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SHORT NOTE

Gastrointestinal helminths of King penguins (*Aptenodytes patagonicus*) at Crozet Archipelago

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Abstract Communities of helminths are known to be related to feeding behaviors of hosts. While climate change and overfishing can impact food availability for Antarctic piscivorous predators, knowledge about infectious and parasitic diseases among Antarctic species is scarce or fragmentary. We studied the helminth community of King penguins (*Aptenodytes patagonicus*) from the Crozet Archipelago, the main breeding area of the species. Based on a sample of 41 individuals found freshly dead from predation or starvation, the gastrointestinal helminth community in King penguins was composed of 1 species of cestode (*Tetrabothrius wrighti*) and 2 species of nematodes (*Tetrameres wetzeli* and *Contracaecum heardi*). Cestodes formed the core of the helminth community (97.5% of worms collected) with a prevalence of infestation of 100% and a mean intensity of 178.6 worms per host. Sources of infestation and pathologies caused by these worms are also discussed.

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Introduction

Within the context of climate changes and threatening of Antarctic animal populations by increasing fishery and tourism activities, few data are available concerning endemic diseases (Kerry et al. 1999; Barbosa and Palacios 2009). Knowledge about diseases and parasites is crucial, however, to understand an ecosystem's health particularly when it concerns isolated areas. Indeed, introduced diseases have already been shown to cause decline or extinction among endemic populations (Warner 1968). New diseases can also be contracted by changes in diet composition as well, through climate change, because infestation by gastrointestinal parasites is highly dependent on feeding habits (Lozano 1991).

Despite previous recommendations (Kerry et al. 1999), knowledge about diseases and parasites from Antarctic birds remains scarce, fragmented, and old (Barbosa and Palacios 2009; Woods et al. 2009). To date, only the nematode *Contracaecum heardi* and the cestode species *Tetrabothrius pauliani* were described in the two unique studies realized in King penguins (*Aptenodytes patagonicus*) respectively 60 and 40 years ago (Mawson 1953; Prudhoe 1969 cited by Barbosa and Palacios 2009). Also, these two reports consist of more general studies on nematodes (for the first) and cestodes (for the second), without values on infestation of birds. So, the aim of the present study is to describe for the first time the entire helminth community in adult and chick King penguins, by investigations made at Crozet Archipelago, the main breeding area of the species (Jouventin et al. 1984).

Methods

We collected 41 entire digestive tracts of King penguins found freshly dead from predation or starvation at Crozet Archipelago, Possession Island, Baie du Marin rookery (46°25′S, 51°52′E). Digestive tracts were collected from 1 adult (male) in January 2007, 1 adult (female) in October 2007, 18 chicks between August and November 2007, and 21 chicks between July and November 2008. Digestive tracts were removed and deep frozen at −20°C within 2–12 h after death, until parasitological examinations could be carried out. Gastrointestinal helminths were removed under binocular, counted, and stored in 70% ethanol. Cestodes were stained with Semichon's carmine, dehydrated in an ethanol series, and mounted in Canada balsam. Nematodes were cleared and studied in temporary mounts of lactophenol and then returned to the preservative. Species were identified under microscope and based on the study by Leiper and Atkinson (1914) and Joyeux and Baer (1954) for cestodes and on the study by Mawson (1953) and Schmidt (1965) for respectively anisakid and tetramerid nematodes. Voucher specimens of helminths were deposited in the United States National Parasite Collection, Beltsville, Maryland, USA, under accession numbers 103307–103308. Prevalence and mean intensity of infestation were defined as described in Bush et al. (1997). Mean intensities are given with their standard error.

In addition, five stomachs and 8 intestines from 8 King penguins (6 chicks, 2 adults) were also analyzed during the present study, but mean intensities of infestation were not calculated because digestive tracts were incomplete, or information about the date and condition of collection was absent. For those birds, only prevalence was given.

The study was approved by the French Ethic Committee of the Institut Polaire Paul-Emile Victor (IPEV) and by the Polar Environment Committee of the Terres Australes et Antarctiques Françaises (TAAF).

Results and discussion

All King penguins examined were infected by at least one gastrointestinal helminth species. Three helminth species were identified: 1 cestode species (*Tetrabothrius wrighti*) in the small intestine and 2 nematode species (*Contracaecum heardi* and *Tetrameres wetzeli*) in the esophagus and stomach. This low gastrointestinal helminth species richness in King penguins is in accordance with those reported in other penguin species (Obendorf and McColl 1980; Ranum and Wharton 1996; Pazos et al. 2003; Fredes et al. 2007).

Tetrabothrius wrighti was identified by the shape and size of the scolex, the number of testes per proglottid (11–12) (Fig. 1), and the morphology of the genital atrium. *Contracaecum heardi* is characterized by the presence of 2 double rows of preanal papillae in the males rather than the 2 single rows found in most *Contracaecum* spp. *Tetrameres wetzeli* males were identified by the absence of spines on the body, the lengths of the spicules (2,355 and 225 microns), the distinctive shape of the short right spicule, and the presence of lateral alae on the tail (Fig. 2).

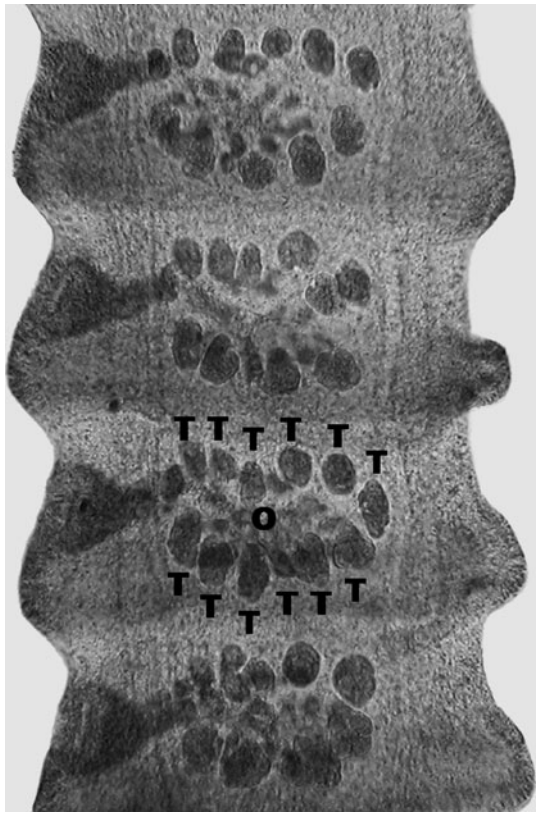

Fig. 1 Section of mature strobila of *Tetrabothrius wrighti* found in the intestine of the King penguin at Crozet Archipelago showing 10–12 testes (*T*) in each segment surrounding a central ovary (*O*)

The helminth community of King penguins was largely dominated by *Tetrabothrius wrighti* that represented 97.5% of the total number of helminths ($n = 7{,}505$, Fig. 1) while we did not find *T. pauliani* mentioned in King penguins previously (Prudhoe 1969 cited by Barbosa and Palacios 2009). *Tetrabothrius wrighti* was characterized by a high prevalence (100%) and mean intensity (178.6 ± 60.4 cestodes per host, range 5–2,068) of infestation. This cestode was also found in 6 out of 8 other intestines examined during the present study (see Methods). The infestation ranged between 5 and 1,355 for the 39 chicks and was 447 and 2,068 for the two adult birds, showing that both chicks and adults can be heavily infested by this cestode. Infestation by the genus *Tetrabothrius* differ among penguin species with prevalence ranging from 12 to 89% and mean intensities varying from 24 cestodes per host up to "enormous numbers", sometimes completely occluding the duodenum

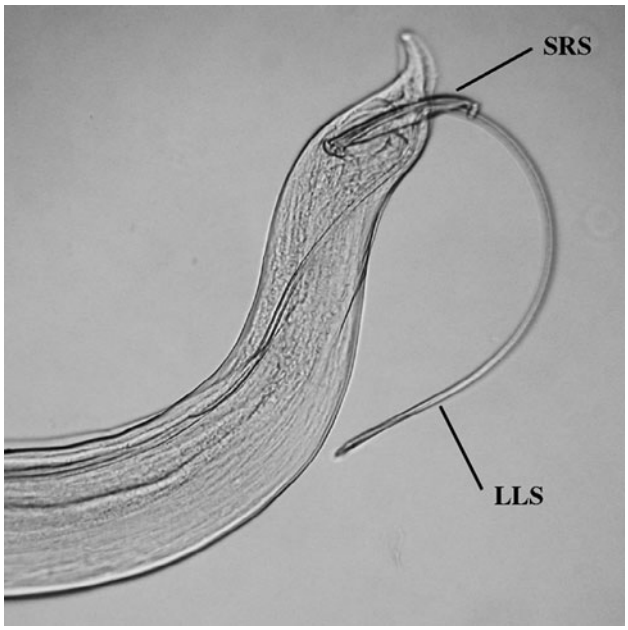

Fig. 2 Posterior end of male of *Tetrameres wetzeli* found in the stomach of the King penguin at Crozet Archipelago showing unequal copulatory spicules (*SRS*: short right spicule, *LLS*: long left spicule)

(Obendorf and McColl 1980; Andersen and Lysfjord 1982; Pazos et al. 2003). This cestode was also reported in Adelie (*Pygoscelis adeliae*) (Leiper and Atkinson 1914) and Emperor penguins (*Aptenodyptes forsteri*) (Prudhoe 1969 cited by Barbosa and Palacios 2009). It is interesting to note that life cycles of *Tetrabothrius* spp. are still unknown, but some authors suggest that fish can serve as intermediate hosts (Ellis and Williams 1978, Hoberg 1987, Mallory et al. 2007). Myctophid fish form the main prey of King penguins at Crozet Archipelago throughout the year (Cherel and Ridoux 1992, Cherel et al. 1993). In spring, the period during which most of our birds were collected, myctophid fish constitute 97% of the number of prey eaten. More precisely, the two fish species *Krefftichthys anderssoni* and *Protomyctophum tenisoni* form respectively 42 and 46% of the total number of prey consumed by birds (Cherel et al. 1993). Regarding the high degree of infestation by cestodes noted in King penguins from Crozet Archipelago, these two fish species could be the main sources of infective larvae of *T. wrighti* occurring within this subantarctic area.

In contrast, values of prevalence and mean intensity of infestation by nematode species were much lower with respectively 14.6 and 4.5 ± 2.7 (range 1–18) for *Contracaecum heardi* and 41.5% and 9.2 ± 6.3 nematodes per host (range 1–110) for *Tetrameres wetzeli*. None of the 5 supplementary stomachs examined during the present study contained these two nematode species. In other penguin species, prevalence and mean intensity of infestation by *Contracaecum* were respectively between 12 and 95% and from 1 to 300 nematodes per host (Obendorf and McColl 1980; Ranum and Wharton 1996; Garbin et al. 2007). At Crozet Archipelago, the infestation by *Contracaecum heardi* ranged from 1 to 18 nematodes per chick. Only one of the two adult penguins harbored one *Contracaecum heardi*. The second intermediate hosts for *Contracaecum* spp. are commonly fish species (Anderson 2000). Myctophid fish could thus be the main sources of the infective stage of *Contracaecum* for King penguins even if Notothenid fish could also be the intermediate hosts for *Contracaecum* larvae in subantarctic areas (Mawson 1953). *Contracaecum heardi* has been found in the two studies realized in King penguins at Heard and Macquarie islands (Mawson, 1953) and at Crozet Archipelago (this work).

The present study in the Crozet Archipelago reports *Tetrameres wetzeli* (Fig. 2) for the first time in the King penguin and in the Crozet Archipelago. While the two adults were not infested, infestation of chicks by *Tetrameres wetzeli* ranged from 1 to 110 nematodes per host. *Tetrameres wetzeli* was only previously described in a Rock-hopper penguin (*Eudyptes chrysocome*) collected in Kerguelen Islands and died in a zoo in Germany (Schmidt 1965). Male and female *T. wetzeli* live in a common cyst included in the wall of the proventriculus, so males might occur accidentally either in the lumen of the proventriculus or in the body cavity when the cyst was ruptured (Schmidt 1965). This could explain why only males were found during our parasitic examination of digestive tracts and it suggests an underestimation of the infestation by this nematode species in King penguins from Crozet Archipelago. *Tetrameres* spp. are common nematodes in columbiform and galliform birds in which female worms are known to ingest blood and to cause compression necrosis and fibroplasias of the glandular epithelial cells of the proventriculus (e.g., Fink et al. 2005; Martínez-Carrasco et al. 2009). Prevalence of infestation is age-related with a higher infestation in young birds (Fink et al. 2005). Similar consequences could occur in King penguins, but further studies should be conducted to determine possible sources of infestation by *T. wetzeli* and if this nematode could be a source of mortality in this host, in spite of the low prevalence we observed in the present study. Because *T. wetzeli* lives in the wall of the proventriculus, the prevalence of this nematode species may have been underestimated with respect to the global population of King penguins.

However, it should be considered that our results may not be totally representative of the infestation of the whole population of King penguins. Firstly, sampled birds were found dead by either starvation or predation. It could not be excluded that the both causes of death were due to a particularly high infestation. Secondly, the sample was mainly composed of chicks that may not have enough time to acquire the entire helminth community of King penguins from Crozet Archipelago. Thirdly, adult birds may harbor a

more important diversity and number of parasites by cumulating contamination during the whole year (for instance during migration in other areas). Also, the seasonal change in diet composition of King penguins (Cherel et al. 1993) may induce changes in the helminth community composition of birds at Crozet Archipelago. Furthermore, it cannot be excluded that the diet of adult and young birds differs during chick rearing. It will therefore be necessary in future studies to take into account all these variables and especially to have a greater number of adult penguins.

To conclude, our study presents a new species of *Tetrabothrius* (*T. wrighti* vs. *T. pauliani*) and of nematodes (*Tetrameres wetzeli*) in King penguins. We report here for the second time *Contracaecum heardi* in King penguins. Climate change, overfishing, sampling effort of helminths, diet of birds, and geographical range of studies could be suggested to explain these differences between helminth community compositions among King penguin populations. We, therefore, cannot conclude that a change has occurred in the helminth community during recent decades. However, the exposure of King penguins to a wider diversity of helminths may be indicated. Our study provides a first reference base for the gastrointestinal helminth community in King penguins at Crozet Archipelago.

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