SHORT COMMUNICATION

Avian malaria parasites (*Plasmodium* spp.) in Dunedin and on the Otago Peninsula, southern New Zealand

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Abstract: There is concern that avian malaria maybe partly responsible for fluctuations in yellow-eyed penguin (*Megadyptes antipodes*) populations in New Zealand. Recent findings, however, have provided no evidence of avian malaria parasites infecting yellow-eyed penguins on the Otago Peninsula, raising questions as to whether this area is currently free of such parasites. To test this possibility we collected blood samples from 109 individuals of five non-native bird species known to carry malarial parasites elsewhere in New Zealand. Molecular screening by polymerase chain reaction revealed 6% of the sampled birds were positive for malarial parasites, indicating that a local reservoir of infection is present. Sequence data revealed a generalist strain of *Plasmodium* is present, one that infects a number of native and non-native bird species elsewhere in the country. The absence of this generalist strain in yellow-eyed penguins, some of which were sampled during the same period as the current study, may be due to low levels of mosquito vectors of disease during the study period, low densities of non-native birds around yellow-eyed penguin colonies, or infected penguins dying before they could be sampled. Continued monitoring of mosquito populations and the factors that affect their densities should be included in the future management of native birds in this area.

Keywords: *Megadyptes antipodes*, yellow-eyed penguins

Introduction

Avian malaria, a mosquito-vectored pathogen, has been responsible for one of the most dramatic losses of avifauna in the world. After its introduction to Hawai'i over two centuries ago, avian malaria (*Plasmodium relictum*) played a key role in the extinction of almost half the endemic bird species (van Riper et al. 1986). In New Zealand, recent findings that malarial parasites are present at high prevalence in non-native birds raise the concern they may be acting as a reservoir of infection to native bird species, with some populations potentially suffering the same disease impact as that seen in Hawai'i (Tompkins & Gleeson 2006). This is particularly worrying for endangered species in small, low-density populations. Although such populations are usually not conducive to virulent pathogens, as infected animals die before the disease can be spread (McCallum & Dobson 1995), the presence of a reservoir means that population-scale impacts of disease are more likely due to spillover events from the reservoir (Daszak et al. 2000; Cleaveland et al. 2002). Such a mechanism has been implicated in a number of population crashes, including that observed in Ethiopian wolves in 1991–92, which was attributed to rabies transmitted from local domestic dogs (Sillero-Zubiri et al. 1996).

One endangered native species thought to be at risk from avian malaria is the yellow-eyed penguin (*Megadyptes antipodes*) (Duignan 2001; Alley et al. 2004), which has a known total population of only about 4000 individuals occurring around the south of New Zealand, and ongoing threats from habitat degradation and chick predation by introduced vertebrate pests. This species is classified as 'endangered' by the International Union for the Conservation of Nature and Natural Resources (IUCN) (BirdLife International 2000). Several lines of evidence suggest transmission of malarial parasites to this species may occur. First, historical observations of malarial parasites in blood smears from yellow-eyed penguins have been documented in wild birds from Foveaux Strait and Stewart Island (Fantham & Porter 1944; Laird 1950). Second, in 2001, histology on a juvenile yellow-eyed penguin found dead without gross external lesions revealed acute necrosis and inflammation of the myocardium, liver and spleen associated with intracytoplasmic *Plasmodium*-like parasites (Alley 2001). Finally, there have been several

reports of high seroprevalence to malarial antibodies in wild New Zealand penguins (Graczyk et al. 1995; McDonald 2003). With a yellow-eyed penguin population crash having occurred in the Dunedin and Otago Peninsula areas in recent years (Gill & Darby 1993), the potential role of avian malaria in these crashes is of concern.

However, despite these findings and concerns, two recent surveys for malarial parasites in yellow-eyed penguins at Boulder Beach on the Otago Peninsula using polymerase chain reaction (PCR) primers known to be sensitive to *Plasmodium* spp. revealed no evidence of malarial parasites (unpubl. data). Hence, the current study was undertaken to investigate whether this finding was simply due to Dunedin and the Otago Peninsula being currently free of malarial parasites, by analysing blood samples from local non-native bird species known to harbour malarial parasites elsewhere in New Zealand.

Methods

Birds were caught using mist nets at dusk and dawn over 33 days from November 2006 to March 2007 at four locations at Dunedin and the Otago Peninsula (Fig. 1). The five locally most abundant non-native bird species were sampled: blackbird (*Turdus merula*); song thrush (*Turdus philomelos*); starling (*Sturnus vulgaris*); chaffinch (*Fringilla coelebs*) and house sparrow (*Passer domesticus*). Malarial parasites were detected in all five of these species in a previous national survey (Tompkins & Gleeson 2006). One hundred microlitres of blood was collected by brachial puncture and stored in 200 µl of Longmire's Lysis buffer (Longmire et al. 1988) until molecular analysis. Sampled birds were marked with white marker on the tail feathers before release to prevent resampling.

DNA was extracted and purified using a QIAamp DNA mini kit (QIAGEN, Hilden, Germany), following the manufacturer's protocol for blood samples. For every extraction run of 20 samples, four were selected randomly and visualised on gels to confirm that genomic DNA was present (a total of 26 samples). DNA from blood samples from blackbirds stored in lysis buffer and known to contain *Plasmodium* malarial parasites was extracted for use as a positive control. A 355-base-pair fragment of the mitochondrial cytochrome-b gene was amplified using PCR and specific malarial parasite (*Plasmodium* and *Haemoproteus*) primers L15368 (5'AAA AAT ACC CTT CTA TCC AAA TCT 3') and H15730 (5'CAT CCA ATC CAT AAT AAA GCA T 3'), as described by Ricklefs et al. (2005). The PCR was carried out in 25-µl volumes containing 10× PCR buffer and 20 mM $MgCl_2$, 2 mM each of dNTPs, 0.2 mM each of the primers, 1.5 U per reaction of Faststart Taq (Roche Diagnostics, Mannheim, Germany) and 5 µl of extracted DNA. Positive and negative (water) controls were added to each PCR run. Amplification was performed using a programme of initial denaturation of 4 min at 95°C, followed by 35 cycles of 30 s at 94°C for denaturation, 60 s at 48°C, extension for 1 min 30 s at 72°C, followed by a final extension for 3 min at 72°C. Amplified products were analysed using 2% agarose gel in Tris acetate EDTA, and visualised under ultraviolet light following gel staining with Sybr-safe (Invitrogen, Carlsbad, USA). PCR products were purified

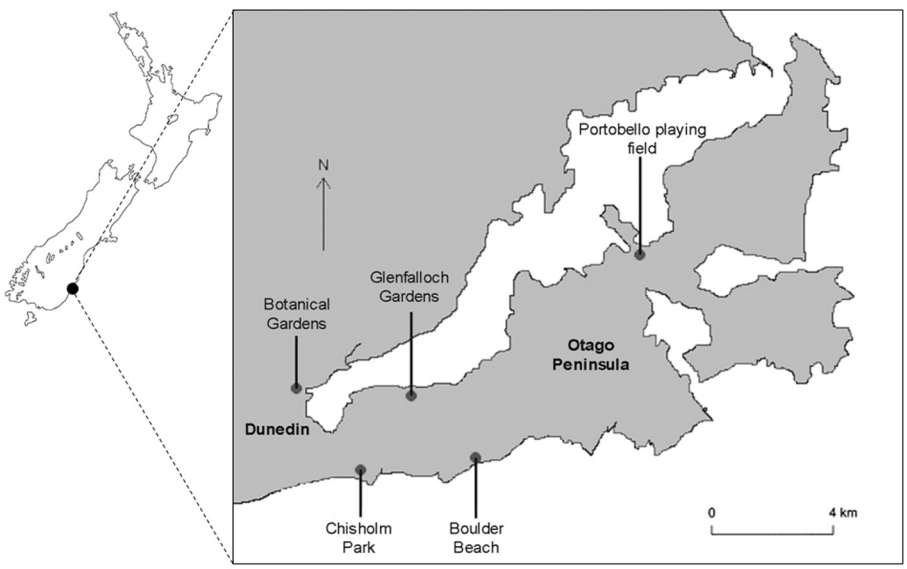

Figure 1. Map of Dunedin and the Otago Peninsula, southern New Zealand (46° S, 170° E), showing the four sampling sites and penguin colony previously sampled at Boulder Beach.

using Invitrogen Purelink (Invitrogen, Carlsbad, USA) following the manufacturer's protocol, and sequenced to confirm pathogen identity.

Results

DNA was visualized on all 26 samples randomly selected to confirm the extraction process was successful. DNA from *Plasmodium* parasites was successfully amplified from the positive controls, whereas none of the negative controls responded to the PCR. Of the 109 birds screened using PCR, 7 (6%) tested positive for the presence of the 355-base-pair cytochrome-b fragment (Table 1).

Sequence data revealed that all the amplified fragments were from *Plasmodium* and were identical. When compared with other strains found in New Zealand, sequences clustered most closely with a strain found in a number of different native and non-native birds throughout New Zealand (D. Gleeson, Landcare Research, Auckland, pers. comm.).

Discussion

Our study confirmed that non-native birds in Dunedin and the Otago Peninsula do harbour a generalist strain of malarial parasite, and suggests that parasite prevalence may be higher in birds from Dunedin city centre (Botanical Gardens). This may be due to differences in ecology, such as mosquito and bird densities, although failure to detect parasites occurring at such low prevalence at other sites is just as likely to be due to sample sizes being too small. This may also be true for apparent differences in prevalence between species, as large sample sizes were only obtained for blackbirds and thrushes.

When compared with the average prevalence of *Plasmodium* infection in New Zealand in the same five species from a recent survey (Tompkins & Gleeson 2006), it is clear that birds from the Dunedin area harbour these parasites at low prevalence (Table 2). It is likely that low densities of two locally recorded mosquito species, *Culex pervigilans* and *Aedes australis* (Holder et al. 1999), is the cause of this difference, a suggestion that is supported by findings of generally low numbers of mosquitoes around yellow-eyed penguin colonies on the Otago Peninsula (McDonald 2003; HJWS unpubl.). Low mosquito density could also account for the lack of evidence of malarial parasites in 143 yellow-eyed penguins (Sturrock & Tompkins 2007, unpubl. data) and 14 Northern royal albatrosses *Diomedea epomophora sanfordi* (unpubl. data) on the Otago Peninsula. Alternatively, the lack of infection of these native birds could be due to the density of non-native birds being lower on the Otago Peninsula than in Dunedin. This possibility is supported by the fact that it was noticeably more difficult to find and catch non-native birds at sites that were increasingly distant from Dunedin city centre. This, coupled with low mosquito numbers, would limit exposure of penguins and albatrosses to malarial parasites occurring in non-native bird species. Alternatively, the lack of infection found in penguins and albatrosses may be a consequence of infected individuals having died before they were sampled. Malarial parasites are known to be highly virulent in captive penguins

Table 2. Prevalence of positive *Plasmodium* responses in non-native birds sampled in Dunedin and the Otago Peninsula in 2006–2007 compared with results of a national survey by Tompkins & Gleeson (2006).

	Prevalence of *Plasmodium* in sampled birds	
	Dunedin and Otago Peninsula	NZ average
Blackbird	4/60 (7%)	40/79 (51%)
Song thrush	3/27 (11%)	7/36 (19%)
House sparrow	0/11	17/147 (12%)
Chaffinch	0/8	2/17 (12%)
Starling	0/3	19/55 (35%)

Table 1. Prevalence of *Plasmodium* in non-native birds sampled in Dunedin and the Otago Peninsula, November 2006 to March 2007.

	Prevalence of *Plasmodium* in sampled birds				
	Chisolm Park	Botanical Gardens	Portobello playing field	Glenfalloch Gardens	All sites
Blackbird	1/12	3/40	0/2	0/6	4/60 (7%)
Song thrush	0/4	3/17		0/6	3/27 (11%)
House sparrow	0/5	0/2	0/4		0/11
Chaffinch	0/1	0/6		0/1	0/8
Starling	0/1	0/2			0/3
Total site	1/23	6/67	0/6	0/13	7/109 (6%)

throughout Europe and the United States (Bennett et al. 1993; Clarke & Kerry 1993; Cranfield et al. 1994), and there is a report of a suspected death from avian malaria in juvenile yellow-eyed penguins on the Otago Peninsula in 2001 (Alley 2001).

Whatever the explanation for the lack of detectable infection in these two native bird species, the fact that a generalist malarial parasite strain is present in the Dunedin area, and that avian malaria is known to cause high mortality in captive penguins, makes the understanding of the disease a priority for native bird management on the Otago Peninsula. Of the three explanations proposed, mosquito density seems the most likely limiting factor as to whether transmission of malarial parasites from non-native birds to penguins occurs. It may be that spillover of avian malaria infection from non-native reservoirs to native bird species in the Dunedin and Otago Peninsula areas only occurs when mosquito abundance is uncharacteristically high, such as during unusually warm and wet summers (Smith et al. 2004). Such dynamics would explain the sporadic nature of the evidence for malarial infection in yellow-eyed penguins in this region, and any population-scale impacts that such infection may have. We recommend that future management of native birds on the Otago Peninsula should therefore include mosquito density surveys and monitoring of temperature and rainfall. This information may help to predict future risk of avian malaria impacts on native bird populations.

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