Experimental assessment of the effects of gastrointestinal parasites on offspring quality in chinstrap penguins (*Pygoscelis antarctica*)

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SUMMARY

Parasites reduce host fitness and consequently impose strong selection pressures on their hosts. It has been hypothesized that parasites are scarcer and their overall effect on hosts is weaker at higher latitudes. Although Antarctic birds have relatively low numbers of parasites, their effect on host fitness has rarely been investigated. The effect of helminth parasitism on growth rate was experimentally studied in chinstrap penguin (*Pygoscelis antarctica*) nestlings. In a total of 22 two-nestling broods, 1 nestling was treated with anthelminthics (for cestodes and nematodes) while its sibling was left as a control. Increased growth rate was predicted in de-wormed nestlings compared to their siblings. As expected, 15 days after treatment, the experimental nestlings had increased body mass more than their siblings. These results show a non-negligible negative effect of helminth parasites on nestling body condition that would presumably affect future survival and thus fitness, and it has been suggested there is a strong relationship between body mass and mortality in chinstrap penguins.

Key words: anthelminthics, Antarctica, chinstrap penguin, helminths, levamisole, parasite effects, praziquantel.

INTRODUCTION

Parasites constitute a large fraction of all living beings. At least at some time during their life, individuals of most organisms are infected with parasites, which exploit their host for the resources required for their reproduction and survival (Price, 1980). These resources could have been used by the host to increase its own fitness, and many experimental studies have demonstrated that it is significantly reduced by parasitism (see reviews by Lehmann, 1993 and Møller, 1997). The costs of parasitism can be direct, through loss of resources drawn from the host by the parasites, or indirect through the resources spent by the host on parasite defence (de Lope *et al*. 1998). In recent years, ecological studies have emphasized the importance of parasites and disease as selection pressures affecting fitness in terms of condition, survival prospects or reproductive success (Loye and Zuk, 1991; Clayton and Moore, 1997; Møller, 1997). Parasitism also affects predation, because predators are known to preferentially select infected prey (Schaller, 1972; Moore, 2002), as infected hosts are more vulnerable (Hudson *et al*. 1992; Murray *et al*. 1997; Packer *et al*. 2003). Parasites could also induce changes in host behaviour (Dobson, 1988; Jog and Watve, 2005).

Knowledge about avian-parasite interaction is biased towards certain kinds of parasites. While there is wide information about effects of ectoparasites and haemoparasites in their avian hosts, little is known about interaction with other kinds, such as gastrointestinal parasites. Reduced metabolizable energy available to the infected animal and reduced growth rates have often been associated with gastrointestinal worm infections in domestic animals (see MacRae, 1993). However, the consequences of gastrointestinal parasitic infection in wild animals have been studied little, despite the possibility that even relatively small effects on energy acquisition could significantly reduce survival and fecundity (Delahay *et al*. 1994). The few studies in this regard show that such parasites may cause important costs to their hosts. For example, Dobson and Hudson (1995) showed that gastrointestinal parasites could increase vulnerability to predators. Langston and Hillgarth (1995) demonstrated that birds with higher infections of nematodes compromised their future reproduction because of an incomplete moult. It has also been shown that intestinal parasite loads affect offspring quality, such as the case of significantly higher chick survival in red grouse (*Lagopus lagopus*) in areas treated with anthelminthics (Newborn and Foster, 2002).

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Penguins offer a good opportunity to test for the effect of gastrointestinal parasites because they would be more important in Antarctic fauna than other parasites such as arthropods or pathogens which are less prevalent (Barbosa and Palacios, 2009 and references therein; Barbosa *et al*. 2011) or haemoparasites which are absent (Merino *et al*. 1997). It is generally thought that parasitism has little impact in polar regions, because parasites seem to be scarcer and their overall effect on hosts is weaker at higher latitudes (Barbosa and Palacios, 2009). Our study model, the chinstrap penguin (*Pygoscelis antarctica*), is infested by at least 4 species of gastrointestinal parasites (see Barbosa and Palacios 2009; Vidal *et al*., unpublished data). Considering all the parasites present in this species, parasite prevalence and intensity are on average 1·6- and 30-fold lower, respectively, than in other non-Antarctic penguins (Barbosa *et al*. *unpublished*).

Our aim is to study the impact of intestinal parasites experimentally in chicks of a species with a low diversity of parasites, such as the chinstrap penguin (*Pygoscelis antarctica*), so that the effects of a specific group of parasites could be detected. Considering that gastrointestinal parasites remove resources from the hosts (Price, 1980), chicks with a heavy parasite load should grow less than individuals with a lighter load. In other words, we predict that chicks treated with antiparasitics should grow more than chicks treated with a placebo. To test this prediction we carried out an experiment in which we administered anthelminthic drugs to remove the parasites present in the chicks and compared their growth with its siblings treated with the placebo. This approach allowed us to control for differences due to parental quality among nests. In chinstrap penguins, a strongly biased mortality operates soon after independence, with higher mortality in lighter chicks (Moreno *et al*. 1999). Therefore, reduction in individual offspring body mass by parasitism could affect its survival, and can therefore be used as a proxy for fitness.

MATERIALS AND METHODS

Study area and species

The study was conducted at the Vapour Col chinstrap penguin rookery on the west side of Deception Island, South Shetlands (63°00′S; 60°40′W) during the breeding season of 2005–2006, in January and February of 2006. The chinstrap penguin breeds in large colonies in the area of the South Shetlands and the Scotia Sea (Williams, 1995). The modal clutch is 2 eggs and the most frequent broods consist of 2 chicks. After a 1-month incubation period, when both parents take turns in covering the eggs, chicks remain in the nest for approximately another month while being protected by one of the parents, that is, the so-called guard phase (Lishman, 1985; Moreno *et al*. 1994; Viñuela *et al*. 1996). After that, the chicks are left unguarded by their parents for most of the day in the crèche or post-guard stage (Williams, 1995; Viñuela *et al*. 1996; Wilson, 2009). Parents continue feeding their own chicks, but neither parent remains with the chick very long after feeding. At 55 days of age, nestlings leave the colonies for the sea (Williams, 1995).

Sampling

Twenty-two nests in one of the largest colonies (600 pairs) with chicks in the guard phase were randomly selected for similar-sized chicks. Only nests with 2 chicks were included. Nests were marked with numbered sticks. In each nest, 1 chick was selected as experimental (to which the treatment was applied) and the remaining chick was the control. When chicks were of a similar size, selection was at random. However, if any small differences in size were perceived, larger and smaller were alternated as the experimental chick in successive nests. Chicks were removed from the nest (one at a time) and individually marked on the flippers with insulation tape. Then they were weighed (to the nearest 50 g with a spring balance), and flipper length (to the nearest 0·1 cm with a metal ruler), and beak length (to the nearest 0·01 mm with a digital calliper) were measured. A feces sample was taken to determine the parasite load. After handling, the chick was replaced in the nest and the adult immediately resumed care of it.

Experimental procedure

The experimental chick was administered anthelminthics by intramuscular injection, Tetravermiven (Levamisole, Iven laboratories) for nematodes and Droncit (Praziquantel, Bayer Laboratories) for cestodes, to eliminate or reduce the gastrointestinal parasite load. Levamisole is widely used as an anthelminthic in cattle, sheep, goats, swine, poultry and birds in zoos. It is effective against lungworms and gastrointestinal nematodes (El-Kholy and Kemppainen, 2005), and has been used in several experiments for assessing the effects of gastrointestinal parasites (Martinez-Padilla *et al*. 2007; Mougeot *et al*. 2010). Praziquantel has been used effectively for cestodes (Thomas and Gonert, 1977) in avian hosts (Jones *et al*. 1996). Following the manufacturer's instructions, we used 0·06 ml/kg of levamisole and 0·17 ml/kg of praziquantel. The control chick was injected with the volume of phosphate-buffered saline (PBS) corresponding to the anthelminthic drugs in relation to its body mass. Fifteen days later, the chicks were recaptured, weighed and measured again. We chose this time lapse on the basis of the reported time when effects of the anthelminthics are at a maximum

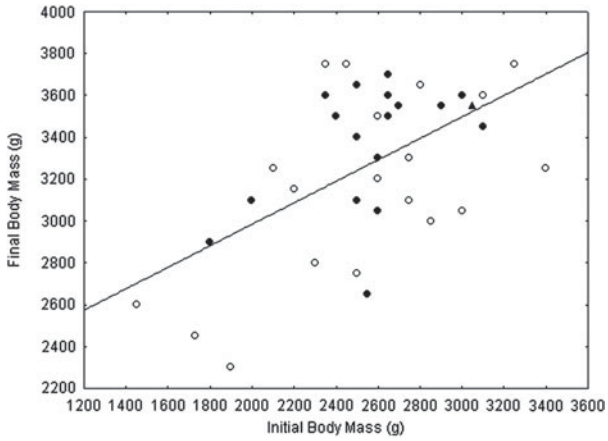

Fig. 1. Plot of final body mass against initial body mass. Black circles are control chicks and white circles are experimental chicks. Black triangle shows 2 chicks one experimental and one control.

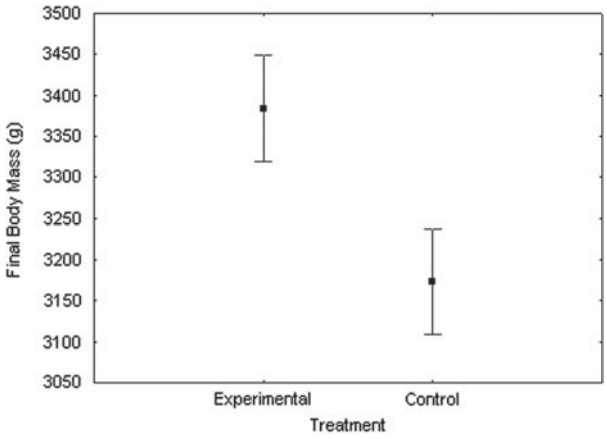

Fig. 2. Differences in final body mass between control and experimental chicks after 15 days of anthelminthic treatment. Bars denote standard errors.

(Rojas and Montero, 1982). Samples of feces were also taken. The final sample was 19 nests due to predation by skuas or lost markers.

Statistics

All the variables were normally distributed. Initial body mass, flipper and beak measurements were highly variable among individuals (body mass, mean ± standard error 2521·84 ± 92·42, range = 1450–3400 g; flipper length, mean = 179·9 ± 1·4, range = 150–196 mm; beak length, mean = 31·70 ± 0·49, range = 25·6–38·9 mm) and were significantly related with the final body mass, flipper and beak measurements ($r = 0{\cdot}57$, $P = 0{\cdot}0001$ (Fig. 1); $r = 0{\cdot}58$, $P = 0{\cdot}0001$; $r = 0{\cdot}66$, $P < 0{\cdot}0001$, respectively). Individuals were therefore expected to grow at different rates depending on their original body mass and measurements. In fact, we found a negative relationship between the increase in body mass (differences between the first and the fifteenth day) and the initial body mass ($r = -0{\cdot}55$, $P < 0{\cdot}001$), a negative relationship between the increase in flipper length (differences between the first and the fifteenth day) and the initial flipper length ($r = -0{\cdot}7$, $P < 0{\cdot}001$) and a negative relationship between the increase in beak length (differences between the first and the fifteenth day) and the initial beak length ($r = -0{\cdot}51$, $P = 0{\cdot}0013$). We then used general linear mixed model (GLMM) analyses (Lair and Ware, 1982) with final body mass, beak length and flipper length as the response variables, and initial body mass, beak length and flipper length, treatment (experimental or control), interaction between initial measurements and treatment as fixed effects, and nest membership as a random effect. Statistical analyses were performed with Statistica 9.0 (Statsoft Inc.).

We estimated the impact of parasites on chick growth as the difference in body mass or morphological measurements between experimental and control chicks from the beginning of the experiment to the final body mass or final morphological measurements 15 days later.

RESULTS

There were no differences between experimental and control chicks before the treatment (body mass: $t_{18} = -0{\cdot}46$, $P = 0{\cdot}65$; flipper length: $t_{18} = -0{\cdot}56$, $P = 0{\cdot}62$; beak length: $t_{18} = 0{\cdot}49$, $P = 0{\cdot}68$).

As expected, when experimental chicks were compared with their siblings injected with PBS (controls), the treated chicks were found to have significantly increased body mass over the controls ($F_{1,16} = 6{\cdot}18$, $P = 0{\cdot}024$; Fig. 2). We also found significant differences among nests ($F_{18,16} = 2{\cdot}39$, $P = 0{\cdot}04$). The results also show a marginal significant relationship between initial body mass and final body mass ($F_{1,16} = 3{\cdot}88$, $P = 0{\cdot}06$), but the interaction between initial body mass and treatment was not significant ($F_{1,16} = 0{\cdot}44$, $P = 0{\cdot}51$). We did not find significant differences either in the increase in flipper length ($F_{1,16} = 0{\cdot}01$, $P = 0{\cdot}92$), or in the increase in beak length ($F_{1,16} = 0{\cdot}08$, $P = 0{\cdot}77$). We did find significant differences among nests (flipper length, $F_{18,16} = 4{\cdot}81$, $P = 0{\cdot}001$; beak length, $F_{18,16} = 2{\cdot}64$, $P = 0{\cdot}02$). Relationships between initial and final measurements was only significant for beak length ($F_{1,16} = 7{\cdot}85$, $P = 0{\cdot}001$). Finally, the interactions between initial measurements and the treatment were not significant flipper length × treatment, $F_{1,16} = 2{\cdot}20$, $P = 0{\cdot}15$; beak length × treatment, $F_{1,16} = 0{\cdot}98$, $P = 0{\cdot}98$). Unfortunately, we were unable to determine the actual effect of the anthelminthic drugs on the parasite burden, even though we had feces samples from before and after treatment. Previous studies on gastrointestinal parasites in the same population based on coprological techniques in dead individuals have shown a high

number of false negatives (Vidal *et al*. *unpublished observations*). In those cases, no eggs were found in the feces of individuals that were found to actually be highly parasitized when necropsies were carried out. This means that coprology could clearly underestimate the presence of parasites in our samples, and therefore we were unable to analyse the differences between experimental and control birds before and after the treatment. However, we did find a high prevalence of gastrointestinal parasites (around 91%, Vidal *et al*. *unpublished observations*), in necropsies of dead chicks collected in the same rookery. Therefore, it is highly probable that most of the birds used in the experiment were parasitized, and that the changes in mass detected could be attributed to an effect of the treatment.

Estimation of the impact of parasites on the significantly affected variable, i.e., body mass, showed a reduction of 6% of the final body mass.

DISCUSSION

We investigated the costs of gastrointestinal parasites to a bird species, the chinstrap penguin, which harbours 4 species of helminth parasites (*Stegophorus* sp., *Parorchites* sp., *Tetrabothrius* sp. and *Corynosoma* sp. (Vidal *et al*. *unpublished observations*)). The absence of blood parasites has been reported in this species (Merino *et al*. 1997; Barbosa and Palacios, 2009), as well as the presence of bacteria, protozoa and ectoparasites, but in low prevalences (Barbosa and Palacios, 2009; Barbosa *et al*. 2011). We used an experimental approach, treating chicks with anthelminthic drugs and comparing them with their siblings injected with PBS as a control. This experimental procedure allowed us to control for differences in the quality of parents attending the nests. Our results show that chicks treated with anti-parasitic drugs increased their body mass more than control birds, supporting our prediction that a gastrointestinal parasite load would deteriorate body condition. Negative impact can be estimated as an average reduction of 200 g in 15 days, which represents 6% of body mass. There are some examples of severe negative effects of parasites on host fitness derived from studies in which the level of parasite infestation has been experimentally changed. For example, fumigation of the nests of cliff swallows (*Hirundo pyrrhonota*) with a weak pesticide reduced the level of infestation with ectoparasites, and this then resulted in an improvement in the quality and quantity of offspring produced by the avian host (Loye and Carrol, 1991). Hannsen *et al*. (2003) found that recruitment of treated non-reproductive females of the common eider (*Somateria mollisima*) was higher in the population the following year. Martinez-Padilla *et al*. (2007) and Mougeot *et al*. (2010) found that individuals dosed with levamisole reduced the presence of a nematode parasite increasing plasma carotenoid concentration and comb redness, and reducing oxidative stress. Furthermore, it is well established that livestock animals fed with anti-parasitic drugs, or reared in germ-free environments, grow more rapidly and larger (Lochmiller and Deerenberg, 2000).

Reduction of parasite levels has at least 4 important implications for the host (de Lope *et al*. 1998). First, reduced parasite intensity means fewer resources drained from the host, because parasites may affect components of the host's energy budget, leading to an energy imbalance. This would reduce the energy available to be allocated to other demands such as activity, thermoregulation or reproduction (Deerenberg *et al*. 1997; Svensson *et al*. 1998; Greenman *et al*. 2005). Second, a reduction in the parasite load could improve host digestive efficiency (see Holmes, 1987). Third, reduced parasite intensity would decrease any toxic effects of the parasites experienced by the host (Holmes, 1987). Finally, a consequence, which may be termed an indirect effect, is that by reducing parasite numbers, immune reactions would be down-regulated. As this reduces immune system requirements, it also leads to resource savings (Sheldon and Verhulst, 1996).

Whatever the reason is in this case, the differential body mass increase between the treated and control individuals, seems to demonstrate a high cost of parasitism. Infection with parasites is often inferred to have significant energetic costs in birds (Bouslama *et al*. 2002; Nilsson, 2003; Møller and Saino, 2004), and intestinal parasites such as digeneans and nematodes have been found to be negatively associated with body condition in common eiders (Warrelius, 1993). There are several different mechanisms that may link parasite infection to ecological consequences for the host. The body mass and the size of offspring at fledging have been shown to be correlated with later survival in several avian species (Perrins, 1965; Garnett, 1981; Nur, 1984; Hochachka and Smith, 1991; Magrath, 1991). In our study species, Moreno *et al*. (1999) showed that a strongly biased mortality operates soon after independence in chinstrap penguins, with higher mortality in lighter chicks. Therefore, effects of parasitism on the offspring reducing individual body mass could affect their survival.

Our results can be interpreted in terms of reduced resources available through food intake in parasitized individuals. However, the mechanism explaining our results may be different if parasites affect host behaviour. Field studies have shown that dosing animals with anti-parasitic drugs can have major effects on their activities. For example, treated heifer cattle spent more time grazing and grew faster than untreated animals (Forbes *et al*. 2000), therefore treated chicks could be fed more by their parents because they beg more or look healthier.

Unfortunately, this possibility remains to be tested. However, in any case, the ultimate effect of a strong cost to fitness remains.

We did not find any differences between dosed and control chicks in flipper length or bill length. One explanation could be because the time from approximately 30 to 45 days of age, i.e., the 15 days of the experiment, was not enough to detect differences due to the slow growth rate in these variables during that period. Moreno *et al*. (1994) showed that body mass growth rate in this period is higher than flipper and bill length in the same species, which would explain why we found effects on body mass but not the other two variables.

The results also show significant differences depending on the nest, which can be explained by the heterogeneity of the sample in spite of the size selection of chicks as the study was carried out during the guard phase.

Our results then show a strong impact of gastrointestinal parasites on the chinstrap penguin in contrast to the generally accepted hypothesis that parasites have a weak effect in polar regions, which would seem not to apply. One consequence of our work is that it suggests this hypothesis should be revised, although direct comparisons with other penguin species are needed to properly test this assumption. The hypothesis that parasites have a lower impact in Antarctica has probably been inferred from 2 main points, the low number of parasite species present in Antarctic organisms (Barbosa and Palacios, 2009) and the absence of mass mortality events due to parasitism in Antarctic birds or marine mammals (Kerry and Riddle, 2009). There are several reasons explaining the high impact of gastrointestinal parasites. First, recent environmental changes, such as climate change, could mean that Antarctic penguins are now confronted with new parasites, and their immunological response is not suitable to this new situation. However, the number of parasites present in pygoscelid penguins, including chinstraps, seems not to have increased in the last 40 years (Barbosa and Palacios, 2009), precluding this explanation. Nevertheless, increased parasite prevalence or intensity due to environmental changes cannot be discarded, because there is unfortunately not enough published information to test this possibility. Second, chinstrap penguins could be more susceptible to gastrointestinal parasite infections than other non-Antarctic seabirds, or gastrointestinal parasites found in the chinstrap penguin could be more virulent than the gastrointestinal parasites found in non-Antarctic seabirds. These explanations also remain to be tested.

Finally, levamisole has immunostimulant properties, increasing the total level of immunoglobulins, enhancing resistance to some pathogenic bacteria or modulating leukocyte cytotoxic activity (Mulero *et al*. 1998; Cuesta *et al*. 2002, 2004), which could be indirectly responsible for the increase in body mass found. Unfortunately there are no data in this study showing whether or not levamisole affects the chinstrap penguin in this way. However, in another study with adult Adélie penguins (*Pygoscelis adeliae*), a closely related species also treated with this drug, we did not find any differences in immunoglobulin levels between experimental birds and controls ($t = -0{\cdot}50$, $P = 0{\cdot}61$, $n_{experimental} = 24$, $n_{control} = 21$, Barbosa *et al*. *unpublished observations*), suggesting that immunostimulation by levamisole probably did not have an important role in our results.

In summary, our results show that individuals dosed with anthelminthic drugs grew more than controls treated with a placebo, suggesting direct negative effects of gastrointestinal parasites in Antarctic penguins. This study shows that gastrointestinal parasites play an important role in the ecology of species.

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