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Comparison of Detection Rates of *Toxoplasma gondii* among Five Host Tissues and Two Primer Sets in Three Bird Species

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ABSTRACT: *Toxoplasma gondii* is a globally distributed parasite that infects a wide range of warm-blooded animals and requires felids as definitive hosts. Although birds are recognized carriers of *T. gondii*, in New Zealand species morbidity and mortality events have been sporadically reported, and systematic data are lacking. The objective of this study was to determine the prevalence and tissue distribution of *T. gondii* in three common aquatic birds in New Zealand: the native Red-billed Gull (*Chroicocephalus scopulinus*) and Black-backed Gull (*Larus dominicanus*), and the introduced Mallard (*Anas platyrhynchos*). Birds were collected between September 2022 and April 2025 and screened using nested PCR with two commonly used primer sets (B1, targeting the B1 gene, and FOOD, targeting the pppk-dhps region). Five organs (liver, lung, heart, brain, and spleen) were tested to compare detection rates across tissues. Overall, prevalence was low but consistent across primers and tissues in all three species. Black-backed Gulls and Mallards showed higher prevalence than Red-billed Gulls, probably reflecting differences in diet, habitat, and behavior. Brain and heart tissues yielded the highest detection rates, and the FOOD primers were approximately twice as sensitive as the B1 set. These findings provide practical guidance for primer and tissue selection in avian *T. gondii* studies and represent the first assessment of infection in these three bird species in New Zealand. They also highlight potential ecologic differences among species that may influence exposure to *T. gondii*.

Key words: *Anas platyrhynchos*, B1 gene, *Chroicocephalus scopulinus*, *Larus dominicanus*, organ-specific detection, pppk-dhps gene, primer comparison, *Toxoplasma gondii*.

INTRODUCTION

Toxoplasma gondii is one of the most widespread parasites globally, capable of infecting all warm-blooded animals. Despite its universality, the parasite requires felids as definitive hosts, and these hosts shed environmentally resistant oocysts in the environment through their feces (Dubey 2002, 2004; Hill and Dubey 2002). Such oocysts can then infect intermediate hosts through ingestion of contaminated soil or water. Infection can also occur through consumption of tissues containing *T. gondii* cysts from infected intermediate hosts (Dubey 2002, 2004; Hill and Dubey 2002; Attias et al. 2020; Wilson et al. 2020; Al-Malki 2021). Although *T. gondii* usually presents as a subclinical infection or with mild clinical signs in warm-blooded animals, severe cases of the disease can result in neurologic issues, and infection can be passed vertically to offspring, causing morbidity and mortality (Hill and Dubey 2002; Hill et al. 2005; Di Guardo et al. 2010).

Avian hosts are known to play an important role in *T. gondii* epidemiology because they can act as intermediate hosts and transmit tissue cysts within the food web, yet they are often overlooked. Birds may also serve as environmental sentinels due to their wide-ranging habitats and diverse diets (Wilson et al. 2020; Dubey et al. 2021; Zaki et al. 2024). Toxoplasmosis has been reported in birds globally (Buschang et al. 2025), and seabirds, particularly gulls, are of interest because of their long lifespans, global distribution, and opportunistic feeding habits. Their diets range from scavenging carcasses and consuming landfill waste in areas with high felid densities to foraging in both aquatic and terrestrial ecosystems (Falla et al. 1970; Mills et al. 2008; Heather and Robertson 2015; Reusch et al. 2025). These behaviors may expose them to multiple infection pathways, including ingestion of cysts from dead

animals (marine or terrestrial), contact with items contaminated by soil, or exposure to *T. gondii* oocysts entering marine ecosystems via runoff.

Although this parasite is known to be a major issue in New Zealand with morbidity and mortality events in key species in New Zealand such as sheep (*Ovis aries*), kiwi (*Apteryx* spp.), and Hector's dolphins (*Cephalorhynchus hectori*), only limited studies have investigate this (Innes 2010; Roe et al. 2013; Howe et al. 2014; Gulliver et al. 2022; Taylor et al. 2023). The only research on *T. gondii* in New Zealand birds has been limited to case studies and a single retrospective study on raptors (Howe et al. 2014; Gartrell et al. 2017; Mirza et al. 2017; Gulliver et al. 2022; Taylor et al. 2023). Therefore, the current prevalence and distribution of *T. gondii* within New Zealand's birds remain poorly understood.

In New Zealand, there are three native species of gulls: Red-billed Gull (*Chroicocephalus novaehollandiae*), Black-billed Gull (*Chroicocephalus bulleri*), and Southern Black-backed Gull (*Larus dominicanus*). This study focuses on the Red-billed Gull and the Black-backed Gull, along with the introduced Mallard (*Anas platyrhynchos*). The Red-billed Gull is widely distributed along New Zealand's coasts, often found in urban and suburban environments. Classified as an omnivore, the Red-billed Gull's diet is dominated by krill and other small marine invertebrates, although they occasionally exploit urban food sources (Falla et al. 1970; Mills et al. 2008; Tobias et al. 2022). The Southern Black-backed Gull is a larger species that shows a stronger association with urban areas. As an omnivorous scavenger, it frequently exploits human-derived food sources, such as landfills and fishery waste, in addition to preying on or scavenging both terrestrial and marine animals, which increases its likelihood of encountering oocysts in urban or anthropogenic environments (Heather and Robertson 2015; Tobias et al. 2022).

Mallards were introduced into New Zealand from Australia in 1867 for game hunting, using both North American and British farm stocks (Dyer and Williams 2010; Heather and Robertson 2015). Today, the Mallard is one of the most widespread waterfowl species in New Zealand and often interbreeds with the native Pacific Black Duck (*Anas superciliosa*), known in New Zealand as the Grey Duck (Heather and Robertson 2015). Mallards occupy freshwater habitats, including rivers, lakes, wetlands, and agricultural landscapes. Although largely herbivorous, they are exposed to *T. gondii* primarily through waterborne oocysts originating from urban and agricultural runoff (Shapiro et al. 2019; Li et al. 2022; Dini et al. 2023). Mallards are classified as having a primarily aquatic lifestyle; they spend most of their time on the water and typically obtain food either while floating or by diving beneath the surface. By contrast, the Black-backed Gull and Red-billed Gull are both generalist species that use multiple lifestyle categories (Tobias et al. 2022). Combined, these three species represent complementary ecologies with close proximities to aquatic ecosystems and urban areas, allowing assessment of the differences between habitat use (marine, coastal, urban, freshwater, and agricultural), diets (herbivore vs. omnivore vs. scavenger), and geographic distributions across New Zealand as potential *T. gondii* exposure routes.

Detection of *T. gondii* in wildlife commonly relies on molecular methods, particularly PCR assays targeting repetitive genetic elements such as the B1 gene, that enable sensitive identification of parasite DNA in host tissues (Mancianti et al. 2013, 2020; Sroka et al. 2019; Taylor et al. 2023). Alternative primer sets, including those developed for foodborne transmission studies, may vary in sensitivity and specificity, but their comparative performance in avian tissues remains unclear. Additionally, although several organs have been screened for *T. gondii* in birds, most studies have examined only one or two tissues, and systematic comparisons among multiple organs within the same individuals remain limited (Dubey 2002; Darwich et al. 2012; Huang et al. 2012; Ammar et al. 2020; Gazzonis et al. 2021; Dini et al. 2023). It is therefore difficult to determine how primer

FIGURE 1. Sampling locations of birds within New Zealand (a) in this study, including (b) hunter donated Mallards (*Anas platyrhyn- chos*) from Canterbury, South Island, New Zealand; and (c) Red-billed Gulls (*Chroicocephalus scopulinus*) and (d) Black-backed Gulls (*Larus dominicanus*) donated to our laboratory from various sites around Otago, South Island, New Zealand, from September 2022 to April 2025.

selection and tissue choice affect detection outcomes in birds.

The aims of this study were to investigate the occurrence of *T. gondii* in Red-billed Gulls, Black-backed Gulls, and Mallards in New Zealand and to evaluate differences in *T. gondii* detection among host species, among organs, and between two commonly used PCR primer sets. Because these species occupy contrasting ecologic niches, we expected that detection patterns would vary among species according to differences in diet and exposure pathways. We also expected that detection probabilities would differ among organs and between primer sets, reflecting potential tissue tropism of the parasite and variation in assay sensitivity.

MATERIALS AND METHODS

Sample collection

In total, 138 dead birds were obtained from two sources: through donations by local hunters (Mallards, n=46) or specimens from the Dunedin Wildlife Hospital, Dunedin, South Island, New Zealand (Red-billed Gulls, n=46 and Black-backed Gulls, n=46). Mallards were collected by hunters in May 2024 from two regions in the South Island of New Zealand (Fig. 1a) in Canterbury (Rangitata Valley and Woodbury; Fig. 1b) and were processed for sample collection within 2 d of death.

The gulls had been found dead or were later euthanized due to injury, illness, or starvation and were stored frozen before analysis. The gull samples had varying time of deaths ranging from September 2022 to April 2025 and originated from various locations around the Otago region, but primarily in the Dunedin area (Fig. 1c, d). Specimens were thawed and dissected in batches over a period of 1 yr.

For all birds, the sex, body condition, date of death, location, age class (adult or juvenile), body length (centimeters; from tip of beak to base of tail with bird fully extended), and body weight were recorded. Tissue samples were taken from each bird when available, including liver (bottom tip of the left lobe), lung (bottom section of the right lung near backbone), heart (bottom tip), brain (~1 mL), and spleen (entire spleen). Brain tissue was collected either by opening the cranium and removing a section of the brain by gently scooping, or by aspiration via the foramen magnum by using an 18-gauge, 38-mm (1.5-in) needle attached to a 3-mL syringe, where up to approximately 1–2 mL of brain material was collected. In most cases, the cranium was not opened, to preserve the pelt for cultural and ethical considerations. Weights of each complete organ were recorded before subsampling, excluding the brain, for which only partial material was obtained. Subsamples of each organ were stored in 70% ethanol or frozen for genetic analyses.

Molecular detection of *T. gondii*

Samples were removed from ethanol or the freezer and a subsample of tissue was taken (25 mg from the liver, lung, heart, and brain

TABLE 1. Primer sequences (PmL and FOOD, respectively) used for nested PCR detection of *Toxoplasma gondii* targeting the B1 and pppk-dhps genes.

Primer	Type	Sequence	Literature cited
Pml/S1	External forward	5′-TGTTCTGTCCTATCGCAACG-3′	Grigg and Boothroyd 2001
Pml/S2	External reverse	5′-ACGGATGCAGTTCCTTTCTG-3′	Grigg and Boothroyd 2001
Pml/AS1	Internal forward	5′-TCTTCCCAGACGTGGATTTC-3′	Grigg and Boothroyd 2001
Pml/AS2	Internal reverse	5′-CTCGACAATACGCTGCTTGA-3′	Grigg and Boothroyd 2001
FOOD1	External forward	5′-GGAACATCCGCTGAAGCTCATGG-3′	Aspinall et al. 2002
FOOD2	External reverse	5′-CAGAGAATCCAGTTGTTTCGAGG-3′	Aspinall et al. 2002
FOOD3	Internal forward	5′-CAGTCCAGACTCGTTCACCGATC-3′	Aspinall et al. 2002
FOOD4	Internal reverse	5′-CCGGAATAGTGATATACTTGTAG-3′	Aspinall et al. 2002

and 10 mg from the spleen) following the recommended quantities specified for the DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany). Tissue samples were cut into small fragments, and DNA was extracted according to the manufacture's protocol, with DNA eluted in 200 µL of buffer following the manufacturer's protocol. The DNA extracts were put through nested PCR (nPCR) using two different sets of primers. The first set targeted the B1 gene (Grigg and Boothroyd 2001), and the second set (FOOD primers) targeted the pppk-dhps gene (Aspinall et al. 2002). These primer sets were selected for comparison following a literature survey of reported *T. gondii* infections in wild birds, in which these two nPCR assays were the most frequently reported molecular detection approaches. Primer sequences are provided in Table 1. The B1 primer set had the following nPCR conditions for both rounds: hold, 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 45 s, finished with a hold at 72 °C for 10 min. The FOOD primer set had the following nPCR conditions for both rounds: hold, 95 °C for 2 min, followed by 40 cycles of 95 °C for 30 s, 55 °C for 45 s, and 72 °C for 60 s, finished with a hold at 72 °C for 7 min. A negative PCR control (water) and a positive control consisting of a previously confirmed and sequenced *T. gondii* sample were included in each PCR run to monitor contamination and amplification performance. The PCR products were run on 2% gel with SYBR Safe DNA Gel Stain (Thermo Fisher Scientific, Waltham, Massachusetts, USA) to identify positive amplifications. All positive gel results were cleaned using EXOSAP Express PCR Product Cleanup Reagent (USB Corporation, Cleveland, Ohio, USA) and sequenced using the Genetic Analysis Service, Department of Anatomy, at the University of Otago, Dunedin, New Zealand. The returned sequences were manually edited and cleaned in Geneious Prime version 2026.1.1 (Biomatters, Inc. 2026) and then run in NCBI BLAST (NCBI 2026) searches to confirm *Toxoplasma* amplification.

Statistical analyses

The datasets were imported into R version 4.3.2 (R Core Team 2023) by using the readxl package (Wickham and Bryan 2023). Following data import, raw data were reconstructed into a long format with each record representing a PCR outcome from a specific organ–primer combination per individual bird. Predictor variables used in the analysis included host species (Red-billed Gull, Black-backed Gull, and Mallard), organ tested (brain, liver, heart, lung, and spleen), and primer set (B1 and FOOD). Information regarding the bird included body weight that was divided into tertile-based categories: light (≤256.9 g), medium (257.0–1055.6 g), and heavy (≥1055.7 g) birds. Although additional demographic variables such as sex and age were recorded during sample collection, these variables were not included in the statistical

analyses due to incomplete records and the limited number of positives detected.

A generalized linear mixed model with binomial structure was used, with a binary response variable (1=positive, 0=negative). For the model, the predictors were as follows: species, bird weight, organ, and primer, with the bird ID being used as a random effect in the model to account for repeated sampling of multiple organs from each individual bird. The model was run using glmer from the lme4 package (Bates et al. 2015; Kuznetsova et al. 2017). Predictor significance was assessed using Wald z-tests, and odds ratios (OR) with 95% confidence intervals (CIs) were obtained from the model coefficients. Mean PCR positivity rates were calculated for each organ–primer–species combination, and detection patterns were visualized as heatmaps by using *ggplot2* (Wickham 2016). Furthermore, we ran a McNemar test for paired binary outcomes to compare the overall detection performance of the two different primer sets by using the base functions in R and tidyverse (Wickham et al. 2019). The data were converted into a 2×2 contingency table to summarize concordant and discordant results between the two primer sets. McNemar's chi-square test was applied to assess whether detection outcomes were significantly different between primers, indicating preference toward one set. Sampling locations were mapped using latitude and longitude coordinates to illustrate the spatial sites of origins of birds with the *leaflet* package (Cheng et al. 2024).

RESULTS

In total, 1,362 PCR outcomes from 684 organs across 138 individual birds were included in the analyses after excluding organs that were unavailable or unsuitable for PCR analysis (see Supplementary Material Table S1). Of these, 61 PCR outcomes tested positive for *T. gondii*, representing a 4.5% positivity rate overall. The 61 positives originated from 40 individual birds, resulting in 29% of all birds testing positive. At the species level, Black-backed Gull had the highest prevalence, with 16/46 birds (35%) testing positive. When PCR outcomes from different tissues were treated as separate samples, Mallards showed a positivity rate of 6% (26/454, heart=4, brain=10, lung=2, liver=5, spleen=5) compared with 5% (23/452, heart=8, brain=8, lung=5, liver=1, spleen=1) in Black-backed Gulls, despite fewer individual birds testing positive overall (15/46, 33%). In Mallards, three individuals showed positive detections in more than one organ, including one individual that was positive across all five organs by using a combination of B1 and FOOD primers. By contrast, Black-backed Gulls were typically positive in a single organ, with two individuals showing detection in more than one organ. In these cases, detection often differed between primer sets. Although a greater percentage of Black-backed Gulls were positive at the individual level, the total number of PCR-positive organ samples was higher in Mallards. Red-billed Gulls had the lowest prevalence, with 20% (9/46) individual birds and 3% (12/456, heart=3, brain=5, lung=2, liver=1, spleen=1) PCR outcomes testing positive.

In the generalized linear mixed-effects model including bird identity as a random effect, the odds of detecting *T. gondii* DNA differed strongly among organs (Table 2). Compared with the brain, detection was significantly less likely in the heart (OR, 0.39; 95% CI, 0.17–0.90; P=0.032), liver (OR, 0.22; 95% CI, 0.08–0.54; P=0.003), lung (OR, 0.26; 95% CI, 0.10–0.63; P=0.006), and spleen (OR, 0.26; 95% CI, 0.10–0.63; P=0.006). Generally, across all three species, the brain and heart remained the most commonly positive organs (Fig. 2), lung was intermediate, and detection was lowest in the liver and spleen (Fig. 3).

Primer type was also associated with detection, with the FOOD primer showing higher sensitivity than the B1 primer (OR, 2.04; 95% CI, 1.07–3.93; P=0.030). Probabilities predicted by the model (Fig. 3a) highlighted these patterns, whereas observed detection rates (Fig. 3b) also indicated that the FOOD primer outperformed B1 across organs and species. Furthermore, the McNemar test confirmed a

TABLE 2. Odds ratios (ORs) with 95% confidence intervals (95% CIs) and *P*-values resulting from the generalized linear mixed-effects model looking at the effects of bird species, body weight, organ type, and PCR primer on *Toxoplasma gondii* detections in Red-billed Gulls (*Chroicocephalus scopulinus*), Black-backed Gulls (*Larus dominicanus*), and Mallards (*Anas platyrynchos*) from South Island, New Zealand.

Term	OR (95% CI)	*P*-value
Baseline (light weight, brain, Mallards, B1 primer)	0.03 (0.00–0.34)	0.00469127
Species: Black-backed vs. Mallard	0.95 (0.19–4.87)	0.954868227
Species: Red-billed vs. Mallard	0.55 (0.06–4.80)	0.588859028
Weight_birdMedium	1.96 (0.29–13.18)	0.488580778
Weight_birdHeavy	1.33 (0.11–15.30)	0.821361088
Organ: heart vs. brain	0.39 (0.16–0.92)	0.031886842
Organ: liver vs. brain	0.22 (0.08–0.60)	0.003172828
Organ: lung vs. brain	0.26 (0.10–0.68)	0.005861519
Organ: spleen vs. brain	0.26 (0.10–0.67)	0.005801865
Primer: FOOD vs. B1	2.04 (1.07–3.89)	0.029797175
sd__(Intercept)	1.46 (NA–NA)[a]	

[a] NA = not applicable.

statistically significant difference between the two primers (χ^2=6.92, *P*=0.0085). Among discordant pairs, 27 samples tested positive only with the FOOD primers, 10 tested positive only with the B1 primers, and 12 organ samples tested positive with both primers. The McNemar analysis included samples for which results from both primer sets were available. This discordance in primer performance is visualized in Figure 3 where the overall prevalence (dotted line) in each organ exceeds the detection rate of either primer alone, except in the liver, indicating the presence of discordant detection. This effect was particularly evident in the brain, heart, and lungs, where a substantial divergence appeared between the detection rate per primer and the overall prevalence. Conversely, detection in the liver was largely concordant across primers, with the FOOD primers detecting all B1-positive infections plus additional cases not identified by B1. Physiologic predictors (weight category) and species identity (e.g., Black-backed Gull vs. Red-billed Gull) were not significantly associated with *T. gondii* detection (*P*>0.4 for all comparisons). Finally, random effects analysis showed substantial heterogeneity among individual birds (SD=1.46), highlighting clustering of positive detections within certain individuals.

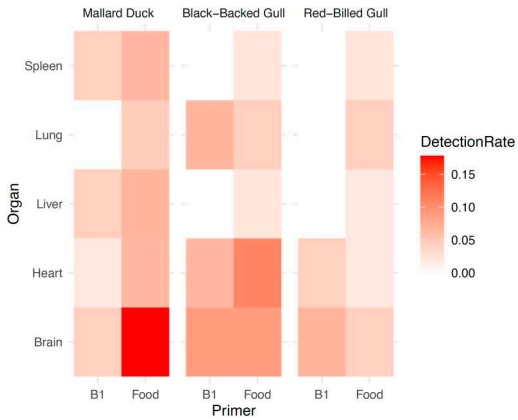

FIGURE 2. Detection rates of *Toxoplasma gondii*, shown as the proportion of PCR-positive samples (white = none, red = high), in organs from Mallards (*Anas platyrhynchos*), Red-billed Gulls (*Chroicocephalus scopulinus*), and Black-backed Gulls (*Larus dominicanus*) from the South Island of New Zealand. Rows represent the sampled organ, whereas columns represent the primer used (B1 or FOOD), grouped by bird species. The heatmap highlights hot spots of detection, with the brain and FOOD primers showing the highest (most red) rates.

DISCUSSION

Our organ-level molecular assessment of *T. gondii* infection in Red-billed Gulls, Black-backed Gulls, and Mallards in New Zealand

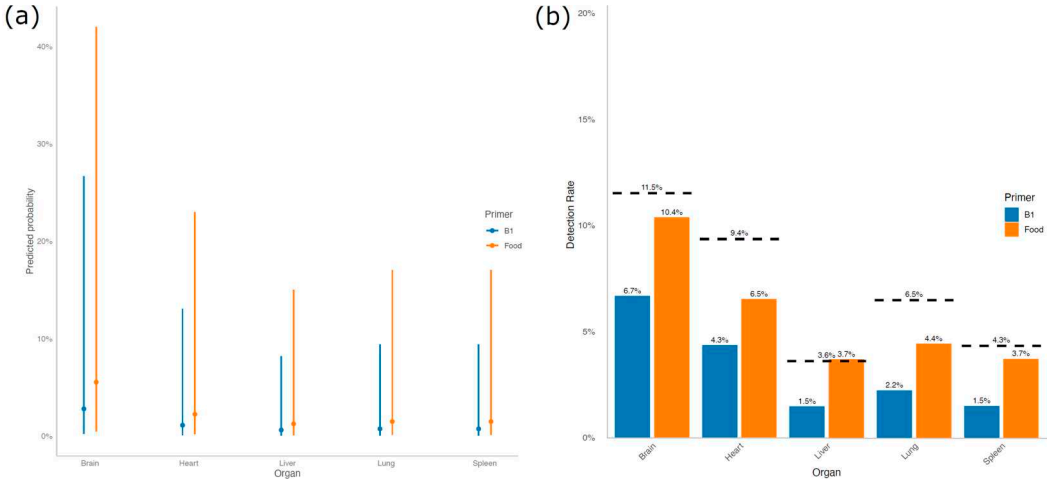

FIGURE 3. Detection of *Toxoplasma gondii* by organ and primer across three bird species, Mallards (*Anas platyrhynchos*), Red-billed Gulls (*Chroicocephalus scopulinus*), and Black-backed Gulls (*Larus dominicanus*), from the South Island of New Zealand. (a) Predicted detection probability (±SE) from generalized linear models, showing that brain and heart were consistently the most commonly positive organs. Detection was lowest in liver and spleen across species. (b) Observed detection rates (percent positives) highlighting the same tissue-specific patterns and illustrating that the FOOD primer outperformed B1 across organs and species. Dashed horizontal bars indicate the overall prevalence in each organ, that is, the proportion of individuals that tested positive by either primer type.

detected low levels of *T. gondii* across different tissues, with the highest detection rates in the brain and heart. This pattern may indicate a tissue-specific predilection for neural and cardiac tissue, as has been reported in previous avian studies (Cooper et al. 2015; Dubey et al. 2021; Dini et al. 2023). However, differences in tissue collection methods should be considered when interpreting this pattern. Brain samples were obtained differently from other organs due to specimen condition and handing constraints; nevertheless, similar tissue masses were used for DNA extraction, suggesting that differences in sampling approach were unlikely to have substantially affected detection probability. Going forward, we would recommend sampling the brain and/or heart and pooling the samples, as previous studies have done with similar organs (Khademvatan et al. 2013; Vitaliano et al. 2014; Karakavuk et al. 2018).

The FOOD primers showed about twofold higher odds of detecting *T. gondii* than the B1 primers. This may reflect differences in the primers' efficiency (Veronesi et al. 2017), variations in the gene copy numbers (Costa and Bretagne 2012), accessibility of the target region within the avian tissue samples, or strain-specific factors affecting detection (Coupe et al. 2019). Importantly, all positive amplifications this study were confirmed as *T. gondii* through sequencing and BLAST analysis, supporting that the higher detection rate observed with the FOOD primers reflects increased sensitivity rather than nonspecific amplification. Although the B1 gene remains widely used as a standard molecular target, these findings indicate that FOOD primers represent a reliable alternative for detection in avian tissues. Other molecular approaches, including quantitative PCR and assays targeting the 529bp repetitive element (e.g., Gerhold et al. 2017; Ammar et al. 2020; Gherman et al. 2025), have also demonstrated high sensitivity and should be considered in future work. Our present study focused on comparing more commonly applied nPCR assays; direct comparisons across a broader range of molecular methods within birds would consequently be a valuable direction for future work.

Among the three species examined, the Black-backed Gull showed the highest proportion of positive individuals, whereas the Red-billed Gull had the lowest. However, species identity was not significantly associated with detection, indicating that these differences should be interpreted cautiously. Variation in exposure risk may nevertheless reflect differences in diet, habitat, and behavior affecting exposure pathways. The marine Black-backed Gulls have a more omnivorous and scavenging diet and rely more on anthropogenic food sources and inland habitats (Falla et al. 1970; Heather and Robertson 2015; Tobias et al. 2022; Reusch et al. 2025), whereas the coastal Red-billed Gulls rely more heavily on marine prey such as krill (Falla et al. 1970; Mills et al. 2008; Heather and Robertson 2015; Tobias et al. 2022). Mallards, in contrast, are considered to be primarily exposed through waterborne oocysts in freshwater ecosystems (Falla et al. 1970; Heather and Robertson 2015; Wilson et al. 2020).

The similar detection pattern observed in Mallards and Black-backed Gulls may reflect shared exposure to urban and agricultural environments, which have been associated with elevated *T. gondii* prevalence in wildlife (Morais et al. 2021; Ledgerwood and Luscier 2025). In addition to ecologic exposure, host specific factors such as immune responses, physiology, and life-history traits, may influence infection dynamics (Innes 2010; Robert-Gangneux and Dardé 2012). Black-backed Gulls may contribute to transmission of the parasite through their widespread distribution and variety of foraging and scavenging behaviors, which may facilitate trophic transfer of *T. gondii* within their food webs (Wilson et al. 2020, 2024). Mallards may also present a potential zoonotic risk as they are commonly hunted for human consumption, and ingesting undercooked meat containing *T. gondii* cysts could represent a transmission pathway (Dubey et al. 2021). By contrast, the comparatively lower prevalence in Red-billed Gulls further supports that species ecology—including diet and habitat use—probably mediate exposure risk (Dubey et al. 2021; Wilson et al. 2020, 2024).

The geographic sampling areas or sampling timeframe was unlikely to have influenced the differences in infection rates between the gull species in our study because these birds were collected in the same regions over the same period (2022–25). Although Mallards were sampled over a shorter timeframe, we do not expect this to have a strongly influenced the observed prevalence patterns, because *T. gondii* tissue cysts can persist for extended periods, reducing the influence of short-term variation (Wilson et al. 2020). Furthermore, Mallards were sampled from a slightly more northern part of the South Island than the gulls, which might have affected exposure risk, but none of these species are long-distance migratory birds, and they reside within New Zealand year-round, making it unlikely that infections were acquired outside the sampled regions (Falla et al. 1970; Heather and Robertson 2015). Finally, the information provided by the Dunedin Wildlife Hospital indicated that no gulls included in this study died from, or were diagnosed with, toxoplasmosis. Although these samples should not be considered representative of completely health populations, opportunistic sampling from wildlife rehabilitation centers is a common and practical approach in wildlife disease studies (Stallknecht 2007).

Several factors should be considered when interpreting our findings. The overall low detection rate limited the statistical power of our analyses, particularly for ORs in contingency analyses. In addition, PCR-based detection cannot distinguish between recent and older infections with this persistent parasite, limiting assumptions that can be made about the current significance of *T. gondii* in the birds. Furthermore, molecular detection depends on the presence of a parasite DNA within the tested material; therefore, infections may be missed if DNA is absent or present at very low concentrations in the analyzed tissue sample due to uneven parasite distribution. By contrast, serologic assays detect host antibodies and may reveal infections even when parasite DNA is

not detected in tissue samples. Future work should incorporate both molecular and serologic methods to obtain a more comprehensive assessment of infection. Additionally, we recommend genotyping, or strain typing, when possible, to provide valuable insights into the lineage of detected parasites. Such analyses typically require additional multilocus genotyping methods (e.g., PCR-restriction fragment-length polymorphism) or single-nucleotide polymorphism–based assays to distinguish among major lineages (Grigg and Boothroyd 2001; Su et al. 2006, 2010; Huang et al. 2012). This process requires additional primers and analyses beyond the B1 gene assay used in this study.

Despite these limitations, this study provides useful guidance for avian *T. gondii* surveillance by supporting the use of the FOOD primers and optimal tissue targets. The consistent detection of *T. gondii* across multiple species highlights the parasite's presence in New Zealand aquatic birds and underscores the importance of continued monitoring, particularly in species associated with urban and freshwater environments.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/JWD-D-25-00189.

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