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***Toxoplasma gondii* Exposure Prevalence in Little Spotted Kiwi (*Apteryx owenii*)**

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ABSTRACT: *Toxoplasma gondii* has been reported as a cause of morbidity and mortality in New Zealand's native avifauna, including the ground-dwelling Kiwi (*Apteryx* spp.). To better understand the extent of *T. gondii* infection in Little Spotted Kiwi (*Apteryx owenii*), a prevalence survey of kiwi living inside a 200-ha predator-proof mainland ecosanctuary (Zealandia Te Māra a Tāne, Wellington, New Zealand) was undertaken. Antibodies to *T. gondii* were detected by a latex agglutination test (LAT) with a cutoff positive titer of $\geq 1:64$, and *T. gondii* DNA was detected by PCR. In total, 16/19 (84.2%) birds tested were positive for *T. gondii* by LAT (10/11), PCR (10/19), or both (4/11). Antibody titers ranged from 1:32 to $\geq 1:2,048$. These results suggest widespread exposure of *T. gondii* in this population of Little Spotted Kiwi and, in conjunction with earlier reports of toxoplasmosis causing mortality in kiwi, raise important questions as to the effect this parasite may be having on this rare endemic species. Further information on the epidemiology of *T. gondii* infections within free-living and managed kiwi populations is urgently needed.

Key words: *Apteryx*, kiwi, prevalence survey, *Toxoplasma gondii*, toxoplasmosis.

Toxoplasmosis has been reported in a wide range of domestic and wild species, including in New Zealand (Hill and Dubey 2002; Howe et al. 2014). Infection occurs from ingestion of *Toxoplasma gondii* oocysts in food or water fecally contaminated by domestic cats (*Felis catus*) and other felid species (Dubey 2021), or by ingestion of uncooked meat containing bradyzoites (Hill and Dubey 2002). Felines are the definitive hosts, and New Zealand's domestic and feral cat population is a source of infection for several native avian and mammalian species (Roberts et al. 2020). A recent small study found 61% seroprevalence

of *T. gondii* antibodies in 200 owned cats in New Zealand (Coupe 2021), suggesting there is likely to be a substantial burden of *T. gondii* oocysts in the New Zealand environment (Roberts et al. 2020).

Two clinical cases of suspected toxoplasmosis had been seen in wild Little Spotted Kiwi (LSK, *Apteryx owenii*) a flightless, nocturnal ratite endemic to New Zealand (Germano et al. 2018). These birds were from Zealandia Te Māra a Tāne ($-41^{\circ}17'26.94''$ S, $174^{\circ}45'12.16''$ E), a 200-ha predator-proof ecosanctuary in Wellington, New Zealand. Both were found during the day in poor body condition, displaying ataxia and weakness (B. Lenting pers. comm.). Serology in one case revealed an initial *T. gondii* antibody titer of 1:256, rising to 1:1,024 when repeated 14 d later. The second bird had an initial *T. gondii* antibody titer of $>1:32,768$, which dropped to 1:8,192 when repeated 18 d later. These results, in conjunction with clinical signs, were considered highly suspicious of toxoplasmosis. Despite intensive veterinary supportive care, neither bird fully recovered, and both were euthanized on welfare grounds. The veterinarians were unable to confirm toxoplasmosis by subsequent histology and PCR postmortem, and a definitive cause of illness was not established.

To define the extent of the infection with *T. gondii* at Zealandia Te Māra a Tāne, 19 LSK (from a total population of approximately 200 at this site) were captured and sampled for serologic and PCR testing 26–29 January 2021. Individuals were located by the use of accredited kiwi tracking dogs and manually extracted from their burrows. Birds were

manually restrained by experienced kiwi handlers, and blood was taken from the medial metatarsal vein as described in the *Kiwi best practice manual* (Robertson et al. 2017). In brief, 0.6–1.0 mL of blood was extracted from each bird with a 25-ga \times 1-inch needle and a 1-mL syringe and placed into a 1.3-mL serum micro blood collection tube (Knight Benedikt, Sydney, Australia). Samples were centrifuged at $769 \times G$ for 10 min, and serum was removed. The remaining blood was placed into a 0.5-mL lithium heparin micro blood collection tube (Knight Benedikt) for PCR analysis. To ensure that the same individual was not sampled twice during the sampling period, birds were either permanently banded, or the right middle toe of the kiwi was marked with liquid paper correction fluid (Wite-Out®, Bic, Shelton, Connecticut, USA).

Individual serum samples ($n=11$) were sent to a commercial veterinary pathology laboratory (Gribbles Laboratory, Auckland, New Zealand) for toxoplasmosis antibody titer testing with a latex agglutination test (LAT; Toxoreagent, Mast Group, Bootle, UK). All samples tested demonstrated an antibody response; with a positive cutoff titer of $\geq 1:64$, 10/11 samples were considered positive for *T. gondii* antibodies on LAT (90.9%; 95% confidence interval [CI] 58.7–99.7%), with antibody titers ranging from 1:32 to $\geq 1:2,048$ (Table 1). Estimated infection prevalence and 95% CI were calculated by Stata version 14 (StataCorp LP, College Station, Texas, USA).

We carried out PCR analysis on remaining whole blood ($n=19$) as described by Roe et al. (2013), with 10/19 samples positive for *T. gondii* DNA (52.6%, 95% CI 28.9–75.6%; Table 1). Overall, 16/19 samples tested positive by either PCR, LAT, or both (Table 1), resulting in an estimated prevalence of exposure to *T. gondii* of 84.2% (95% CI 60.4–96.6%). Genotyping was attempted as described previously (Roe et al. 2013; Coupe 2021) for B1, Sag1, and Sag2 genes; however, products were too weak to sequence or to perform restriction fragment length polymorphism.

TABLE 1. *Toxoplasma gondii* latex agglutination test (LAT) antibody titer and PCR results for 19 Little Spotted Kiwi (*Apteryx owenii*) sampled from an ecosanctuary (Zealandia Te Māra a Tāne, Wellington, New Zealand) 26–29 January 2021. For the LAT, antibody endpoint titers of $\geq 1:64$ were considered seropositive.

Bird ID	LAT titer	PCR
No tag 27	$\geq 1:2,048$	–
Chick 1–28	1:512	–
Male chick 2–28	1:64	+
28514	1:64	–
28549	nt ^a	+
32016	nt	–
35525	nt	+
35528 R1 1	nt	+
35529	1:32	+
35530	$\geq 1:2,048$	+
28544	1:64	–
28545	1:64	+
28550	1:64	+
30545	nt	+
31405	1:64	–
35522	nt	+
35523	1:128	–
35524	nt	–
62978	nt	–

^a nt = not tested.

Serologic studies of *T. gondii* in live kiwi have been lacking; however, toxoplasmosis has previously been reported at postmortem examination in 0.32% (5/1,586) of all kiwi recorded in the School of Veterinary Science pathology database at Massey University (Palmerston North, New Zealand; Roberts et al. 2020). In four of these cases, toxoplasmosis was considered the primary cause of death. Although all species of kiwi were included in that dataset, toxoplasmosis was only recorded in Brown Kiwi (*Apteryx mantelli*; 3/1,195, 0.25%) and Little Spotted Kiwi (2/45, 4.4%). Grossly, affected birds were in poor body condition with hepatosplenomegaly, and infection was characterized by hepatocellular necrosis with protozoal organisms within hepatocytes and Kupffer cells (Orr and Black 1996; Hunter and Alley 2014). One bird showed necrosis of multiple organs, with free

tachyzoites in the lungs and protozoal cysts in the other organs (Orr and Black 1996).

Our results suggest that LSK at Zealandia Te Māra a Tāne are widely exposed to *T. gondii*. These results are not unexpected because ground feeders such as kiwi are at high risk of exposure to *T. gondii* and may serve as indicators of environmental contamination with oocysts (Dubey et al. 2010, 2021). Exposure of kiwi to *T. gondii* probably coincided with the introduction of cats to New Zealand by early European explorers in the late 1700s. The route of exposure to *T. gondii* for the kiwi surveyed in the present study remains unknown. Cats have been excluded from the sanctuary since 1999 with a predator-proof fence. Oocysts survive in the environment for a maximum of 18 mo in ideal conditions (Dubey et al. 1998). Waterborne transmission by urban runoff from neighboring land (Miller et al. 2002; Dubey et al. 2021), windborne transmission (Shapiro et al. 2019), or ingestion of invertebrates that are carrying oocysts (Hill and Dubey 2002; Mazzillo et al. 2013) are possibilities. In contrast to predator-free sanctuaries such as Zealandia Te Māra a Tāne, many populations of kiwi share habitat with cats, and these populations likely will have equal or higher levels of exposure to *T. gondii*.

Previous surveys in other ratite species have showed a range of *T. gondii* seroprevalence from 2.9% to 80% by a modified agglutination test (Dubey et al. 2000, 2021). That test is not available in New Zealand. The LAT that we used detects both immunoglobulin G and immunoglobulin M and is not host species specific. The manufacturers recommend antibody endpoint titers for the LAT in animals of $<1:32$ as negative, $1:32$ as weak seropositive, and $\geq 1:64$ as seropositive. We used a cutoff titer of $\geq 1:64$ as positive according to recommendations by Patel et al. (2017): in a study on red deer (*Cervus elaphus*), they found substantial agreement between the LAT and the “gold standard” western blot when a cutoff titer of $\geq 1:64$ was used, but only moderate agreement at a cutoff of $\geq 1:32$. Additionally, Patel et al. (2017) found a higher specificity for the LAT at a cutoff titer of

$\geq 1:64$ than at $\geq 1:32$ (89.7% vs. 74.3%), although sensitivity did drop for the higher cutoff titer (88.7% reduced to 76.2%). Caution should be used when considering the results in avian species, because the LAT has not been validated to the same extent in this taxon, and the effect of other related apicomplexan parasites on cross-reactivity is unknown. Nevertheless, the results of the highly specific PCR support the LAT results in LSK. Additionally, the higher estimated prevalence seen in kiwi when compared with previous prevalence surveys in other ratite species (Dubey et al. 2000, 2021) may be a reflection of our use of two diagnostic methodologies (PCR and LAT) for *T. gondii* exposure detection.

The main threat to kiwi (*Apteryx* spp.) remains predation from introduced predators; nevertheless, disease should not be overlooked as a risk to kiwi populations (Holzapfel et al. 2008; Germano et al. 2018). Toxoplasmosis has already been documented as causing mortality in kiwi (Orr and Black 1996; Howe et al. 2014; Hunter and Alley 2014; Roberts et al. 2020), and our study provides evidence of high rates of exposure to *T. gondii* in a kiwi population with no cohabitation with cats. The potential effect of toxoplasmosis in wild and captive kiwi is yet to be fully understood, and further research in other populations of kiwi is required.

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