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Parasites in soil samples from Signy island, Antarctica

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Abstract. Studies on parasite populations in Antarctic soils are scarce and thus little is known about the threat of these parasites towards either the natural fauna or human visitors. However, human presence in Antarctica, mainly through research and tourism, keeps increasing over time, potentially exposing visitors to zoonotic infections from Antarctic wildlife and environment. Most available literature to date has focused on faecal samples from Antarctic vertebrates. Therefore, this study addressed the possible presence of parasites in Antarctic soil that may be infectious to humans. Soil samples were obtained from five locations on Signy Island (South Orkney Islands, maritime Antarctic), namely North Point and Gourlay Peninsula (penguin rookeries), Pumphouse (relic coal-powered pump house), Jane Col (barren high altitude fellfield) and Berntsen Point (low altitude vegetated fellfield close to current research station). Approximately 10% of the soil samples (14/135) from 3 out of the 5 study sites had parasites which included Diphyllobotridae spp. eggs, *Cryptosporidium* sp., an apicomplexan protozoa (gregarine), *Toxoplasma gondii*, helminths (a cestode, *Tetrabothrius* sp., and a nematode larva) and mites. The presence of parasites in the 3 sites are most likely due to the presence of animal and human activities as two of these sites are penguin rookeries (North Point and Gourlay Peninsula) while the third site (Pumphouse Lake) has human activity. While some of the parasite species found in the soil samples appear to be distinctive, there were also parasites such as *Cryptosporidium* and *Toxoplasma gondii* that have a global distribution and are potentially pathogenic.

INTRODUCTION

Parasitic infections caused by protozoa, helminths and ectoparasites are a major health problem in tropical and subtropical regions. Parasites may exert negative effects on their host's population growth, altering their competitive relationships with other species, and also leading to the emergence of new infectious diseases (Dunn, 2009). Although parasites are generally regarded as having negative impacts on ecosystem health, some do exert positive impacts on communities by acting as ecosystem engineers and modifying energy budgets and nutrient cycling, thus influencing biodiversity (Hatcher *et al.*, 2012; Wood & Johnson, 2015). Protozoal parasites that have been reported in seabirds from the Antarctica that can potentially cause disease in humans include *Cryptosporidium* sp., *Isospora* sp. and *Sarcocystis* sp. (Barbosa & Palacios, 2009) while *Cryptosporidium* sp. has also been reported in a Southern Elephant seal from the Antarctic Peninsula (Rengifo-Herrera *et al.*, 2011).

Little is known about the presence and associated threats of parasites in Antarctica because studies on parasite populations are scarce (Akhurst & Bedding, 1975; Rengifo-Herrera *et al.*, 2014; Santoro *et al.*, 2013;

Vidal *et al.*, 2012). However, human contact with and presence in Antarctica, mainly through research and tourism, continues to increase over time (Jones & Shellam, 1999; Tin *et al.*, 2009), potentially exposing visitors to zoonotic infections from Antarctic wildlife and the environment. Given the continent's isolation and the limited information available, it is not clear whether any parasite species present are closely related to those from other parts of the world, or display Antarctic-specific bio geography (Barbosa & Palacios, 2009).

Parasites may have been introduced to Antarctic wildlife through two main sources: i) via migratory species of animals such as albatrosses, polar skuas, giant petrel, elephant seals etc. that disperse over great distances between the Antarctic and other areas (Lewis *et al.*, 2006; Kopp *et al.*, 2011) or ii) through human activity such as scientific activities and tourism (Barbosa *et al.*, 2015). In the Antarctic region, there are large marine vertebrate populations (various seals, penguins, other birds) that breed and rest on land, often inevitably close to areas of human stations and activity. They produce much waste/faeces etc. which enter the local terrestrial ecosystem. If parasites have intermediate or resting stages, then they can exist in terrestrial habitats for extended periods until they are picked up by another host or a human. Some of these parasites and their vector hosts may have sufficient stress tolerance to do so, for example, the *Ixodes* ticks found in penguin colonies. Many terrestrial habitats are well buffered from the extremes of Antarctic air temperatures hence allowing both vectors and parasites to survive extreme conditions (Convey *et al.*, 2018).

To date, studies on parasites in Antarctica have largely focused on native marine vertebrate populations such as penguins, seals, seabirds and fish (Akhurst & Bedding, 1975; Rengifo-Herrera *et al.*, 2014; Santoro *et al.*, 2013; Vidal *et al.*, 2012). The British Antarctic Expedition from (1910-1913) provided the earliest records of parasitic helminthes recovered from Antarctic birds such as the emperor penguin (*Aptenodytes forsteri*), Adélie penguin (*Pygoscelis adeliae*), south polar skua (*Catharacta maccormicki*) and black-browed albatross (*Thalassarche melanophris*). They discovered three species of tapeworm, *Anomotaenia zederi*, *Tetrabothrius wrighti*, *Tetrabothrius cylindraceus* and a nematode, *Kathleena scotti* (Lieper & Atkinson, 1914). However, *Tetrabothrius* sp. which has been found in a range of dissimilar marine hosts ranging from large whales (Hermosilla *et al.*, 2015) to fish-eating birds such as albatrosses and penguins, has not been reported to have any medical importance to humans.

Most studies of the Antarctic parasite fauna have relied on faecal samples. Recent data are available from Antarctic and sub-Antarctic species including emperor and Adélie penguins, sheathbills (*Chionis alba*) and southern giant petrels (*Macronetes giganteus*) (Barbosa & Palacios, 2009). The most commonly reported parasites were cestodes (e.g. *Parorchites zederi* and *Tetrabothrius pauliani*), nematodes (*Stegophorus macronectes*), thorny-headed worms (*Corynosoma hamanni*) and trematodes (*Gymnophallus deliciosus*) (Barbosa & Palacios, 2009). A study by Santoro *et al.* (2013) of five species of fish including icefish (*Chionodraco hamatus*), dragonfish (*Cygnodraco mawsoni*), emerald rock cod (*Trematomus bernachii*), striped rock cod (*T. hansoni*) and dusty rock cod (*T. newnesi*) recorded the nematodes *Contracaecum osculatum* and *Ascarophis nototheniae*, acanthocephalans *Metacanthocephalus* spp. and *Corynosoma* spp. and the digeneans *Elythrophalloides oatesi* and *Genolinea bowersi*. Rengifo-Herera *et al.* (2014) reported high prevalence of Anisakidae nematodes and Diphyllobothridae cestodes in the Antarctic and sub-Antarctic phocids seals including Weddell (*Leptonychotes weddellii*), Leopard (*Hydrurga leptonyx*) and southern elephant (*Mirounga leonina*), while Vidal *et al.* (2012) reported two cestode species (*Parorchites zederi* and *Tetraabothrius pauliani*), one nematode species (*Stegophorus macronectus*) and immature acanthocephalans (*Corynosoma* sp.) in chinstrap penguin (*Pygoscelis Antarctica*) faecal samples.

Faecal examination is a most simple and reliable method of detecting parasites or parasitic stages, and thus most epidemiological studies on parasitic infections utilise this approach (Griffin *et al.*, 1990). In Atka Bay, Antarctica, faecal analysis of 50 individual emperor penguins using the sodium-acetate formaldehyde concentration method revealed a high prevalence of four different types of gastrointestinal parasites (Kleinertz *et al.*, 2014). Parasitological analysis of faecal samples from Antarctic gentoo penguins (*Pygoscelis papua*) recovered eggs of *Tetrabothrius* spp. by using flotation and sedimentation techniques (Fredes *et al.*, 2007). Other than faeces, Griffin *et al.* (1990) examined soil samples in Husvik, South Georgia (sub-Antarctic) and Signy Island, South Orkney Islands (maritime Antarctic), for the presence of insect parasitic nematodes of the families Heterorhabditidae and Steinernematidae by baiting the samples with live insects, but failed to demonstrate their presence in Antarctic soil. Recognising the lack of studies on soil contaminated with parasites in Antarctica, the present study examined soil samples recently obtained from Signy Island to increase knowledge of Antarctic parasite diversity, and their potential impact on both Antarctic animals and human visitors.

MATERIALS AND METHODS

Study sites and sampling procedures

Samples were obtained from Signy Island, a maritime Antarctic island within the South Orkney Island archipelago, in collaboration with the British Antarctic Survey (BAS). Systematic sampling was carried out at five sites, namely North Point (S 60º 40.528', W 45º 37.398') and Gourlay Peninsula (S 60º 43.649', W 45º 34.993') (penguin rookeries), Pumphouse (S 60º 41.974', W 45º 36.725') (relic coal-powered pump house with human activity), Jane Col (S 60º 41.897', W 45º 37.739') (a pristine, barren high altitude fellfield) and Berntsen Point (S 60º 42.452', W 45º 35.636') (low altitude vegetated fellfield close to the existing research station with human activity) (Figure 1). These 5 sites were chosen to represent the distinct ecological habitats on Signy Island.

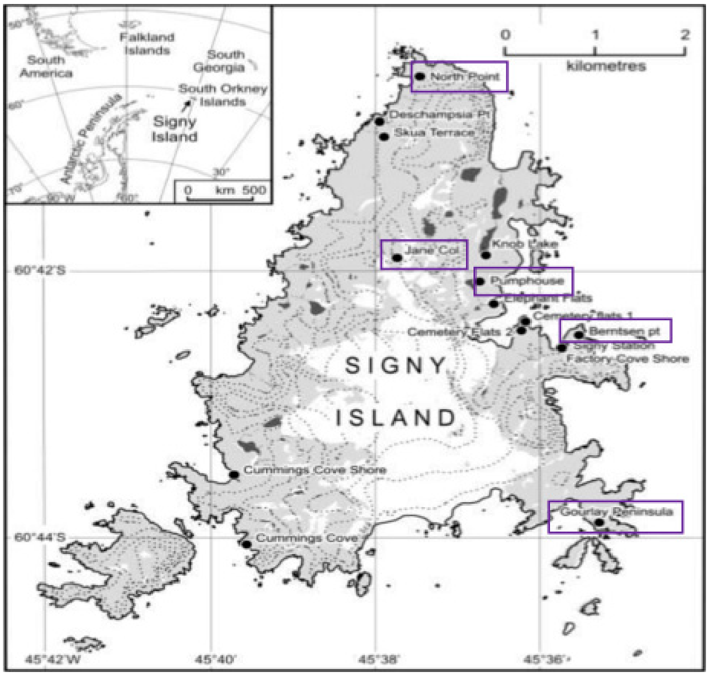

Figure 1. Map of Signy Island, Antarctica.

The sampling regime was as follows: Within a 2 km radius of each point, a station was set every 200 meters along a transect away from the point in a north-south direction. At each station, five soil samples (each separated by 50 m) were collected randomly. The soil samples were collected using sterile methodology, stored in sterile 50 mL Falcon tubes and transported back to Malaysia under refrigerated/frozen conditions and stored at -80°C for about 3 months before further analysis.

Direct Smear

Examination of soil samples for parasites was performed using direct smear. A small amount (~0.1 g) of soil sample was mixed with 50 µL of 70% ethanol to sterilize any infectious agents present in the soil sample, and then mixed with an equal amount of normal saline. The mixture was then smeared on microscopic slides pre-treated with Mayer's albumin and observed under a light microscope.

Formalin Ethyl-Acetate Sedimentation Technique

Formalin ethyl-acetate sedimentation (Cheesborough, 1998) was used to attempt to detecting small numbers of eggs, cysts and larvae. Four layers can be seen clearly in the mixture. The first layer is ethyl acetate, the second a plug of debris, the third a clear layer, and the fourth the sediment containing parasites. After discarding the unwanted solution, the sediment with parasites was withdrawn and mixed with normal saline. Then, the mixture was smeared on microscopic slides with Mayer's albumin and allowed to dry overnight at room temperature. Gomori's Trichrome staining procedure (Gomori, 1950) was carried out on the following day. The stained smears were observed under a light microscope and/or Nikon Eclipse 80i digital microscope with camera attachment at 100X, 400X and 1000X magnification.

RESULTS

Table 1 summarises the presence of endo-parasites and ectoparasites in samples from the five sampling sites. At North Point, 4/ 35 samples (11.4%) contained parasites; three samples were positive for Diphyllobotridae spp. eggs (Figure 2-A) while one sample contained a mite (Figure 2-B) which could not be identified given its poor condition. At Pumphouse, 3/35 of the samples (8.6%) were also positive for Diphyllobotridae spp. eggs. All samples from Jane Col and Berntsen Point were negative for both endo- and ectoparasites. At Gourlay Peninsula, 7/35 soil samples contained one or more parasites. These included one intestinal protozoa (*Cryptosporidium* sp.), one apicomplexan protozoa (gregarine), one

Table 1. Parasites present in soil samples obtained from the five sampling sites on Signy Island

Site	Number and % of positive soil samples	Parasites (No. of soil samples)
North Point	4/35 (11.4%)	Diphyllobotridae spp. (3) Mite (1)
Jane Col	0/35 (0%)	–
Pumphouse Lake	3/35 (8.6%)	Diphyllobotridae spp. (3)
Berntsen Point	0/35 (0%)	–
Gourlay Peninsula	7/35 (20%)	*Cryptosporidium* spp. (2) Gregarine (3) *Toxoplasma gondii* (3) *Tetrabothrius* spp. (2) Nematode larva (1) Mite (2)

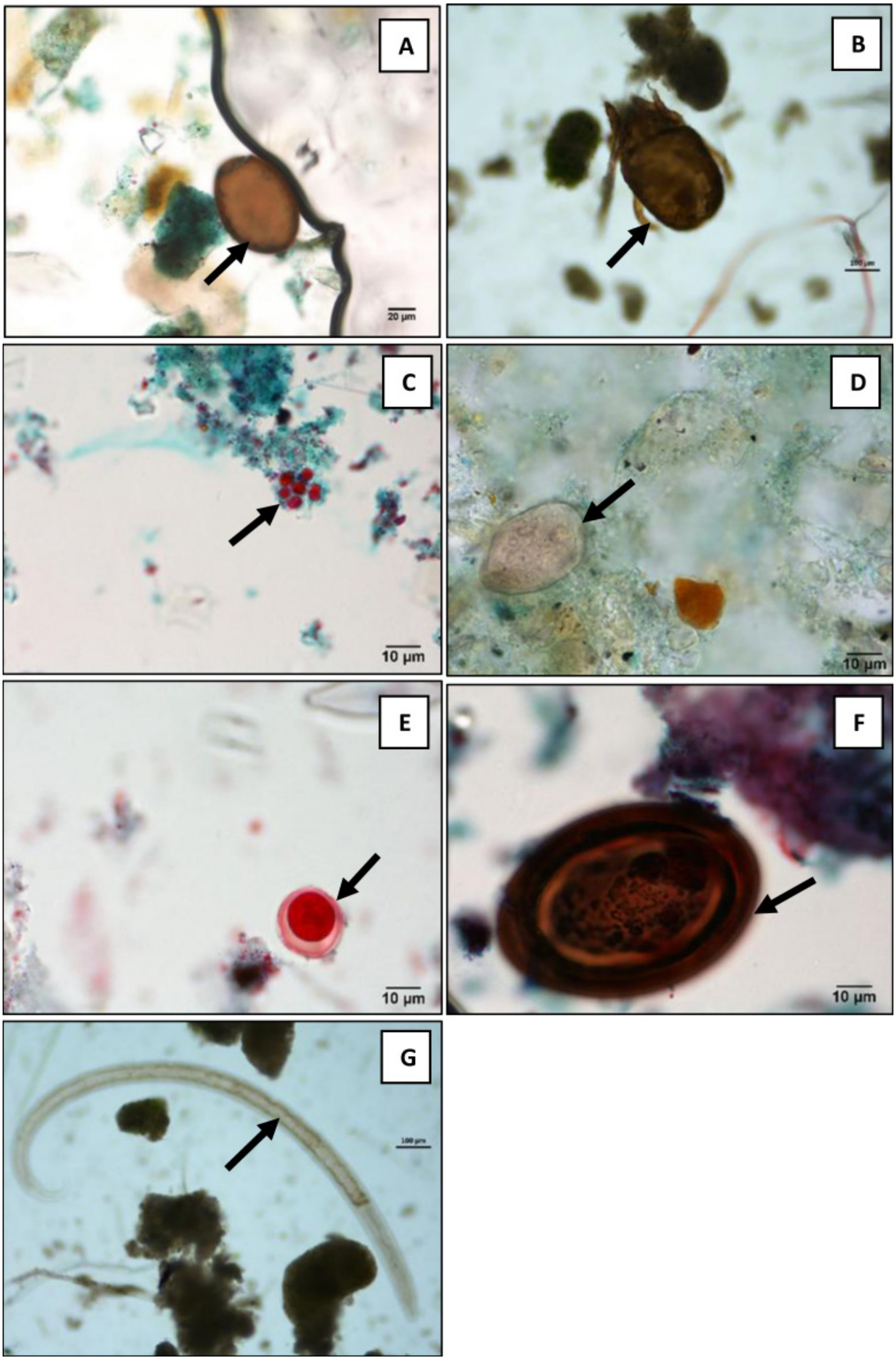

Figure 2. Protozoa and helminths eggs observed in the soil samples. A: Diphyllobotridae spp.; B: Mite; C: *Cryptosporidium* spp. oocyst; D: Gregarine; E: *Toxoplasma gondii* oocyst; F: *Tetrabothrius* spp. egg; G: Nematode larva.

tissue protozoa (*Toxoplasma gondii*), two helminths (a cestode, *Tetrabothrius* sp., and a nematode larva) and ectoparasites (mites).

Cryptosporidium spp. oocysts with size approximately 4-6 µm were identified in two soil samples (Figure 2-C) while a gregarine, a fish parasite (approximately 20 µm in length), was also found in three samples (Figure 2-D). *Toxoplasma gondii* oocysts (Figure 2-E) were observed in three soil samples. In addition, *Tetrabothrius* spp. eggs (Figure 2-F) approximately 80 µm in length were found in two soil samples while two nematode larvae (Figure 2-G) were found in one soil sample. Mites were observed in the direct smear of two soil samples.

DISCUSSION

The findings of this study showed that only 5 species of endoparasites were found in the soil samples collected from Signy Island with an overall prevalence of about 10%. Soil samples from other studies in the tropics have shown higher prevalence rates for soil transmitted parasites. For example, the prevalence for *Toxocara* ranged from 50 - 97.5% is soil samples collected from Malaysia, Japan and Greece (Loh & Israf, 1998; Zibaei & Uga, 2008; Himonas *et al.*, 1992). In terms of species richness, several studies in tropical and sub-tropical regions have reported the presence of a number of helminth and protozoan parasites in soil samples that are potentially harmful to humans. These include *Ascaris*, *Ancylostoma*, *Trichuris* (Noor Azian *et al.*, 2008) as well as *Cryptosporidium*, *Giardia intestinalis*, *Entamoeba histolytica* and *Toxoplama* (Kafle *et al.*, 2014, Olsen *et al.*, 1999). This low parasite species richness at Signy Island is believed to be associated with the highly specific diet, also known as the stenophagic diet, of the host animals (Vidal *et al.*, 2012). Invertebrate fauna present at North Point and Pumphouse Lake included southern elephant seal, Adélie penguin, chinstrap penguin and gentoo penguin (Chong *et al.*, 2009), species that typically feed on silverfish (*Pleuragramma antarcticum*) and crustaceans (e.g. *Euphausia superba*) (Santoro *et al.*, 2013; Kleinertz *et al.*, 2014), which are possible primary or secondary hosts of parasites. Diphyllobothridae spp. are commonly found in various species of Antarctic bony fish secondary hosts, and this family normally infects crustaceans as primary host. Various species of bird or mammal are the definitive hosts. Diphyllobothridae spp. eggs were the most common parasite detected in the soil samples in this study.

Cryptosporidium spp. has been reported in Antarctic mammals including seals and whales (e.g. Rengifo-Herrera, 2011). The cyst of *Toxoplasma gondii*, commonly known from the intestine of domestic cats, can also survive in seawater at 4°C and lives within harsh environments that experience freezing temperatures (Dubey *et al.*, 1970; Lindsay *et al.*, 2003). *Toxoplasma* antibodies have been reported in Antarctic pinnipeds (seals) (Jensen *et al.*, 2012). *Tetrabothrius* spp. eggs were found in soil samples collected from Gourlay Peninsula in this study and this parasite has been reported in other studies conducted in Antarctica (Cilecka *et al.*, 1992; Hoberg, 1987; Vidal *et al.*, 2012). Both *Toxoplasma* and *Cryptosporidium* have a global distribution and are known to cause disease in humans and therefore may pose a health risk to people exposed to these parasites. The presence of gregarine parasites at Gourlay Peninsula is not unexpected as other studies have demonstrated their presence in the Antarctic. For instance, Takahashi *et al.* (2008) reported gregarines in Antarctic krill, the primary prey of the large penguin colonies located on the Gourlay Peninsula.

We found no evidence of parasites in soil samples from Berntsen Point and Jane Col. This may be a function of the limited development of soil in the maritime Antarctic and the extreme environmental conditions, with soil experiencing repeated freezing as well as periods of desiccation, conditions unfavourable for survival of most parasites. In addition, Jane Col is a pristine site with no animal or human activity. The typically loose and unconsolidated structure of the soil combined with very low levels of carbon and nitrogen and low pH buffering capacity may also have contributed to the failure to recover parasites from some locations (Fisher, 2014). The low prevalence of parasites found in this study may also be a function of the limited amount of soil sample available for examination, with only 3 g of soil being used for the sedimentation analyses. Other soil parasite studies have used larger amounts of soil ranging from 20-500 g (Ghashghaei *et al.*, 2016; Nwoke *et al.*, 2013; Tavalla *et al.*, 2012). In this study, parasites were primarily found at locations on the island where there is significant marine vertebrate presence and influence, such as the penguin rookeries, indicating their transfer from faeces to the soil habitat.

While the nematode and mite specimens could not be identified with confidence, there is a possibility that the nematodes are free living native species and the mites could possibly be oribatid mites based on overviews of typical maritime Antarctic soil arthropods and nematodes (Convey & Smith, 1997; Maslen & Convey, 2006).

Further and more extensive studies of different Antarctica soil types and different regions are clearly required to properly explore the parasitic fauna of this continent. As methodologies continue to develop, approaches such as PCR with specific primers, or environmental genomics may provide a better alternative to morphological approaches for more definitive identification of both protozoan and helminth parasites (Tavares *et al.*, 2011).

CONCLUSION

This study confirmed the presence of Diphyllobotridae spp., *Cryptosporidium* spp., gregarine, *Toxoplasma gondii*, *Tetrabothrius* spp., nematode larvae and mites in samples of soil from Signy Island. Two of the parasites found in one of the penguin rookeries (Gourlay Peninsula), namely *Cryptosporidium* spp. and *Toxoplasma gondii*, are known to cause disease in humans and therefore may pose a risk to human health.

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