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Short communication

Epidemiology of an avian malaria outbreak in a native bird species (*Mohoua ochrocephala*) in New Zealand

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Abstract Emerging infectious diseases pose a considerable threat to wildlife globally. One such disease that has apparently emerged in recent years in New Zealand is avian malaria, with *Plasmodium* infections being detected in numerous species for the first time. Although the overall significance of this apparent emergence is not yet known, infection by *Plasmodium* has been diagnosed as a cause of mortality in several native species in captivity. Here we investigate the epidemiology of the most recently confirmed case, with our results having potentially important implications for native bird management. Avian malaria caused the death of five mohua or yellowheads (*Mohoua ochrocephala*) at Orana Wildlife Park in Canterbury during 2003–05, after their translocation from the Blue Mountains (Otago) in 2003. A lack of detectable *Plasmodium* infection in wild mohua in both the Blue Mountains and the nearby Catlins region, in contrast to an unusually high prevalence in wild bird populations at Orana Park at the time of the outbreak, indicates that infection was most likely acquired by the birds after translocation. This evidence, although not conclusive, strongly argues for assessment of the risk of greater (and potentially deleterious) exposure to malarial parasites to be undertaken prior to native bird translocation. A mosquito investigation carried out at Orana Wildlife Park identified the ubiquitous indigenous mosquito *Culex pervigilans* as the likely disease vector. Hence, management of this mosquito species (in addition to the exotic *Cx. quinquefasciatus*, a known vector of avian malaria in other countries) is a potentially useful preventative measure against disease outbreaks in native bird populations of conservation value in New Zealand.

Keywords avian malaria; captive breeding; *Culex pervigilans*; emerging infectious disease; *Mohoua ochrocephala*; mosquito; *Plasmodium*; translocation

INTRODUCTION

Emerging infectious diseases (EIDs)—those diseases whose geographic range, host range, or prevalence have increased in recent years—pose a considerable threat to wildlife globally (Daszak et al. 2000). Daszak & Cunningham (2003) described numerous wildlife EIDs that

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have resulted in “mass mortalities, population declines, and even extinctions” (p. S37). One recent high profile example is the outbreak of West Nile virus in North America (Daszak et al. 2004), where it is estimated that more than 200 avian species have been affected, and hundreds of thousands of birds have died (Marra et al. 2004).

A significant disease affecting wildlife that has apparently emerged in recent decades in New Zealand is avian malaria, an arthropod-borne disease where protozoan blood parasites (*Plasmodium* spp.) are transmitted by mosquitoes (Diptera: Culicidae). The introduction of *Plasmodium relictum* and the mosquito vector *Culex quinquefasciatus* to Hawaii had dramatic consequences, with avian malaria estimated to have played a key role in the extinction of approximately half of Hawaii’s endemic bird species (van Riper III et al. 1986). Host-specificity of *Plasmodium* spp. varies, but most species are generalists, being able to infect a large number of bird species (Jones & Shellam 1999). This low level of host-specificity has the potential to increase disease impact on susceptible species through the provision of reservoirs of infection (Cleaveland et al. 2002; Tompkins & Poulin 2006). In New Zealand, for example, widespread exotic birds such as house sparrows (*Passer domesticus*), song thrushes (*Turdus philomelos*) and blackbirds (*Turdus merula*) are believed to be asymptomatic carriers of malarial parasites that are not greatly impacted by disease themselves, but may act as sources of infection to more susceptible species (Laird 1950; Tompkins & Gleeson 2006).

Although the overall significance of avian malaria in New Zealand is not yet known, two lines of evidence suggest that impacts on native bird populations are likely (Derraik 2006). First, avian malaria has now been reported in the wild from many native bird species, including Fiordland crested penguin (*Eudyptes pachyrhynchus*) (Laird 1950), New Zealand pipit (*Anthus novaeseelandiae*) and grey duck (*Anas superciliosa*) (Dore 1920), South Island saddleback (*Philesturnus carunculatus carunculatus*) (Hale 2008), weka (*Gallirallus australis*), silvereye (*Zosterops lateralis*), bellbird (*Anthornis melanura*), keruru (*Hemiphaga novaeseelandiae*), saddleback (*Philesturnus carunculatus*), yellow-crowned parakeet (*Cyanoramphus auriceps*) and New Zealand robin (*Petroica australia*) (Tompkins et al. 2008). Second, infection by *Plasmodium* spp. has been confirmed as a cause of mortality in captive individuals of two native species in recent years. In 1996, an outbreak of avian malaria affected New Zealand dotterel chicks (*Charadrius obscurus*) in two captive-rearing institutions in the North Island (Auckland Zoological Park and Otorohanga Kiwi House), causing the death of at least one captive bird and the subsequent death of another 10 with opportunistic infections (Jakob-Hoff et al. unpubl. data; Reed 1997). More recently, infection by *Plasmodium* spp. has been diagnosed as the cause of mortality of five mohua or yellowheads (*Mohoua ochrocephala*; Passeriformes: Pachycephalidae) at Orana Wildlife Park (OWP) in Canterbury during 2003–05 (Alley et al. 2008).

AVIAN MALARIA IN MOHUA

Mohoua ochrocephala is an indigenous bird, endemic to the South Island of New Zealand, whose populations have been steadily declining as a result of human-induced habitat destruction and predation by exotic mammalian pests (O’Donnell 1993). It is a threatened species that is considered to be “endangered” by The World Conservation Union (IUCN 2008). On 10 September 2003, eight wild-caught individuals were transferred from the Blue Mountains (Otago, 45°54′S, 169°23′E) into the Orana Wildlife Park (OWP) (43°28′S, 172°28′E) near Christchurch, as part of a captive-rearing program. The mohua population at OWP steadily declined over approximately 19 months and all birds were eventually lost, including three chicks born at the site. Histopathological evidence showed that at least five of the eight adult birds died as a result of haemoparasite infection and transmission electron microscopy confirmed that these organisms were *Plasmodium* spp. (Alley et al. 2008).

To investigate when and where infection was most likely acquired by the mohua (i.e., prior to, or post-translocation), two host surveys for blood parasites were carried out—wild mohua in the Blue Mountains (source of the translocation) and the nearby Catlins region, and non-native birds (potential infection sources post-translocation) at both OWP and the nearby Willowbank Wildlife Reserve. The sample at OWP consisted of 19 house sparrows, 4 starlings (*Sturnus vulgaris*), 3 goldfinches (*Carduelis carduelis*) and 2 greenfinches (*Carduelis chloris*); while the sample at Willowbank consisted of 13 house sparrows, 4 chaffinches (*Fringilla coelebs*), 4 starlings, 2 blackbirds, 2 greenfinches, 2 song thrushes and 1 goldfinch. Samples were representative of relative abundance at both sites. For both surveys, birds were caught in mist-nets and had peripheral blood samples collected from the brachial vein and stored in lysis buffer (more detailed methods as described in Tompkins & Gleeson 2006). DNA was extracted from samples using the QIAamp® DNA mini kit (QIAGEN), following the manufacturer's protocol for blood samples. Parasite detection was carried out using PCR primers L15368 (5′ AAA AAT ACC CTT CTA TCC AAA TCT 3′) and H15730 (5′ CAT CCA ATC CAT AAT AAA GCA 3′), designed to amplify an approximately 355-base-pair fragment of the mitochondrial cytochrome-*b* gene of *Plasmodium* and *Haemoproteus* parasites, as described in Ricklefs et al. (2004). PCR protocol was as described in Tompkins & Gleeson (2006), with blood from a *Plasmodium* infected blackbird used as a positive control.

Eighteen mohua in the Blue Mountains (3 in December 2005, and 15 in December 2006), and 12 mohua in the Catlins (during January–February 2007) were caught and sampled. All individuals were negative for the PCR diagnostic test, indicating that (assuming a test sensitivity of 90%; Tompkins & Gleeson 2006) actual prevalence was 95% likely to be 11% or less. Given that at least five of the eight adult mohua (63%) that died after translocation to Orana Wildlife Park were infected with *Plasmodium* at the time of death (Alley et al. 2008), infection with this parasite was most likely not acquired by the mohua prior to translocation. This is unsurprising given that the incidence of avian malaria infection in non-native birds is far below the national average in the regions from which the birds were sourced (Tompkins & Gleeson 2006).

Previous work has shown that wild non-native birds at OWP were infected with an unusually high prevalence of *Plasmodium* spp. parasites at the tail end of the mohua outbreak in the summer of 2004/05 (Tompkins & Gleeson 2006), with 53% of sampled birds positive using PCR analysis, compared with an average of 9% for the Christchurch region (including Willowbank). In the current study, birds caught and sampled a year later (in the summer of 2005/06) had a prevalence of infection at Willowbank similar to the average (3/28 or 11%; all house sparrows), and one at OWP much reduced from the previous year's high (8/28 or 29%; 6 house sparrows and 2 starlings). This indicates that malaria prevalence was unusually high both temporally and spatially in wild birds at OWP at the time of the mohua outbreak. Both this, and the lack of detectable infection in wild mohua in the Blue Mountains, strongly suggests that infection of the captive birds occurred post-translocation.

MOSQUITO FAUNA AT ORANA WILDLIFE PARK

We surveyed the mosquito fauna breeding at OWP, and the available records of the mosquito fauna in the general area, to investigate what potential vectors of avian malaria occur at this site. A preliminary survey was carried out at OWP in late spring (November) 2005, at which time mosquitoes were only present at relatively low densities. This was followed up with a more thorough investigation in mid summer (February) 2006, the time of year when mosquito populations seem to peak in New Zealand (Derraik & Slaney 2005). Mosquito larvae were searched for in all available water-holding containers and other potential larval mosquito habitats (including ground pools, drains and drainage sumps) over 2 consecutive days. Whenever larvae were encountered, these were collected and taken to the laboratory for later identification. Most identifications were carried out on larval stages, but some specimens were reared to adults for confirmation.

A total of 60 potential mosquito larval habitats were observed, which could be classified into three types: artificial containers (44), other artificial habitats (11) and natural ground pools (5). Of these, 12 (27%), 6 (54%) and 2 (40%), respectively, were found to harbour mosquito larvae (Table 1). The single mosquito species recorded at OWP was the native *Culex pervigilans*, the presence of which was not surprising given that this is New Zealand's most widespread mosquito. The second most common mosquito in the country, the exotic *Aedes notoscriptus*, although present in Christchurch, currently has a distribution that is restricted to the main urban centre (P. Holder pers. obs.).

Two notable absences in the mosquito survey are *Cx. quinquefasciatus* (van Riper III et al. 1986) and *Aedes australis* (Lawrence 1946), exotic species that are potential vectors of *Plasmodium*. *Culex quinquefasciatus* is the mosquito species responsible for the impact of avian malaria in Hawaii (van Riper III et al. 1986), and its southwards spread in New Zealand over the last three decades is one hypothesised cause of avian malaria emergence (Tompkins & Gleeson 2006), while *Ae. australis* is described as a potential vector of the disease in Australia (Lawrence 1946). The absence of *Ae. australis* in the current survey at OWP is not surprising, given that its distribution in New Zealand appears to be limited to the Otago coast, where it breeds in saline rock pools, with no records as far north as Christchurch (Snell 2005).

The absence of *Cx. quinquefasciatus* in the current survey at OWP is likewise not surprising. The last comprehensive mosquito survey carried out across New Zealand in 1993–94 yielded no evidence of this species anywhere in the South Island (Laird 1995). In addition, despite the very large number of larval and adult samples collected by public health services at various sites, this species has only been recorded three times in the Christchurch region

Table 1 The mosquito larval habitats investigated in February 2006 at Orana Wildlife Park and the respective distribution of *Culex pervigilans*.

Habitat type	Larval habitat description	No. positive for *Cx. pervigilans*	No. negative for mosquitoes
Artificial containers	Plastic container	6	11
	Metal container	–	4
	Glass container	–	1
	Concrete container	–	1
	Pool on polythene sheet	–	2
	Metal wheel	1	1
	Assorted debris	1	–
	Used tyre	4	12
Artificial larval habitats	Large fish tank (c. 6 m^2 base)	2	1
	Drain	1	–
	Gully trap	1	1
	Drainage sump	1	–
	Blocked gutter	–	3
	Pool on concrete floor	1	–
Natural larval habitats	Grass pool (leaky tap)	1	–
	Temporary pools (grass)	–	3
	Pooled stream	1	–

since July 2001 (Mark Disbury pers. comm. 2006). Two of these records comprise the collection of larvae and one adult at Port Lyttleton, while the third is the collection of one adult at Christchurch Airport. There is little doubt that these are cases of human-aided dispersal, where the specimens have arrived in aircraft and ships from northern parts of the country. Hence, there is no evidence that *Cx. quinquefasciatus* is established in the region.

CONCLUSIONS

The pathogenesis of avian malaria includes two post-infection phases: a short acute phase during which most impacts on bird health occur, and a subsequent chronic phase that can last for the lifetime of the bird, and from which relapses to the acute phase can occur (Valkiunas 2005). Hence, although the first mohua confirmed to be infected with *Plasmodium* spp. died nearly 7 months after arriving at OWP (Alley et al. 2008), one cannot discard the possibility that infection of these birds occurred in the Blue Mountains prior to translocation. However, the absence of PCR detectable *Plasmodium* infection in mohua sampled from this region, documented here, indicates that this is unlikely. In contrast, the high prevalence of avian malaria in wild non-native bird populations at OWP at the time of the mohua outbreak makes it likely that infection was acquired after translocation to the park. In light of this, and given the wide variation in avian malaria prevalence that has been documented in wild bird populations across New Zealand (Tompkins & Gleeson 2006), and the impacts on susceptible individuals that this disease can have, we recommend that the relative exposure of birds to malarial parasites at both source and destination locations, and the risk of disease impacts, be considered for future native bird translocations.

With only the indigenous *Cx. pervigilans* being found in the mosquito survey at OWP (and also the only culicid known to occur in the area), this species is the likely vector of avian malaria at this site. Three additional lines of evidence support its role as such. First, *Cx. pervigilans* seems to be primarily a bird-feeder (Derraik & Slaney 2007). Second, it is closely-related to other competent vectors of avian malaria, such as *Cx. quinquefasciatus*. Third, PCR diagnostics have recently detected *Plasmodium* spp. parasites inside the blood meal of an individual female *Cx. pervigilans* elsewhere in New Zealand (Massey et al. 2007). Although the study of Massey et al. (2007) does not prove that *Cx. pervigilans* is a competent vector of *Plasmodium* (non-competent vectors can be PCR-positive after taking a blood meal from an infected bird), it adds to the mounting evidence. Therefore, management of this mosquito species (in addition to the exotic *Cx. quinquefasciatus*) is likely to be an important preventative measure against avian malaria outbreaks in native bird populations of conservation value in New Zealand.

The occurrence of native bird mortality due to avian malaria in association with the indigenous mosquito *Cx. pervigilans*, as opposed to the exotic mosquito *Cx. quinquefasciatus*, raises additional concern regarding the current and potential future impact of this disease on wild populations. In particular, these findings indicate that the influence of factors such as climate and land-use change on avian malaria incidence in New Zealand in the future (for example, by increasing mosquito abundance or vector competence), will most likely not operate solely through the exotic mosquito species (mainly *Cx. quinquefasciatus*) that still seem to be patchily distributed. Rather, by operating through the more widespread indigenous mosquito species, their potential deleterious effects on native bird populations are far wider-reaching.

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