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Differences in the faecal microbiome of non-diarrhoeic clinically healthy dogs and cats associated with *Giardia duodenalis* infection: impact of hookworms and coccidia ☆

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ABSTRACT

The protozoan parasite *Giardia duodenalis* causes a waterborne diarrhoeal disease in animals and humans, yet many *Giardia*-infected hosts remain asymptomatic. Mixed parasite infections are common in both animals and humans with unknown consequences for *Giardia* or other parasites. We compared the composition and diversity of bacterial communities from 40 dogs, including free-roaming dogs, and 21 surrendered cats from Australia. The dog cohort included 17 (42.5%) dogs positive for *Giardia* and 13 (32.5%) dogs positive for dog hookworm (*Ancylostoma caninum*). The cat samples included eight positive for *Giardia* and eight positive for *Cystoisospora*. The V4 region of 16S rRNA was sequenced at an average of 36,383 high quality sequences (>200 bp) per sample using the Ion Torrent PGM™ platform. In dogs we found significant ($P < 0.05$, AnoSim) difference between the *Giardia*-positive and -negative groups when evaluating bacterial genera. No such difference was demonstrated between *Ancylostoma*-positive and -negative dogs. However, there was a modest but not significant separation of the *Giardia*-negative and -positive dogs ($P = 0.09$, UniFrac) using principal coordinate analysis. Removal of dogs with hookworms further separated *Giardia*-positive and -negative groupings ($P = 0.06$, UniFrac). In cats, the presence of *Giardia* was not associated with a significant difference based on bacterial genera ($P > 0.05$, AnoSim). *Cystoisospora*-positive cats, however, exhibited significantly different profiles from *Cystoisospora*-negative cats ($P = 0.02$, AnoSim) and UniFrac showed significant separation of *Cystoisospora*-positive and -negative samples ($P < 0.01$). The results suggest that in clinically heathy dogs and cats, helminths and protozoa are associated with different microbiomes and possibly variable gut microbiota functions. Understanding the association of parasites and microbiomes has important consequences for the administration of antiparasitic drugs in animals and humans.

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1. Introduction

Giardia duodenalis causes a waterborne diarrhoeal disease in animals and humans (Geurden et al., 2010; Hanevik et al., 2014; Tysnes et al., 2014). The parasite is widely prevalent worldwide and is included in the World Health Organisation Neglected Disease Initiative (Savioli et al., 2006). The extracellular trophozoite of the parasite strongly attaches to upper small intestine enterocytes using a highly specialised ventral adhesive disc (House et al., 2011). One of the more perplexing aspects of *Giardia* infection is the range of symptoms and signs in affected humans and animals. Presence of *Giardia* in the gut may cause diarrhoea, however many hosts remain asymptomatic despite shedding environmentally-resistant cysts (Tysnes et al., 2014).

The pathogenesis of giardiasis and associated sequelae is a concerted affair associated with parasite attachment and loss of epithelial barrier function (Cotton et al., 2011; Bartelt et al., 2013). Penetration of intestinal bacteria into the inflamed intestinal wall and permanent damage to the intestinal epithelium in experimentally *Giardia*-infected mice has been documented (Chen et al., 2013). Singer and Nash (2000) postulated that changes to resident intestinal microflora are responsible for the disease outcome with giardiasis, because resistance to *Giardia* in mice was observed after treatment with an antibiotic, neomycin.

☆ *Note:* Nucleotide sequence data from this study are available in the GenBank database under accession numbers KP844728–KP844736 and the SRA database under accession number PRJNA276586.

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Experimental laboratory models using *Giardia* are now enabling an understanding of the interplay between parasite factors and intestinal microbiota (Gerbaba et al., 2015). Humans and animals, however, are exposed to a diverse range of intestinal pathogens and diets that influence the composition of intestinal microflora (Hooda et al., 2012; Yatsunenko et al., 2012; Andersen et al., 2013).

Parasites including the intestinal hookworms, roundworms, *Giardia*, *Cryptosporidium* and coccidia (*Cyclospora*, *Cystoisospora*) are major global burdens of human disease (Pierce and Kirkpatrick, 2009; McCarty et al., 2014; Checkley et al., 2015). Learning and understanding the complex interactions of parasitic eukaryotes with the bacterial microbiome in humans can be challenging due to diverse diets and climatic conditions (Cooper et al., 2013; Cantacessi et al., 2014; Lee et al., 2014). We can, however, gain comparative knowledge from domestic animals such as dogs and cats, which are parasitized with an analogous array of co-evolving parasites. Dogs and cats offer the opportunity of a more defined diet and even if they live in close association with humans, they retain a much closer link with their environment through free-roaming or hunting. Furthermore, both domestic dogs and cats suffer from parasitic diseases such as giardiasis, coccidiosis and hookworm infection (Kalkofen, 1987; Litster et al., 2014; Tysnes et al., 2014). In fact, *Giardia* is currently the most common cause of parasitic disease in domestic dogs and cats, closely followed by hookworms and coccidia (Palmer et al., 2008; Epe et al., 2010; Gates and Nolan, 2014). Hookworms are important in parasitic diseases of dogs and cats in most tropical and subtropical areas of the world (Bowman et al., 2010). Hookworms are blood sucking intestinal worms causing anaemia, and in puppies the disease caused by large numbers of *Ancylostoma caninum* are often fatal (Kalkofen, 1987). Coccidiosis in dogs and cats is caused by intracellular *Cystoisospora* spp. and manifests as a mild to severe diarrhoea (Dubey et al., 2009). Asymptomatic and clinically healthy dogs and cats shedding *Giardia*, *Cystoisospora* or hookworms occur and represent important reservoirs as direct or indirect sources of infection for other hosts (Lappin, 2005).

The aim of the current study was to discover whether the relationship between the host and *Giardia* is reflected in the faecal bacterial microbiome. Our hypothesis was that *Giardia*-positive dogs and cats have a different microbiome structure and composition from animals not infected with *Giardia*. For this purpose, we used naturally infected cohorts of dogs and cats infected not only with *Giardia*, but also with hookworms and coccidia. The use of naturally infected non-diarrhoeic dogs enabled us to investigate whether other eukaryotic parasites represented confounding factors.

2. Material and methods

2.1. Sampling

Samples from 366 free-roaming Aboriginal community dogs were collected from six remote Aboriginal communities in the Northern Territory, Western Australia, north-western New South Wales and tropical north Queensland, Australia, between 2007 and 2012 (Fig. 1, Supplementary Data S1). All communities except Collarenebri had had no veterinary intervention or worming. Ti Tree, Northern Territory, is a remote desert community in central Australia. Bidyadanga is a sub-tropical coastal community in Western Australia. Ngiui is a tropical community in the Tiwi Islands, 80 km north of Darwin, Northern Territory. Yarrabah is a coastal sub-tropical community in Queensland. Collarenebri and Goodooga are inland communities in north-western New South Wales. Dogs 0–3 months of age were considered puppies, 3–12 months of age were considered juveniles and those over

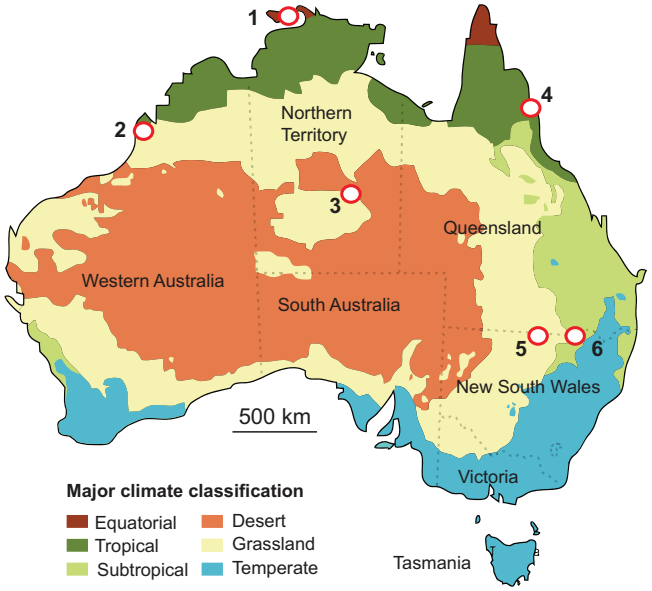

Fig. 1. Locations of Aboriginal community dog sampling sites in Australia. Nguiu, Tiwi Islands (1); Bidyadanga (2); Ti Tree (3); Yarrabah (4); Goodooga (5); and Collarenebri (6). Six major climate zones, based on the Köppen classification and native vegetation, are shown on the map (based on data from the Australian Government, Bureau of Meteorology (http://www.bom.gov.au/).

12 months were adult dogs. For each site, summary meteorological data was retrieved from the Australian Government, Bureau of Meteorology (http://www.bom.gov.au/; March 2015).

For microbiome analysis, samples from dogs and cats were selected from those submitted for routine diagnostic parasitology (Faculty of Veterinary Science, University of Sydney, Australia) (Tables 1 and 2). Dog samples (total $n = 40$) from clinically healthy animals originated from tropical north Queensland and were collected in March 2014, including free-roaming community dogs in Yarrabah ($n = 10$), pound dogs from Cairns ($n = 20$) and boarded privately owned dogs in Cairns ($n = 10$) (Supplementary Table S1). Cat samples (total $n = 22$) originated from either surrendered clinically healthy animals waiting to be re-homed by a shelter in western Sydney, New South Wales ($n = 19$) collected between 2011 and 2012, from a diarrhoeic cat suffering from chronic giardiasis from the same shelter (two consecutive samples, $n = 2$), and from a client-owned cat adopted from a breeder (CAT.C.KT, $n = 1$) (Supplementary Table S2). The diet of the pound and boarding dogs, and shelter cats was commercial pet food whereas the free-roaming community dogs were left to scavenge food scraps. The surrendered cats and boarded dogs were de-wormed and vaccinated and had received good veterinary care. The Yarrabah community dogs had not been de-wormed nor had any veterinary intervention. The pound dogs were stray dogs with minimal veterinary care; pound dogs were de-wormed on admission.

All samples were collected by veterinarians as part of veterinary health checks and provided opportunistically for this study. The cat samples were diagnostic parasitology samples not specifically collected for this study. The dog samples were collected with the approval of the University of Sydney Animal Ethics Committee, Australia. Faecal aliquots were stored at −20 °C.

2.2. Diagnostic parasitology

Faecal samples were examined microscopically at The University of Sydney for the presence of protozoal and helminth parasites by standard flotation technique as previously described

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