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Multi-scale occupancy approach to estimate *Toxoplasma gondii* prevalence and detection probability in tissues: an application and guide for field sampling

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ABSTRACT

Increasingly, birds are recognised as important hosts for the ubiquitous parasite *Toxoplasma gondii*, although little experimental evidence exists to determine which tissues should be tested to maximise the detection probability of *T. gondii.* Also, Arctic-nesting geese are suspected to be important sources of *T. gondii* in terrestrial Arctic ecosystems, but the parasite has not previously been reported in the tissues of these geese. Using a domestic goose model, we applied a multi-scale occupancy framework to demonstrate that the probability of detection of *T. gondii* was highest in the brain (0.689, 95% confidence interval = 0.486, 0.839) and the heart (0.809, 95% confidence interval = 0.693, 0.888). Inoculated geese had an estimated *T. gondii* infection probability of 0.849, (95% confidence interval = 0.643, 0.946), highlighting uncertainty in the system, even under experimental conditions. Guided by these results, we tested the brains and hearts of wild Ross's Geese (*Chen rossii*, $n = 50$) and Lesser Snow Geese (*Chen caerulescens*, $n = 50$) from Karrak Lake, Nunavut, Canada. We detected 51 suspected positive tissue samples from 33 wild geese using real-time PCR with melt-curve analysis. The wild goose prevalence estimates generated by our multi-scale occupancy analysis were higher than the naïve estimates of prevalence, indicating that multiple PCR repetitions on the same organs and testing more than one organ could improve *T. gondii* detection. Genetic characterisation revealed Type III *T. gondii* alleles in six wild geese and *Sarcocystis* spp. in 25 samples. Our study demonstrates that Arctic nesting geese are capable of harbouring *T. gondii* in their tissues and could transport the parasite from their southern overwintering grounds into the Arctic region. We demonstrate how a multi-scale occupancy framework can be used in a domestic animal model to guide resource-limited sample collection and tissue analysis in wildlife. Secondly, we confirm the value of traditional occupancy in optimising *T. gondii* detection probability in tissue samples.

1. Introduction

Knowing what tissue is best for testing can make the difference between clinical detection and non-detection of a pathogen or disease process (McClintock et al., 2010). In most settings, where space, time and financial resources are limited, sampling and testing for disease or pathogen presence from all possible tissues is not feasible. Further, tissue selection during post-mortem analysis is not always straightforward because reports regarding parasite tissue predilection sites can be conflicting and detection in a specific tissue type might be sensitive to laboratory methodology, the species involved, or the stage of infection. Tissue coccidian parasites such as *Toxoplasma gondii* can be difficult to detect because their distribution might not be diffuse throughout an organ or muscle (Berenreiterová et al., 2011). More information on tissue predilection sites and detection probability of coccidian tissue stages is

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needed to improve diagnostics, especially in wildlife and food animal species.

Toxoplasma gondii is ubiquitous among vertebrates including humans (Dubey, 2009). Hosts can become infected by three different parasite life stages: oocysts, tissue cysts (containing bradyzoites) or tachyzoites, and transmission occurs through both direct and indirect routes. Felids are the only known definitive hosts of *T. gondii*; sexual reproduction of the parasite occurs in the intestinal epithelium and oocysts are then passed in faeces. A wide range of vertebrates can serve as intermediate hosts, developing tissue cysts in their organs and musculature (Dubey, 2009).

Previous studies in mammals provide comparisons of detection frequency in tissues, suggesting that *T. gondii* tissue cysts are consistently found in the brain and heart of infected animals (Jurànková et al., 2014), although these sites are often the only ones tested (Feitosa et al., 2014; Krijger et al., 2014). Despite this assertion, other reports provide evidence of tissue tropism (the tissues of a host that support the growth and propagation of a pathogen), and findings also differ by host species, parasite genotype or inoculation route (Bangoura et al., 2013; Juránková et al., 2013, 2014; Zöller et al., 2013). *Toxoxplasma gondii* can also be detected in less traditionally tested soft tissue such as tongue and skeletal muscle (Burrells et al., 2013; VanWormer et al., 2014). Occurrence of *T. gondii* in wild or domestic birds is not reported as often as in mammals, but numerous avian species worldwide are recognised as intermediate hosts and little is known about tissue tropism in these animals (Dubey, 2002).

Another difficulty surrounding tissue diagnostics for cyst-forming coccidian parasites (e.g., *T. gondii*) is the size of the organ in relation to the amount of tissue screened in the laboratory analysis. Because it is not usually feasible to test an entire organ, uncertainty exists when an infectious organism (e.g., *T. gondii* cyst) is not detected. While some DNA extraction methods effectively extract DNA from up to 1 g of tissue, most commercial kits allow testing of much less (e.g., 25 mg for the Qiagen (Canada) Blood and Tissue Kit, following kit instructions), possibly decreasing the chances that the tissue sample will contain the parasite; *T. gondii* might be unevenly distributed in an organ and processing less tissue might reduce the probability of detection (Berenreiterová et al., 2011).

Occupancy modelling approaches are traditionally used to estimate the probability of occurrence of a wildlife species within a habitat, and are especially useful for rare or cryptic species (MacKenzie et al., 2006). Occupancy approaches also allow for the estimation of the detection probability (denoted p), or, the probability that a species of interest will be detected, given that an area is occupied by that species. These methods are increasingly used in wildlife disease ecology because pathogens can be difficult to detect or are unevenly distributed among different organs within a host and throughout the tissue of infected organs. If so, imperfect detection can add to uncertainty in prevalence estimates (e.g., McClintock et al., 2010; Lachish et al., 2012; Eads et al., 2014; Elmore et al., 2014). To address this concern of imperfect detection, we used a multi-scale occupancy approach (Nichols et al., 2008) to determine whether goose organs vary in their likelihood of containing *T. gondii,* and to determine which infected organ(s) have the highest detection probability of *T. gondii* using molecular methods. To accomplish this, we used an experimental goose model from which detection probability results could be applied to a wildlife field question – which tissues should we be collecting and testing?

Our first objective was to determine the probability of detecting *T. gondii* DNA in infected tissues from experimentally inoculated domestic geese. We hypothesised that *T. gondii* would be most likely to occur in the brain of experimentally inoculated birds. We also expected that each organ would have a different probability of *T. gondii* occupancy and detection because previous research has demonstrated variable tissue predilection and detection of *T. gondii* among tissues of both mammals and birds (Bangoura et al., 2013; Juránková et al., 2013, 2014, 2015). We also predicted that the sex of the geese would not influence occupancy probability in the primary site (a goose), given the absence of this effect in a previous serological study (Elmore et al., 2014).

The results from the first objective helped to guide our second objective, which was to collect and test tissue samples from wild geese for *T. gondii.* We hypothesised that free-ranging geese from Karrak Lake, Nunavut, Canada would be infected with *T. gondii*, based on previous serological data that showed that Ross's Geese (*Chen rossii*) and Lesser Snow Geese (*Chen caerulescens*) were exposed to the parasite at some point in their lives (Elmore et al., 2014). We also examined whether the primary site (goose) occupancy probability varied by species, due to weak evidence for a species effect in a previous study (Elmore et al., 2014). Identifying *T. gondii* in wild geese would support the idea that migratory birds transport the parasite from temperate regions to Arctic ecosystems and would suggest that wild geese are a potential source of *T. gondii* for wildlife predators such as Arctic foxes. The demonstration of parasite DNA in the soft tissues of a migratory prey species would support the hypothesis that ingestion of *T. gondii* by predators could perpetuate the parasite's asexual life cycle in the Arctic region.

2. Materials and methods

2.1. Experimental infection of domestic geese with T. gondii

We obtained domestic goose goslings (*Anser anser domesticus*) on the day of hatching from a local hatchery in Saskatoon, Saskatchewan, Canada. To avoid contamination with coccidian oocysts in the environment, goslings were kept from contact with the ground until they were released in a Biosecurity level 1 room in the Animal Care Unit at the University of Saskatchewan Western College of Veterinary Medicine, Saskatchewan, Canada. Goslings were offered water and chick starter feed (without antibiotics) ad libitum and maintained under a 12 h light/12 h dark light cycle. At 7 weeks of age, 25 of the geese were inoculated with 500 sporulated *T. gondii* oocysts in 1.0 mL of 0.9% saline (Genotype Type III; originally isolated from swine at the Canadian Food Inspection Agency, Centre for Food-Borne and Animal Parasitology, Saskatoon, Canada) by oral gavage with an 8 French red rubber feeding tube into the distal oesophagus (Bartova et al., 2004). We chose the dosage of 500 oocysts because, in a pilot study with domestic ducks ($n = 14$; *Anas platyrhynchos domesticus*), all study animals given between 100 and 1000 oocysts developed strong serological titers against *T. gondii*, indicating a good probability of reliable infection (S.A. Elmore, unpublished data). Two geese were given 1.0 mL of 0.9% saline to serve as negative controls. To prevent contamination with undigested oocysts from the faeces of experimentally infected geese, the control geese were isolated by a plastic barrier for 36 h, and then allowed to co-mingle with the rest of the geese. Faeces were removed, but the floor was not sprayed to prevent the spread of oocysts. We obtained blood samples from all geese prior to inoculation and at weekly intervals thereafter. At 49 days p.i., all geese were anesthetized with isoflurane and euthanised with an i.v. injection of T-61 (0.5 mL/kg; Merck Animal Health, Intervet Canada Corp., Kirkland, Quebec, Canada). Upon necropsy, we collected the entire liver, spleen, brain, heart, kidneys and lungs, and stored tissues separately at −20 °C until further analysis. The experimental infection, daily care and maintenance were performed in compliance with institution regulations

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