basis of parasitism in the *Strongyloides* clade of nematodes. *Nature Genetics*.

HUSAIN, K., ANSARI, R. A. & FERDER, L. 2010. Pharmacological agents in the prophylaxis/treatment of organophosphorous pesticide intoxication. *Indian Journal of Experimental Biology,* 48**,** 642.

HUTCHINSON, P. Strongyloidiasis an investigation into prevalence. 2nd National Workshop on Strongyloides, Royal Brisbane Hospital, Herston, Australia, 25-26 June, 2003.

IGRA-SIEGMAN, Y., KAPILA, R., SEN, P., KAMINSKI, Z. C. & LOURIA, D. B. 1981. Syndrome of hyperinfection with *Strongyloides stercoralis*. *Review of Infectious Diseases,* 3**,** 397-407.

IGUAL-ADELL, R., OLTRA-ALCARAZ, C., SOLER-COMPANY, E., SÁNCHEZ-SÁNCHEZ, P., MATOGO-OYANA, J. & RODRÍGUEZ-CALABUIG, D. 2004. Efficacy and safety of ivermectin and thiabendazole in the treatment of strongyloidiasis. *Expert opinion on pharmacotherapy,* 5**,** 2615-2619.

INPANKAEW, T., SCHÄR, F., DALSGAARD, A., KHIEU, V., CHIMNOI, W., CHHOUN, C., SOK, D., MARTI, H., MUTH, S. & ODERMATT, P. 2014. High prevalence of *Ancylostoma*

ceylanicum hookworm infections in humans, Cambodia, 2012. *Emerging Infectious Diseases,* 20**,** 976.

JALETA, T. G., ZHOU, S., BEMM, F. M., SCHÄR, F., KHIEU, V., MUTH, S., ODERMATT, P., LOK, J. B. & STREIT, A. 2017. Different but overlapping populations of *Strongyloides stercoralis* in dogs and humans—Dogs as a possible source for zoonotic strongyloidiasis. *PLoS Neglected Tropical Diseases,* 11**,** e0005752.

JAMES, C. E. & DAVEY, M. W. 2009. Increased expression of ABC transport proteins is associated with ivermectin resistance in the model nematode *Caenorhabditis elegans*. *International Journal for Parasitology,* 39**,** 213-220.

JEX, A. R., LIM, Y. A., BETHONY, J. M., HOTEZ, P. J., YOUNG, N. D. & GASSER, R. B. 2011. Soil-transmitted helminths of humans in Southeast Asia—towards integrated control. *Advances in Parasitology.* Elsevier.

JOHNSTON, F. H., MORRIS, P. S., SPEARE, R., MCCARTHY, J., CURRIE, B., EWALD, D., PAGE, W. & DEMPSEY, K. 2005. Strongyloidiasis: a review of the evidence for Australian practitioners. *Australian Journal of Rural Health,* 13**,** 247-254.

JOHNSTON, T. H. A census of the endoparasites recorded as occurring in Queensland, arranged under their hosts. 1916. Queensland, Australia: Royal Society of Queensland.

JONES, H. I. 1980. Intestinal parasite infections in Western Australian Aborigines. *The Medical Journal of Australia,* 2**,** 375-380.

JONGSUKSUNTIGUL, P., INTAPAN, P., WONGSAROJ, T., NILPAN, S., SINGTHONG, S., VEERAKUL, S. & MALEEWONG, W. 2003. Prevalence of *Strongyloides stercoralis* infection in northeastern Thailand (agar plate culture detection). *Journal of the Medical Association of Thailand,* 86**,** 737-741.

JOSEPH, L., GYORKOS, T. W. & COUPAL, L. 1995. Bayesian estimation of disease prevalence and the parameters of diagnostic tests in the absence of a gold standard. *American Journal of Epidemiology,* 141**,** 263-272.

JÚNIOR, A. F., GONCALVES-PIRES, M., SILVA, D., GONCALVES, A. & COSTA-CRUZ, J. 2006. Parasitological and serological diagnosis of *Strongyloides stercoralis* in domesticated dogs from southeastern Brazil. *Veterinary Parasitology,* 136**,** 137-145.

KAPLAN, R. M. 2004. Drug resistance in nematodes of veterinary importance: a status report. *Trends in Parasitology,* 20**,** 477-481.

KAPLAN, R. M., KLEI, T. R., LYONS, E. T., LESTER, G., COURTNEY, C. H., FRENCH, D. D., TOLLIVER, S. C., VIDYASHANKAR, A. N. & ZHAO, Y. 2004. Prevalence of anthelmintic resistant cyathostomes on horse farms. *Journal of the American Veterinary Medical Association,* 225**,** 903-910.

KAPLAN, R. M. & VIDYASHANKAR, A. N. 2012. An inconvenient truth: global worming and anthelmintic resistance. *Veterinary Parasitology,* 186**,** 70-78.

KEARN, J., LUDLOW, E., DILLON, J., O'CONNOR, V. & HOLDEN-DYE, L. 2014. Fluensulfone is a nematicide with a mode of action distinct from anticholinesterases and macrocyclic lactones. *Pesticide Biochemistry and Physiology,* 109**,** 44-57.

KEARNS, T. M., CURRIE, B. J., CHENG, A. C., MCCARTHY, J., CARAPETIS, J. R., HOLT, D. C., PAGE, W., SHIELD, J., GUNDJIRRYIRR, R. & MULHOLLAND, E. 2017. *Strongyloides* seroprevalence before and after an ivermectin mass drug administration in a remote Australian Aboriginal community. *PLoS Neglected Tropical Diseases,* 11**,** e0005607.

KEARNS, T. M., SPEARE, R., CHENG, A. C., MCCARTHY, J., CARAPETIS, J. R., HOLT, D. C., CURRIE, B. J., PAGE, W., SHIELD, J., GUNDJIRRYIRR, R., BUNDHALA, L., MULHOLLAND, E., CHATFIELD, M. & ANDREWS, R. M. 2015. Impact of an ivermectin Mass Drug Administration on scabies prevalence in a remote Australian Aboriginal community. *PLoS Neglected Tropical Diseases,* 9**,** 1-13.

KEISER, P. B. & NUTMAN, T. B. 2004. *Strongyloides stercoralis* in the immunocompromised population. *Clinical Microbiology Reviews,* 17**,** 208-217.

KHIEU, V., SCHÄR, F., FORRER, A., HATTENDORF, J., MARTI, H., DUONG, S., VOUNATSOU, P., MUTH, S. & ODERMATT, P. 2014. High prevalence and spatial distribution of *Strongyloides stercoralis* in rural Cambodia. *PLoS Neglected Tropical Diseases,* 8**,** e2854.

KIKUCHI, T., HINO, A., TANAKA, T., HTWE, M. P. P. T. H., AFRIN, T., NAGAYASU, E., TANAKA, R., HIGASHIARAKAWA, M., WIN, K. K. & HIRATA, T. 2016. Genome-wide analyses of individual *Strongyloides stercoralis* (Nematoda: Rhabditoidea) provide insights into population structure and reproductive life cycles. *PLOS Neglected Tropical Diseases,* 10**,** e0005253.

KILPATRICK, C. W. 2002. Noncryogenic preservation of mammalian tissues for DNA extraction: an assessment of storage methods. *Biochemical Genetics,* 40**,** 53-62.

KNOPP, S., MOHAMMED, K. A., KHAMIS, I. S., MGENI, A. F., STOTHARD, J. R., ROLLINSON, D., MARTI, H. & UTZINGER, J. 2008. Spatial distribution of soil-transmitted helminths, including *Strongyloides stercoralis,* among children in Zanzibar. *Geospatial Health,* 3**,** 47-56.

KOEHLER, A. V., BRADBURY, R. S., STEVENS, M. A., HAYDON, S. R., JEX, A. R. & GASSER, R. B. 2013. Genetic characterization of selected parasites from people with histories of gastrointestinal disorders using a mutation scanning-coupled approach. *Electrophoresis,* 34**,** 1720-1728.

KROLEWIECKI, A. J., RAMANATHAN, R., FINK, V., MCAULIFFE, I., CAJAL, S. P., WON, K., JUAREZ, M., DI PAOLO, A., TAPIA, L. & ACOSTA, N. 2010. Improved diagnosis of *Strongyloides stercoralis* using recombinant antigen-based serologies in a community-wide study in northern Argentina. *Clinical and Vaccine Immunology,* 17**,** 1624-1630.

LERNER, H. & BERG, C. 2015. The concept of health in One Health and some practical implications for research and education: what is One Health? *Infection Ecology & Epidemiology,* 5**,** 25300.

LICHTENBERGER, P., ROSA-CUNHA, I., MORRIS, M., NISHIDA, S., AKPINAR, E., GAITAN, J., TZAKIS, A. & DOBLECKI-LEWIS, S. 2009. Hyperinfection strongyloidiasis in a liver transplant recipient treated with parenteral ivermectin. *Transplant Infectious Disease,* 11**,** 137-142.

LIM, S., KATZ, K., KRAJDEN, S., FUKSA, M., KEYSTONE, J. S. & KAIN, K. C. 2004. Complicated and fatal *Strongyloides* infection in Canadians: risk factors, diagnosis and management. *Canadian Medical Association Journal,* 171**,** 479-484.

LITTLE, M., HALSEY, N. A., CLINE, B. L. & KATZ, S. P. 1983. *Ancylostoma* larva in a muscle fiber of man following cutaneous larva migrans. *The American Journal of Tropical Medicine and Hygiene,* 32**,** 1285-1288.

LIVAK, K. J. & SCHMITTGEN, T. D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. *Methods,* 25**,** 402-408.

LOK, J. B. 2006. Strongyloides stercoralis: a model for translational research on parasitic nematode biology. *WormBook: the online review of C. elegans biology***,** 1-18.

LORD, R. *Strongyloides* serology from Woorabinda. Third National Workshop on *Strongyloides*, Rydges Capricorn International Resort, Yeppoon, Australia, 10-11 June 2005.

MAGNAVAL, J.-F., MANSUY, J.-M., VILLENEUVE, L. & CASSAING, S. 2000. A retrospective study of autochthonous strongyloidiasis in Region Midi-Pyrenees (Southwestern France). *European Journal of Epidemiology,* 16**,** 179-182.

MANSFIELD, L. S., ALAVI, A., WORTMAN, J. A. & SCHAD, G. A. 1995. Gamma camera scintigraphy for direct visualization of larval migration in *Strongyloides stercoralis*-infected dogs. *The American Journal of Tropical Medicine and Hygiene,* 52**,** 236-240.

MARCOS, L. A., TERASHIMA, A., DUPONT, H. L. & GOTUZZO, E. 2008. Strongyloides hyperinfection syndrome: an emerging global infectious disease. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 102**,** 314-318.

MAROTO, R., JIMÉNEZ, A., ROMERO, J., ALVAREZ, V., DE OLIVEIRA, J. & HERNÁNDEZ, J. 2011. First report of anthelmintic resistance in gastrointestinal nematodes of sheep from Costa Rica. *Veterinary Medicine International,* 2011.

MARTI, H., HAJI, H. J., SAVIOLI, L., CHWAYA, H. M., MGENI, A. F., AMEIR, J. S. & HATZ, C. 1996. A comparative trial of a single-dose ivermectin versus three days of albendazole for treatment of *Strongyloides stercoralis* and other soil-transmitted helminth infections in children. *American Journal of Tropical Medicine and Hygiene,* 55**,** 477-484.

MASCARELLO, M., GOBBI, F., ANGHEBEN, A., GOBBO, M., GAIERA, G., PEGORARO, M., LANZAFAME, M., BUONFRATE, D., CONCIA, E. & BISOFFI, Z. 2011. Prevalence of

Strongyloides stercoralis infection among HIV-positive immigrants attending two Italian hospitals, from 2000 to 2009. *Annals of Tropical Medicine & Parasitology,* 105**,** 617-623.

MCCARTHY, J. & MOORE, T. A. 2000. Emerging helminth zoonoses. *International Journal for Parasitology,* 30**,** 1351-1359.

MILLER, A., YOUNG, E., TYE, V., CODY, R., MUSCAT, M., SAUNDERS, V., SMITH, M., JUDD, J. & SPEARE, R. 2018. A community-directed integrated *Strongyloides* control program in Queensland, Australia. *Tropical Medicine and Infectious Disease,* 3**,** 48.

MILLER, M., DEEBLE, M., ROCHE, P. & SPENCER, J. 2004. Evaluation of Australia's National Notifiable Disease Surveillance System. *Communicable Diseases Intelligence Quarterly Report,* 28**,** 311.

MOHAMED, E. & AL-FARWACHI, M. 2008. Evaluation the effect of albendazole against nematodes in sheep in Mosul, Iraq. *Iraqi Journal of Veterinary Sciences,* 22**,** 5-7.

MOLENTO, M. B. 2009. Parasite control in the age of drug resistance and changing agricultural practices. *Veterinary Parasitology,* 163**,** 229-234.

MONTES, M., SAWHNEY, C. & BARROS, N. 2010. *Strongyloides stercoralis*: there but not seen. *Current Opinion in Infectious Diseases,* 23**,** 500.

MOUNSEY, K., KEARNS, T., RAMPTON, M., LLEWELLYN, S., KING, M., HOLT, D., CURRIE, B. J., ANDREWS, R., NUTMAN, T. & MCCARTHY, J. 2014. Use of dried blood spots to define antibody response to the *Strongyloides stercoralis* recombinant antigen NIE. *Acta Tropica,* 138**,** 78-82.

MUENNIG, P., PALLIN, D., SELL, R. L. & CHAN, M.-S. 1999. The cost effectiveness of strategies for the treatment of intestinal parasites in immigrants. *New England Journal of Medicine,* 340**,** 773-779.

MUKERJEE, C. M., CARRICK, J., WALKER, J. C. & WOODS, R. L. 2003. Pulmonary strongyloidiasis presenting as chronic bronchitis leading to interlobular septal fibrosis and cured by treatment. *Respirology,* 8**,** 536-540.

NAGAYASU, E., HTWE, M. P. P. T. H., HORTIWAKUL, T., HINO, A., TANAKA, T., HIGASHIARAKAWA, M., OLIA, A., TANIGUCHI, T., WIN, S. M. T. & OHASHI, I. 2017. A possible origin population of pathogenic intestinal nematodes, *Strongyloides stercoralis*, unveiled by molecular phylogeny. *Scientific Reports,* 7**,** 4844.

NAMISATO, S., MOTOMURA, K., HARANAGA, S., HIRATA, T., TOYAMA, M., SHINZATO, T., HIGA, F. & SAITO, A. 2004. Pulmonary strongyloidiasis in a patient receiving prednisolone therapy. *Internal Medicine,* 43**,** 731-736.

NAVES, M. M. & COSTA-CRUZ, J. M. 2013. High prevalence of *Strongyloides stercoralis* infection among the elderly in Brazil. *Revista do Instituto de Medicina Tropical de São Paulo,* 55**,** 309-313.

NICOLL, W. 1917. The conditions of life in tropical Australia. *Epidemiology & Infection,* 16**,** 269-290.

NONTASUT, P., MUENNOO, C., SA-NGUANKIAT, S., FONGSRI, S. & VICHIT, A. 2005. Prevalence of *Strongyloides* in Northern Thailand and treatment with ivermectin vs albendazole. *Southeast Asian Journal of Tropical Medicine and Public Health,* 36**,** 442.

NSUBUGA, A. M., ROBBINS, M. M., ROEDER, A. D., MORIN, P. A., BOESCH, C. & VIGILANT, L. 2004. Factors affecting the amount of genomic DNA extracted from ape faeces and the identification of an improved sample storage method. *Molecular Ecology,* 13**,** 2089-2094.

OKA, Y., SHUKER, S. & TKACHI, N. 2009. Nematicidal efficacy of MCW-2, a new nematicide of the fluoroalkenyl group, against the root-knot nematode Meloidogyne javanica. *Pest Management Science,* 65**,** 1082-1089.

OLSEN, A., VAN LIESHOUT, L., MARTI, H., POLDERMAN, T., POLMAN, K., STEINMANN, P., STOTHARD, R., THYBO, S., VERWEIJ, J. J. & MAGNUSSEN, P. 2009. Strongyloidiasis–the most neglected of the neglected tropical diseases? *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 103**,** 967-972.

OPIE, K. M., HEENAN, P. J., DELANEY, T. A. & ROHR, J. B. 2003. Two cases of eosinophilic pustular folliculitis associated with parasitic infestations. *Australasian Journal of Dermatology,* 44**,** 217-219.

OPPERMAN, C. & CHANG, S. 1991. Effects of aldicarb and fenamiphos on acetycholinesterase and motility of *Caenorhabditis elegans*. *Journal of Nematology,* 23**,** 20.

OTIENO, P. O., LALAH, J. O., VIRANI, M., JONDIKO, I. O. & SCHRAMM, K.-W. 2010. Carbofuran and its toxic metabolites provide forensic evidence for Furadan exposure in vultures (Gyps africanus) in Kenya. *Bulletin of Environmental Contamination and Toxicology,* 84**,** 536-544.

OTTESEN, E. A. & CAMPBELL, W. 1994. Ivermectin in human medicine. *Journal of Antimicrobial Chemotherapy,* 34**,** 195-203.

PAGE, W., SHIELD, J., O'DONAHOO, F., MILLER, A., JUDD, J. & SPEARE, R. 2016. Strongyloidiasis in Oceania. *Neglected Tropical Diseases-Oceania.* Springer.

PAGE, W. & SPEARE, R. 2016. Chronic strongyloidiasis-Don't look and you won't find. *Australian Family Physician,* 45**,** 40.

PAGE, W. A., DEMPSEY, K. & MCCARTHY, J. S. 2006. Utility of serological follow-up of chronic strongyloidiasis after anthelminthic chemotherapy. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 100**,** 1056-1062.

PAKDEE, W., THAENKHAM, U., DEKUMYOY, P., SA-NGUANKIAT, S., MAIPANICH, W. & PUBAMPEN, S. 2012. Genetic differentiation of *Strongyloides stercoralis* from two different climate zones revealed by 18S ribosomal DNA sequence comparison. *Southeast Asian Journal of Tropical Medicine and Public Health,* 43**,** 1333.

PALMER, C. S., THOMPSON, R. A., TRAUB, R. J., REES, R. & ROBERTSON, I. D. 2008. National study of the gastrointestinal parasites of dogs and cats in Australia. *Veterinary Parasitology,* 151**,** 181-190.

PALMER, C. S., TRAUB, R. J., ROBERTSON, I. D., HOBBS, R. P., ELLIOT, A., WHILE, L., REES, R. & THOMPSON, R. A. 2007. The veterinary and public health significance of hookworm in dogs and cats in Australia and the status of *A. ceylanicum*. *Veterinary Parasitology,* 145**,** 304-313.

PAMPIGLIONE, S. & RICCIARDI, M. 1971. The presence of *Strongyloides fiilleborni* von Linstow, 1905, in Man in Central and East Africa. *Parassitologia,* 13.

PASQUALI, V. 2015. Countries by Income Group. *Global Finance.*

PAULA, F. & COSTA-CRUZ, J. 2011. Epidemiological aspects of strongyloidiasis in Brazil. *Parasitology-Cambridge,* 138**,** 1331.

PAULOS, D., ADDIS, M., FROMSA, A. & MEKIBIB, B. 2012. Prevalence of gastrointestinal helminthes among dogs and owners perception about zoonotic dog parasites in Hawassa Town, Ethiopia. *Journal of Public Health and Epidemiology,* 4**,** 205-209.

PEETERS, E., MEEUS, M., RAMET, J., VAN GOMPEL, A. & WOJCIECHOWSKI, M. 2010. 163 Health Status of Children Adopted From Kazakhstan in Belgium. *Pediatric Research,* 68**,** 85-86.

PIDWIRNY, M. 2011. Köppen climate classification system. *Retrieved January,* 6**,** 2015.

POSEY, D. L., BLACKBURN, B. G., WEINBERG, M., FLAGG, E. W., ORTEGA, L., WILSON, M., SECOR, W. E., SANDERS-LEWIS, K., WON, K. & MAGUIRE, J. H. 2007. High prevalence and presumptive treatment of schistosomiasis and strongyloidiasis among African refugees. *Clinical Infectious Diseases,* 45**,** 1310-1315.

PRICHARD, R. 1994. Anthelmintic resistance. *Veterinary Parasitology,* 54**,** 259-268.

PRICHARD, R. K. 2007. Markers for benzimidazole resistance in human parasitic nematodes? *Parasitology,* 134**,** 1087-1092.

PROCIV, P. 1998. Zoonotic hookworm infections (Ancylostomosis). *In Zoonoses,* 1.

PROCIV, P. & CROESE, J. 1990. Human eosinophilic enteritis caused by dog hookworm Ancylostoma caninum. *The Lancet,* 335**,** 1299-1302.

PROCIV, P. & CROESE, J. 1996. Human enteric infection with *Ancylostoma caninum*: hookworms reappraised in the light of a “new” zoonosis. *Acta Tropica,* 62**,** 23-44.

PROCIV, P. & LUKE, R. 1993. Observations on strongyloidiasis in Queensland aboriginal communities. *The Medical Journal of Australia,* 158**,** 160-163.

PROCIV, P. & LUKE, R. A. 1995. The changing epidemiology of human hookworm infection in Australia. *The Medical Journal of Australia,* 162**,** 150-154.

PROKHOROV, A. & GOLOVAN, T. 1983. Epidemiology of strongyloidiasis in northern Caucasus, USSR. *Meditsinskaya Parazitologiya i Parazitarnye Bolezni,* 61**,** 34-38.

PULLAN, R. L., SMITH, J. L., JASRASARIA, R. & BROOKER, S. J. 2014. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. *Parasites & Vectors,* 7**,** 37.

PUTLAND, R., THOMAS, S., GROVE, D. & JOHNSON, A. 1993. Analysis of the 18S ribosomal RNA gene of *Strongyloides stercoralis*. *International Journal for Parasitology,* 23**,** 149-151.

RAHMANIAN, H., MACFARLANE, A. C., ROWLAND, K. E., EINSIEDEL, L. J. & NEUHAUS, S. J. 2015. Seroprevalence of *Strongyloides stercoralis* in a South Australian Vietnam veteran cohort. *Australian and New Zealand Journal of Public Health,* 39**,** 331-335.

REPETTO, S., SOTO, C. A., CAZORLA, S., TAYELDIN, M., CUELLO, S., LASALA, M., TEKIEL, V. & CAPPA, S. G. 2013. An improved DNA isolation technique for PCR detection of *Strongyloides stercoralis* in stool samples. *Acta Tropica,* 126**,** 110-114.

REQUENA-MÉNDEZ, A., CHIODINI, P., BISOFFI, Z., BUONFRATE, D., GOTUZZO, E. & MUÑOZ, J. 2013. The laboratory diagnosis and follow up of strongyloidiasis: a systematic review. *PLoS Neglected Tropical Diseases,* 7**,** e2002.

RIGGIO, F., MANNELLA, R., ARITI, G. & PERRUCCI, S. 2013. Intestinal and lung parasites in owned dogs and cats from central Italy. *Veterinary Parasitology,* 193**,** 78-84.

RIVERA-ROIG, V., SÁNCHEZ, J. L. & HILLYER, G. V. 2008. Hookworm folliculitis. *International Journal of Dermatology,* 47**,** 246-248.

ROBERTS, A. L., SCHNEIDER, A. E., YOUNG, R. L., HINRICHS, S. H. & IWEN, P. C. 2013. *Strongyloides stercoralis* Infection in a Non-Endemic Area. *Laboratory Medicine,* 44**,** 339-343.

ROBERTSON, G. 2016. Routine testing of clinical isolates for *Strongyloides stercoralis*. *unpublished data*.

ROBERTSON, I. D. & THOMPSON, R. 2002. Enteric parasitic zoonoses of domesticated dogs and cats. *Microbes and Infection,* 4**,** 867-873.

ROBINSON, R. D., LINDO, J. F., NEVA, F. A., GAM, A. A., VOGEL, P., TERRY, S. I. & COOPER, E. S. 1994. Immunoepidemiologic studies of Strongyloides stercoralis and human T lymphotropic virus type I infections in Jamaica. *Journal of Infectious Diseases,* 169**,** 692-696.

ROCK, M., BUNTAIN, B. J., HATFIELD, J. M. & HALLGRÍMSSON, B. 2009. Animal–human connections,“one health,” and the syndemic approach to prevention. *Social Science & Medicine,* 68**,** 991-995.

RODRIGO, C. & RAJAPAKSE, S. 2010. HIV, poverty and women. *International Health,* 2**,** 9-16.

ROMAN-SANCHEZ, P., PASTOR-GUZMAN, A., MORENO-GUILLEN, S., IGUAL-ADELL, R., ER-GENEROSO, S. S. & TORNERO-ESTEBANEZ, C. 2003. High prevalence of *Strongyloides stercoralis* among farm workers on the Mediterranean coast of Spain: analysis of the

predictive factors of infection in developed countries. *The American Journal of Tropical Medicine and Hygiene,* 69**,** 336-340.

RUSDI, B., LAIRD, T., ABRAHAM, R., ASH, A., ROBERTSON, I. D., MUKERJI, S., COOMBS, G. W., ABRAHAM, S. & O'DEA, M. A. 2018. Carriage of critically important antimicrobial resistant bacteria and zoonotic parasites amongst camp dogs in remote Western Australian indigenous communities. *Scientific Reports,* 8**,** 8725.

RUSSELL, E. S., GRAY, E. B., MARSHALL, R. E., DAVIS, S., BEAUDOIN, A., HANDALI, S., MCAULIFFE, I., DAVIS, C. & WOODHALL, D. 2014. Prevalence of *Strongyloides stercoralis* Antibodies among a Rural Appalachian Population—Kentucky, 2013. *The American Journal of Tropical Medicine and Hygiene,* 91**,** 1000-1001.

SAFDAR, A., MALATHUM, K., RODRIGUEZ, S. J., HUSNI, R. & ROLSTON, K. V. 2004. Strongyloidiasis in patients at a comprehensive cancer center in the United States. *Cancer,* 100**,** 1531-1536.

SAMPSON, I., SMITH, D. & MACKENZIE, B. Serological diagnosis of *Strongyloides stercoralis* infection. Second National Workshop on Strongyloidiasis, Royal Brisbane Hospital, Hernston, Australia, 25-26 June 2003. 25-26.

SÁNCHEZ, P. R., GUZMAN, A. P., GUILLEN, S. M., ADELL, R. I., ESTRUCH, A. M., GONZALO, I. N. & OLMOS, C. R. 2001. Endemic strongyloidiasis on the Spanish Mediterranean coast. *An International Journal of Medicine,* 94**,** 357-363.

SANTINI, A., LUCCHINI, V., FABBRI, E. & RANDI, E. 2007. Ageing and environmental factors affect PCR success in wolf (*Canis lupus*) excremental DNA samples. *Molecular Ecology Notes,* 7**,** 955-961.

SATO, Y., KOBAYASHI, J., TOMA, H. & SHIROMA, Y. 1995. Efficacy of stool examination for detection of *Strongyloides* infection. *The American Journal of Tropical Medicine and Hygiene,* 53**,** 248-250.

SATOH, M., TOMA, H., SATO, Y., KIKUCHI, M., TAKARA, M., SHIROMA, Y., KIYUNA, S. & HIRAYAMA, K. 1999. Production of a high level of specific IgG4 antibody associated with resistance to albendazole treatment in HLA-DRB1* 0901-positive patients with strongyloidiasis. *The American journal of tropical medicine and hygiene,* 61**,** 668-671.

SCHAD, G. 1989. Morphology and life history of *Strongyloides stercoralis*. *In:* GROVE, D. (ed.) *Strongyloidiasis a major roundworm infection of man.* London: Taylor and Francis.

SCHAD, G., AIKENS, L. M. & SMITH, G. 1989. *Strongyloides stercoralis*: is there a canonical migratory route through the host? *The Journal of Parasitology***,** 740-749.

SCHAD, G., THOMPSON, F., TALHAM, G., HOLT, D., NOLAN, T., ASHTON, F., LANGE, A. & BHOPALE, V. 1997. Barren female *Strongyloides stercoralis* from occult chronic infections are rejuvenated by transfer to parasite-naive recipient hosts and give rise to an autoinfective burst. *Journal of Parasitology,* 83**,** 785-791.

SCHAD, G. A., HELLMAN, M. E. & MUNCEY, D. W. 1984. *Strongyloides stercoralis:* hyperinfection in immunosuppressed dogs. *Experimental Parasitology,* 57**,** 287-296.

SCHAD, G. A. & PAGE, M. R. 1982. *Ancylostoma caninum*: adult worm removal, corticosteroid treatment, and resumed development of arrested larvae in dogs. *Experimental Parasitology,* 54**,** 303-309.

SCHÄR, F., GUO, L., STREIT, A., KHIEU, V., MUTH, S., MARTI, H. & ODERMATT, P. 2014a. *Strongyloides stercoralis* genotypes in humans in Cambodia. *Parasitology International,* 63**,** 533-536.

SCHÄR, F., INPANKAEW, T., TRAUB, R. J., KHIEU, V., DALSGAARD, A., CHIMNOI, W., CHHOUN, C., SOK, D., MARTI, H. & MUTH, S. 2014b. The prevalence and diversity of intestinal parasitic infections in humans and domestic animals in a rural Cambodian village. *Parasitology International,* 63**,** 597-603.

SCHÄR, F., ODERMATT, P., KHIEU, V., PANNING, M., DUONG, S., MUTH, S., MARTI, H. & KRAMME, S. 2013a. Evaluation of real-time PCR for *Strongyloides stercoralis* and hookworm as diagnostic tool in asymptomatic schoolchildren in Cambodia. *Acta Tropica,* 126**,** 89-92.

SCHÄR, F., TROSTDORF, U., GIARDINA, F., KHIEU, V., MUTH, S., MARTI, H., VOUNATSOU, P. & ODERMATT, P. 2013b. *Strongyloides stercoralis:* global distribution and risk factors. *PLoS Neglected Tropical Diseases,* 7**,** e2288.

SCHAUB, N. A., PERRUCHOUD, A. P. & BUECHNER, S. A. 2002. Cutaneous larva migrans associated with Löffler's syndrome. *Dermatology,* 205**,** 207-209.

SCHRADER, C., SCHIELKE, A., ELLERBROEK, L. & JOHNE, R. 2012. PCR inhibitors–occurrence, properties and removal. *Journal of Applied Microbiology,* 113**,** 1014-1026.

SCOWDEN, E. B., SCHAFFNER, W. & STONE, W. J. 1978. Overwhelming strongyloidiasis: an unappreciated opportunistic infection. *Medicine,* 57**,** 527-544.

SEUTIN, G., WHITE, B. N. & BOAG, P. T. 1991. Preservation of avian blood and tissue samples for DNA analyses. *Canadian Journal of Zoology,* 69**,** 82-90.

SEYBOLT, L. M., CHRISTIANSEN, D. & BARNETT, E. D. 2006. Diagnostic evaluation of newly arrived asymptomatic refugees with eosinophilia. *Clinical Infectious Diseases,* 42**,** 363-367.

SHIELD, J., ALAND, K., KEARNS, T., GONGDJALK, G., HOLT, D., CURRIE, B. & PROCIV, P. 2015. Intestinal parasites of children and adults in a remote Aboriginal community of the Northern Territory, Australia, 1994–1996. *Western Pacific Surveillance and Response Journal,* 6**,** 44.

SHIELD, J. M., KEARNS, T. M., GARŊGULKPUY, J., WALPULAY, L., GUNDJIRRYIRR, R., BUNDHALA, L., DJARPANBULUWUY, V., ANDREWS, R. M. & JUDD, J. 2018. Cross-cultural, Aboriginal language, discovery education for health literacy and informed consent

in a remote Aboriginal community in the Northern Territory, Australia. *Tropical Medicine and Infectious Disease,* 3**,** 15.

SHIELDS, A. M., GODERYA, R., ATTA, M. & SINHA, P. 2014. *Strongyloides stercoralis* hyperinfection presenting as subacute small bowel obstruction following immunosuppressive chemotherapy for multiple myeloma. *BMJ Case Reports,* 2014**,** bcr2013202234.

SHIKIYA, K., ZAHA, O., NIIMURA, S., UEHARA, T., OHSHIRO, J., KINJO, F., SAITO, A. & ASATO, R. 1994. [Clinical study on ivermectin against 125 strongyloidiasis patients]. *Kansenshogaku zasshi. The Journal of the Japanese Association for Infectious Diseases,* 68**,** 13-20.

SILVESTRE, A. & CABARET, J. 2004. Nematode parasites of animals are more prone to develop xenobiotic resistance than nematode parasites of plants. *Parasite. Jun,* 11**,** 119-29.

SITHITHAWORN, P., SRISAWANGWONG, T., TESANA, S., DAENSEEKAEW, W., SITHITHAWORN, J., FUJIMAKI, Y. & ANDO, K. 2003. Epidemiology of *Strongyloides stercoralis* in north-east Thailand: application of the agar plate culture technique compared with the enzyme-linked immunosorbent assay. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 97**,** 398-402.

SITTA, R., MALTA, F., PINHO, J., CHIEFFI, P., GRYSCHEK, R. & PAULA, F. 2014. Conventional PCR for molecular diagnosis of human strongyloidiasis. *Parasitology,* 141**,** 716-721.

SMITS, H. L. 2009. Prospects for the control of neglected tropical diseases by mass drug administration. *Expert Review of Anti-Infective Therapy,* 7**,** 37-56.

SMOUT, F., SKERRATT, L., JOHNSON, C., BUTLER, J. & CONGDON, B. 2018a. Zoonotic helminth diseases in dogs and dingoes utilising shared resources in an Australian Aboriginal community. *Tropical Medicine and Infections DIsease* 3**,** 110.

SMOUT, F. A., SKERRATT, L. F., BUTLER, J. R., JOHNSON, C. N., CONGDON, B. C. & THOMPSON, R. A. 2017. The hookworm *Ancylostoma ceylanicum:* An emerging public health risk in Australian tropical rainforests and Indigenous communities. *One Health,* 3**,** 66-69.

SMOUT, F. A., SKERRATT, L. F., JOHNSON, C. N., BUTLER, J. R. A. & CONGDON, B. C. 2018b. Zoonotic Helminth Diseases in Dogs and Dingoes Utilising Shared Resources in an Australian Aboriginal Community. *Trop Med Infect Dis,* 3.

SOULSBY, H. M., HEWAGAMA, S. & BRADY, S. 2012. Case series of four patients with strongyloides after occupational exposure. *The Medical Journal of Australia,* 196**,** 444.

SPEARE, R. 1989. Identification of species of *Strongyloides*. *In:* GROVE, D. (ed.) *Strongyloidiasis a Major Roundworm Infection of Man.* London: Taylor and Francis.

SPEARE, R. July 2011. Criteria for notifiability status of strongyloidiasis. 6th National Workshop on *Strongyloides*. *Annals of the ACTM: An International Journal of Tropical and Travel Medicine,* 12**,** 44-47.

SPEARE, R., BRADBURY, R. S. & CROESE, J. 2016. A case of *Ancylostoma ceylanicum* infection occurring in an Australian soldier returned from Solomon Islands. *The Korean Journal of Parasitology,* 54**,** 533.

SPEARE, R., MILLER, A. & PAGE, W. A. 2014. Strongyloidiasis: a case for notification in Australia. *Acta Tropica,* 138**,** 78-82.

STEINMANN, P., ZHOU, X.-N., DU, Z.-W., JIANG, J.-Y., WANG, L.-B., WANG, X.-Z., LI, L.-H., MARTI, H. & UTZINGER, J. 2007. Occurrence of *Strongyloides stercoralis* in Yunnan Province, China, and comparison of diagnostic methods. *PLoS Neglected Tropical Diseases,* 1**,** e75.

STEWART, L. 1994. *The taxonomy of Ancylostoma species in the Townsville region of North Queensland.* M. Sc. thesis, James Cook University.

STREIT, A. 2008. Reproduction in Strongyloides (Nematoda): a life between sex and parthenogenesis. *Parasitology,* 135**,** 285-294.

SUDARSHI, S., STÜMPFLE, R., ARMSTRONG, M., ELLMAN, T., PARTON, S., KRISHNAN, P., CHIODINI, P. L. & WHITTY, C. J. 2003. Clinical presentation and diagnostic sensitivity of laboratory tests for *Strongyloides stercoralis* in travellers compared with immigrants in a non-endemic country. *Tropical Medicine & International Health,* 8**,** 728-732.

SULTANA, Y., GILBERT, G. L., AHMED, B.-N. & LEE, R. 2012. Strongyloidiasis in a high risk community of Dhaka, Bangladesh. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 106**,** 756-762.

SULTANA, Y., JEOFFREYS, N., WATTS, M. R., GILBERT, G. L. & LEE, R. 2013. Real-time polymerase chain reaction for detection of *Strongyloides stercoralis* in stool. *The American Journal of Tropical Medicine and Hygiene,* 88**,** 1048-1051.

SUTHERLAND, I. A. & LEATHWICK, D. M. 2011. Anthelmintic resistance in nematode parasites of cattle: a global issue? *Trends in Parasitology,* 27**,** 176-181.

SWAMINATHAN, A., TORRESI, J., SCHLAGENHAUF, P., THURSKY, K., WILDER-SMITH, A., CONNOR, B. A., SCHWARTZ, E., KEYSTONE, J. & O'BRIEN, D. P. 2009. A global study of pathogens and host risk factors associated with infectious gastrointestinal disease in returned international travellers. *Journal of Infection,* 59**,** 19-27.

TAKANO, Y., MINAKAMI, K., KODAMA, S., MATSUO, T. & SATOZONO, I. 2009. Cross infection of *Strongyloides* between humans and dogs in the Amami Islands, Japan. *Tropical Medicine and Health,* 37**,** 149-152.

TAYLOR, M. J., GARRARD, T. A., O'DONAHOO, F. J. & ROSS, K. E. 2014. Human strongyloidiasis: identifying knowledge gaps, with emphasis on environmental control. *Research and Reports in Tropical Medicine,* 3**,** 55-63.

THAMSBORG, S. M., KETZIS, J., HORII, Y. & MATTHEWS, J. B. 2017. *Strongyloides* spp. infections of veterinary importance. *Parasitology,* 144**,** 274-284.

THANCHOMNANG, T., INTAPAN, P. M., SANPOOL, O., RODPAI, R., TOURTIP, S., YAHOM, S., KULLAWAT, J., RADOMYOS, P., THAMMASIRI, C. & MALEEWONG, W. 2017. First molecular identification and genetic diversity of *Strongyloides stercoralis* and Strongyloides fuelleborni in human communities having contact with long-tailed macaques in Thailand. *Parasitology Research,* 116**,** 1917-1923.

THOMPSON, R. A. & ROBERTS, M. G. 2001. Does pet helminth prophylaxis increase the rate of selection for drug resistance? *Trends in Parasitology,* 17**,** 576-578.

TOLEDO, R., MUÑOZ-ANTOLI, C. & ESTEBAN, J.-G. 2015. Chapter five-strongyloidiasis with emphasis on human infections and its different clinical forms. *Advances in Parasitology,* 88**,** 165-241.

TOMA, H., SATO, Y., SHIROMA, Y., KOBAYASHI, J., SHIMABUKURO, I. & TAKARA, M. 2000. Comparative studies on the efficacy of three anthelmintics on treatment of human strongyloidiasis in Okinawa, Japan. *Southeast Asian Journal of Tropical Medicine and Public Health,* 31**,** 147-151.

TRAUB, R. J. 2013. *Ancylostoma ceylanicum*, a re-emerging but neglected parasitic zoonosis. *International Journal for Parasitology,* 43**,** 1009-1015.

TRAUB, R. J., INPANKAEW, T., SUTTHIKORNCHAI, C., SUKTHANA, Y. & THOMPSON, R. A. 2008. PCR-based coprodiagnostic tools reveal dogs as reservoirs of zoonotic ancylostomiasis caused by *Ancylostoma ceylanicum* in temple communities in Bangkok. *Veterinary Parasitology,* 155**,** 67-73.

TRAUB, R. J., ROBERTSON, I. D., IRWIN, P., MENCKE, N. & THOMPSON, R. A. 2004a. Application of a species-specific PCR-RFLP to identify *Ancylostoma* eggs directly from canine faeces. *Veterinary Parasitology,* 123**,** 245-255.

TRAUB, R. J., ROBERTSON, I. D., IRWIN, P., MENCKE, N. & THOMPSON, R. A. 2004b. The prevalence, intensities and risk factors associated with geohelminth infection in tea-growing communities of Assam, India. *Tropical Medicine & International Health,* 9**,** 688-701.

TREWIN, D. & MADDEN, R. 2005. The health and welfare of Australia's Aboriginal and Torres Strait Islander peoples. *Canberra, Australian Bureau of Statistics*.

TURNER, S. A., MACLEAN, J. D., FLECKENSTEIN, L. & GREENAWAY, C. 2005. Parenteral administration of ivermectin in a patient with disseminated strongyloidiasis. *The American Journal of Tropical Medicine and Hygiene,* 73**,** 911-914.

UMUR, S., MERAL, Y., BOLUKBAS, C. S., GURLER, A. T. & ACICI, M. 2017. First clinical *Strongyloides stercoralis* case in a dog in Turkey. *Turkish Journal of Veterinary and Animal Sciences*, 41.

UN 2016. Worlds Economic Situation and Prospects 2016. New York: United Nations

UPARANUKRAW, P., PHONGSRI, S. & MORAKOTE, N. 1999. Fluctuations of larval excretion in *Strongyloides stercoralis* infection. *The American Journal of Tropical Medicine and Hygiene,* 60, 967-973.

VADLAMUDI, R. S., CHI, D. S. & KRISHNASWAMY, G. 2006. Intestinal strongyloidiasis and hyperinfection syndrome. *Clinical and Molecular Allergy,* 4, 1.

VALERIO, L., ROURE, S., FERNÁNDEZ-RIVAS, G., BASILE, L., MARTÍNEZ-CUEVAS, O., BALLESTEROS, Á.-L., RAMOS, X., SABRIÀ, M. & DISEASES, N. M. W. G. O. I. 2013. *Strongyloides stercoralis*, the hidden worm. Epidemiological and clinical characteristics of 70 cases diagnosed in the North Metropolitan Area of Barcelona, Spain, 2003–2012. *Transactions of The Royal Society of Tropical Medicine and Hygiene,* 107, 465-470.

VAUGHAN, T. 1998. Cutaneous larva migrans complicated by erythema multiforme. *Cutis,* 62, 33-35.

VENKATESAN, P. 1998. Albendazole. *Journal of Antimicrobial Chemotherapy,* 41, 145-147.

VERWEIJ, J. J., CANALES, M., POLMAN, K., ZIEM, J., BRIENEN, E. A., POLDERMAN, A. M. & VAN LIESHOUT, L. 2009. Molecular diagnosis of *Strongyloides stercoralis* in faecal samples using real-time PCR. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 103, 342-346.

WAGHORN, T., LEATHWICK, D., RHODES, A., LAWRENCE, K., JACKSON, R., POMROY, W., WEST, D. & MOFFAT, J. 2006. Prevalence of anthelmintic resistance on sheep farms in New Zealand. *New Zealand Veterinary Journal,* 54, 271-277.

WALKER-SMITH, J., MCMLLLAN, B., MLDDLETON, A., ROBERTSON, S. & HOPCROFT, A. 1969. Strongyloidiasis causing small-bowel obstruction in an Aboriginal infant. *Medical Journal of Australia*, 1263-5.

WALKER, N. I., CROESE, J., CLOUSTON, A. D., PARRY, M., LOUKAS, A. & PROCIV, P. 1995. Eosinophilic enteritis in northeastern Australia. Pathology, association with *Ancylostoma caninum*, and implications. *The American Journal of Surgical Pathology,* 19, 328-337.

WALZER, P. D., MILDER, J. E., BANWELL, J. G., KILGORE, G., KLEIN, M. & PARKER, R. 1982. Epidemiologic features of *Strongyloides stercoralis* infection in an endemic area of the United States. *The American Journal of Tropical Medicine and Hygiene,* 31, 313-319.

WANG, C., XU, J., ZHOU, X., LI, J., YAN, G., JAMES, A. A. & CHEN, X. 2013. Strongyloidiasis: an emerging infectious disease in China. *The American Journal of Tropical Medicine and Hygiene,* 88, 420-425.

WANG, H., DONG, J. S., LIN, Y., LI, X. Z., FENG, G., LIU, W., MENG, X. & LI, J. J. 2015. Study on cauliflower-like mass in rectum mucosa Induced by *Strongyloides stercoralis* in beagle dogs. *Hemoglobin (g/L),* 98**,** 110-190.

WATERS, A.-M. 2001. *Do Housing Conditions Impact on Health Inequalities Between Australia's Rich and Poor?: Final Report*, Australian Housing and Urban Research Institute.

WHILEY, H., ROSS, K. & BEKNAZAROVA, M. 2017. Strongyloidiasis is a deadly worm infecting many Australians, yet hardly anybody had heard of it. *Conversation.* Melbourne, Australia: The Conversation Trust.

WHO 2005. Deworming for Health and Development: Report of the Third Global Meeting of the Partners for Parasite Control. Geneva, Switzerland: World Health Organisation.

WHO 2006. Preventive Chemotherapy in Human Helminthiasis. *Coordinated Use of Anthelminthic Drugs in Control Interventions: A Manual for Health Professionals and Programme Managers.* Geneva, Switzerland: WHO Press.

WHO 2013. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the ENvironment and the WHO Core Assesment Group on Pesticide Residues. Geneva, Switzerland: Wolrd Health Organisation, Food and Agriculture Organisation of the United Nations.

WILLIS, E. & ROSS, K. 2019. Review of principles governing dog health education in remote Aboriginal communities. *Australian Veterinary Journal,* 97**,** 4-9.

WILLIS, H. H. 1920. A note on the value of oil of chenopodium in the treatment of *Strongyloides* Infection. *Medical Journal of Australia*.

WOODRING, J. H., HALFHILL 2ND, H. & REED, J. C. 1994. Pulmonary strongyloidiasis: clinical and imaging features. *American Journal of Roentgenology,* 162**,** 537-542.

XU, M., MOLENTO, M., BLACKHALL, W., RIBEIRO, P., BEECH, R. & PRICHARD, R. 1998. Ivermectin resistance in nematodes may be caused by alteration of P-glycoprotein homolog. *Molecular and Biochemical Parasitology,* 91**,** 327-335.

YELIFARI, L., BLOCH, P., MAGNUSSEN, P., VAN LIESHOUT, L., DERY, G., ANEMANA, S., AGONGO, E. & POLDERMAN, A. M. 2005. Distribution of human *Oesophagostomum bifurcum*, hookworm and *Strongyloides stercoralis* infections in northern Ghana. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 99**,** 32-38.

YODER, M., DE LEY, I. T., KING, I. W., MUNDO-OCAMPO, M., MANN, J., BLAXTER, M., POIRAS, L. & DE LEY, P. 2006. DESS: a versatile solution for preserving morphology and extractable DNA of nematodes. *Nematology,* 8**,** 367-376.

ZAHA, O., HIRATA, T., KINJO, F. & SAITO, A. 2000. Strongyloidiasis-Progress in Diagnosis and Treatment. *Internal Medicine,* 39**,** 695-700.

ZAHA, O., HIRATA, T., KINJO, F., SAITO, A. & FUKUHARA, H. 2002. Efficacy of ivermectin for chronic strongyloidiasis: two single doses given 2 weeks apart. *Journal of Infection and Chemotherapy,* 8**,** 94-98.

ZAHEDI, A., GOFTON, A. W., JIAN, F., PAPARINI, A., OSKAM, C., BALL, A., ROBERTSON, I. & RYAN, U. 2017. Next Generation Sequencing uncovers within-host differences in the genetic diversity of *Cryptosporidium* gp60 subtypes. *International Journal for Parasitology,* 47**,** 601-607.

ZAKI, M. H., MORAN, D. & HARRIS, D. 1982. Pesticides in groundwater: the aldicarb story in Suffolk County, NY. *American Journal of Public Health,* 72**,** 1391-1395.

Acts

The Quarantine Act 1832 No 16a, New South Wales, Australia

Available at: http://classic.austlii.edu.au/au/legis/nsw/num_act/qa1832n16158/ (accessed: 10.05.2019)