



## The hookworm *Ancylostoma ceylanicum*: An emerging public health risk in Australian tropical rainforests and Indigenous communities



Felicity A. Smout<sup>a,d,\*</sup>, Lee F. Skerratt<sup>a</sup>, James R.A. Butler<sup>b</sup>, Christopher N. Johnson<sup>c</sup>, Bradley C. Congdon<sup>d</sup>, R.C. Andrew Thompson<sup>e</sup>

<sup>a</sup> One Health Research Group, College of Public Health, Medical and Vet Sciences, James Cook University, Townsville, Queensland 4811, Australia

<sup>b</sup> CSIRO Land and Water, EcoSciences Precinct, GPO Box 2583, Brisbane, Queensland 4001, Australia

<sup>c</sup> School of Biological Sciences and Australian Research Council Centre of Excellence for Biodiversity and Heritage, University of Tasmania, Private Bag 55, Hobart, Tasmania 7001, Australia

<sup>d</sup> College of Science & Engineering, James Cook University, Cairns, Queensland 4870, Australia

<sup>e</sup> School of Veterinary and Life Sciences, Murdoch University, Murdoch, Western Australia 6150, Australia

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### ABSTRACT

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

### 1. Introduction

Human hookworm infections have been attributed mainly to *Necator americanus* and *Ancylostoma duodenale* [1,2] while *Ancylostoma ceylanicum*, a common hookworm of domestic dogs and cats throughout Asia [3–5], has been largely ignored. This is despite knowledge that *A. ceylanicum* can cause patent enteric infections in humans [6–8]. Concern about this parasite in tropical Australia has been growing following its recent discovery in humans in Western Australia [9], domestic dogs in Western Australia, Victoria, Queensland and the Northern Territory [10] and dingoes in Far North Queensland [11].

The Wet Tropics bioregion of Far North Queensland contains remnant rainforest which holds globally-significant biodiversity and cultural values, and these are recognised by its designation as the Wet Tropics World Heritage Area (WTWHA) [12,13]. The WTWHA is a major tourist attraction and many locations within or on its periphery are both culturally important for Indigenous communities and also

visited by tourists [14–16]. Free-ranging domestic dogs and dingoes (or 'wild dogs') are widespread, and interact in close proximity to people in the region [17]. Indigenous Australians in tropical communities are at particular risk from *A. ceylanicum* and *A. caninum* infection due to the limited health management of domestic dogs and the presence of free-roaming community dogs that may have been exposed to parasite eggs and larvae in soil contaminated by dingoes [11]. Along with the faecal oral route for infection, the larvae can also penetrate the skin of humans, as well as their canine or feline host. Therefore people coming into contact with contaminated soil or sand also risk infection [18]. Consequently, when developing public health protocols in Indigenous communities the role of the dog in the transmission of hookworm infection to humans should also be considered, since successful control of infection may require better management and treatment of dogs. Chemotherapy focusing on the human population alone is unlikely to be successful [19].

The recent development of advanced, PCR-based techniques capable

\* Corresponding author.

E-mail address: [felicity.smout@my.jcu.edu.au](mailto:felicity.smout@my.jcu.edu.au) (F.A. Smout).



Fig. 1. The Wet Tropics World Heritage Area in Far North Queensland, and the location of study sites.

of differentiating between hookworm species using DNA isolated from eggs in faeces and soil [20] enables a better understanding of the epidemiology of *A. ceylanicum* infection. Here, we investigate the geographical distribution of *A. caninum* and *A. ceylanicum* in tropical Far North Queensland using this latest technology. We report for the first time the presence of *A. ceylanicum* in domestic dogs and soil in Indigenous communities, and locations also frequented by tourists.

## 2. Materials and methods

### 2.1. Study area and collection of specimens

The study area was conducted at six localities within the WTWHA of north-east Queensland, Australia. The region was further sub-divided into six localities, three Indigenous communities (Mossman, Yarrabah and Jumbun) and three tourist locations (Mossman Gorge, Lake Placid and Murray Upper) (Fig. 1).

In total, 130 soil samples were collected from the study sites, and 86 faecal samples were collected from free-ranging domestic dogs in these sites in November and December 2011 (Table 1). In addition, 27 domestic dog carcasses were supplied by Yarrabah Aboriginal Council Animal Control Officers between December 2010 and December 2011. No domestic dogs were killed specifically for this study. All of the specimens were necropsied immediately and faecal samples collected. All protocols were reviewed and approved by James Cook University Animal Ethics Committee (Approval no. A1546).

Table 1

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

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

(f) faeces, (s) soil.

### 2.2. Necropsy technique and parasite preservation

The stomach and intestines of the 27 domestic dog carcasses (9 males and 18 females) ranging in age from 10 weeks to over eight years of age (mean age approximately 2.5 years), were excised. The stomach, small intestine, and large intestine were each ligated at the junctions and examined separately. The intestinal lumen was exposed via an incision along its length and the contents washed into a 250-µm aperture sieve. Stomach washings were also examined for the presence of helminths. Intestines were then passed between the examiner's thumb and forefinger several times to scrape off any attached worms whilst a visual inspection was made of the mucosa. All contents were washed thoroughly and preserved in 70% ethanol for later microscopical examination. Faecal samples were also collected directly from the large bowel and preserved in 5% SAF for microscopy and 80% ethanol for molecular procedures [11].

### 2.3. Microscopic examination

All specimens were transported to the School of Veterinary and Life Sciences, Murdoch University, Western Australia. Intestinal contents were examined under dissecting and compound microscopes. Positive identification of *Ancylostoma* species was established using criteria documented in Biocca's [21] paper. Where present, at least fifty individual hookworms were identified before deciding on the species present. Faecal scats, necropsy-collected faeces and soil were examined by simple smear technique, where faeces or soil were mixed on a slide with a small volume of water, and those samples positive for strongyle eggs noted. Given the high number of positive samples detected it was decided to include all samples for molecular analysis [11].

### 2.4. Genomic DNA extraction

DNA was extracted directly from faeces using a Promega Maxwell® 16 research instrument system and tissue kit. The final DNA elution was prepared in 300 µl of elution solution and stored at -20 °C until required. In order to confirm morphological identification, male *A. caninum* specimens also underwent molecular identification. Worms were washed and DNA was extracted using an Epicentre MasterPure™ Complete DNA and RNA Purification Kit according to the manufacturer's instructions [11]. To save on analysis time and costs batches of five closely collected, individual soil samples were pooled resulting in 26 pooled samples. DNA was extracted using a PowerMax® Soil DNA isolation kit and stored at -20 °C until required.

### 2.5. Molecular methods – PCR

A direct PCR assay modified from Traub et al. [20] was used for the DNA amplification of hookworm species. A forward primer RTHWIF (5'-GATGAGCATTGCWTGAATGCCG-3') and reverse primer RTHWIR (5'-GCAAGTRCCGTTTCGACAAACAG-3') were used to amplify an approximately 485 bp and 380 bp section of the internal transcribed spacer-1 (ITS-1), 5.8S and internal transcribed spacer-2 (ITS-2) regions of *Ancylostoma* spp. The PCR assay was prepared in a volume of 25 µl consisting of 1 X PCR buffer, 2 mM MgCl<sub>2</sub>, 0.4 mM of each dNTP, 10 pmol of each primer, 1.0 U *Taq* DNA polymerase (Biotech International, Perth, Australia) and 1 µl of template genomic DNA. Due to the presence of inhibitors, DNA template often needed to be diluted to 1:2 or 1:4 concentration. PCR cycling conditions consisted of a pre-heating step at 95 °C for 5 min. This was followed by 40 cycles of 95 °C for 30 s (denaturing), 60 °C for 30 s (annealing), 72 °C for 30 s (extension), a final extension of 72 °C for 7 min and a holding temperature of 14 °C. Cycling was performed on an Applied Biosystems 2720 Thermal Cycler. The verification of the PCR product was established on a 1.5% agarose gel dyed with SYBR®Safe DNA gel stain [11].

## 2.6. DNA sequencing of canine hookworm

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

## 2.7. Data analysis

Prevalence of a species of hookworm within positive samples was calculated by dividing the number of samples positive for each hookworm species by the total number of samples positive for hookworm in each location (Table 1). The significance ( $p < 0.05$ ) of the difference between hookworm species and location was determined using the two-sided Fisher's Exact test [22].

## 3. Results

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

BLAST results showed a 99% or greater homology to previously published sequences with GenBank accession nos. DQ78009 for *A. ceylanicum* and JQ812694 for *A. caninum*. Positively identified *A. caninum* from sample NYB14 (from Yarrabah) was 100% homologous with DQ438073 for *A. caninum* confirming that morphological identification of samples was correct.

From the 63 positive canine faecal samples examined, 62 were infected with *A. caninum* (98.4%), one had a sole infection of *A. ceylanicum* (1.6%) and four (6.3%) contained a mixed infection of *A. ceylanicum* and *A. caninum* together. These dual infections occurred only in dogs from the Mossman community while Yarrabah dogs were solely infected with *A. caninum* (100%) (Table 1). The prevalence of *A. ceylanicum* in soil was highest at Mossman Gorge and Lake Placid (100%) followed by Mossman (55.6%) and Yarrabah communities (25%). Fisher's exact test on frequency analysis showed that prevalence of hookworm species in dog faeces varied significantly with location ( $p = 0.018$ ). No significant difference was seen for soil in relation to location ( $p = 0.171$ ).

## 4. Discussion

Prevalence of hookworm found in domestic dogs in the study sites was high. The most predominant species, *A. caninum* is not currently perceived as a severe zoonotic threat as the resultant cutaneous larval migrans infection is self-limiting in humans. Although, *A. caninum* infection can also result in eosinophilic enteritis and associated intestinal hypersensitivity [23]. This study reports, for the first time, *A. ceylanicum* infection in domestic dogs and soil in a Queensland Indigenous community and in soil in protected areas frequented by tourists. Due to this parasite's potential for patent enteric infection in humans, this is a public health concern, and consideration should be given to addressing and mitigating risks for infection.

The local Mossman Indigenous community, Kuku Yalanji, is situated 75 km north of Cairns (Fig. 1) and 4 km from Mossman (the nearest town). It has an estimated resident population of 100 people [24]. The

community has built an eco-tourism centre which attracts 350,000 visitors annually who come to see the rainforest in this part of the Wet Tropics World Heritage Area. Visitors can swim in the local water holes and sunbake on the banks of the Mossman River. *A. ceylanicum* is able to infect a host through percutaneous penetration along with the faecal-oral route. Hence the warm, moist conditions at this site may provide ideal conditions for contracting soil-transmitted helminth infections.

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

Located at the base of the Barron Gorge 15 km north-west of Cairns, Lake Placid is a large natural lake formed by the Barron River (Fig. 1). It is popular amongst local and international tourists as well as kayaking enthusiasts. The, pet-friendly, Lake Placid Tourist Park is located next to Lake Placid recreational area, where a positive sample for *A. ceylanicum* was collected. Although the entrance to the recreational area is clearly signposted that domestic dogs are prohibited, these rules are not always adhered to (F. Smout, pers. obs.).

Jumbun is a small Indigenous community with a population of 104 people [25], located at Murray Upper approximately 160 km south of Cairns (Fig. 1). Due to a recent local council animal management program, only 10 dogs were present in the community at the time of the study, although the dog numbers can vary greatly (A. Miller, pers. comms.). All faecal samples collected here were positive for *A. caninum* (100%).

Yarrabah Aboriginal community is located to the south-east of Cairns (Fig. 1), with a population of 2340 [26]. Free-ranging dogs are abundant in the community and are often seen wandering in the surrounding WTWHA rainforest, where dingoes are also present (F. Smout, pers. obs.). Interestingly, none of the dogs necropsied, nor any of the canine faecal samples were positive for *A. ceylanicum*. However, two of the soil samples collected here did return positive results, and these came from the primary school and the high school playgrounds. Further investigation should be undertaken here of the *A. ceylanicum* status of cats in the community, since they may have been the source of infection and may have used the sandy playground of the schools for defaecation. A previous study in a Western Australia Aboriginal community found poor sanitation and promiscuous defaecation by children to also be factors contributing to hookworm infection [27]. Therefore, if *A. ceylanicum* is present in humans in the community, infection could be spread by similar behaviour.

While *A. ceylanicum* may not be ubiquitous throughout Far North Queensland, further research needs to be undertaken to determine the true extent of infection in this region. Previous research has shown *A. ceylanicum* to be the predominant hookworm infection in the dingo population approximately 40 km north-west of Mossman Gorge [11]. It is possible that *A. ceylanicum* is the more abundant hookworm in these rainforest areas, and that in locations where domestic dogs, the reservoir host of *A. caninum*, are present such as in the Indigenous communities here, there may be a spill-over of infection from domestic dogs that influences the hookworm species present in the dingo population [11].

Our understanding of the epidemiology involved in the transmission of helminth infections in Indigenous Australians has been greatly enhanced through the use of molecular techniques. The Australian Government's ongoing commitment to encouraging tourism in the WTWHA [28] and to "closing the gap" in chronic disease in Indigenous communities [29] requires that dog health, a potentially hazardous environmental issue, needs to be addressed. Current public health approaches to helminth infections are directed at investigating anthroponotic routes of infection [30]. Our results suggest that addressing zoonotic origins may be more appropriate and dogs must be considered as a reservoir of human infections when using mass drug administration

for controlling hookworm infection in the human population [31].

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

#### Conflict of interest statement

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

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