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

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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

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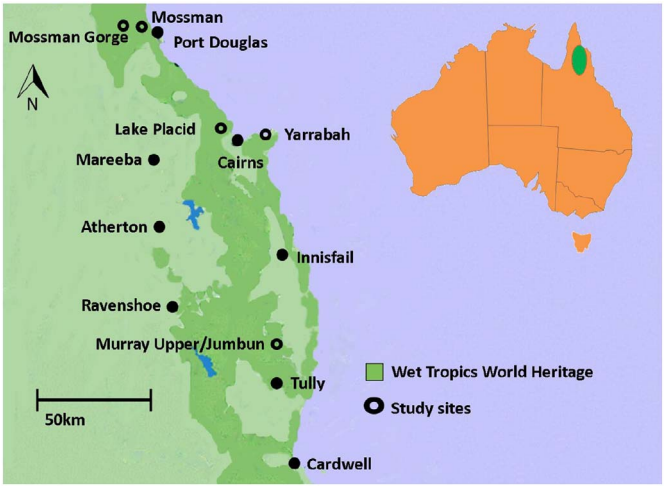

Fig. 1. The Wet Tropics World Heritage Area in Far North Queensland, and the location of study sites.

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

(f) faeces, (s) soil.

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′-GCAAGTRCCGTTCGACAAACAG-3′) were used to amplify an approximately 485 bp and 380 bp section of the internal transcribed spacer-1 (ITS-1), 5.8S and internal transcribed spacer-2 (ITS-2) regions of *Ancylostoma* spp. The PCR assay was prepared in a volume of 25 μl consisting of 1 X PCR buffer, 2 mM $MgCl_2$, 0.4 mM of each dNTP, 10 pmol of each primer, 1.0 U *Taq* DNA polymerase (Biotech International, Perth, Australia) and 1 μl of template genomic DNA. Due to the presence of inhibitors, DNA template often needed to be diluted to 1:2 or 1:4 concentration. PCR cycling conditions consisted of a pre-heating step at 95 °C for 5 min. This was followed by 40 cycles of 95 °C for 30 s (denaturing), 60 °C for 30 s (annealing), 72 °C for 30 s (extension), a final extension of 72 °C for 7 min and a holding temperature of 14 °C. Cycling was performed on an Applied Biosystems 2720 Thermal Cycler. The verification of the PCR product was established on a 1.5% agarose gel dyed with SYBR®Safe DNA gel stain [11].