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Toxoplasma gondii exposure in arctic-nesting geese: A multi-state occupancy framework and comparison of serological assays

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ABSTRACT

The zoonotic parasite, *Toxoplasma gondii*, has a worldwide distribution and a cosmopolitan suite of hosts. In arctic tundra regions, the definitive felid hosts are rare to absent and, while the complete transmission routes in such regions have yet to be fully elucidated, trophic and vertical routes are likely to be important. Wild birds are common intermediate hosts of *T. gondii*, and in the central Canadian arctic, geese are probable vectors of the parasite from temperate latitudes to the arctic regions. Our objective was to estimate seroprevalence of *T. gondii* in Ross's and Lesser Snow Geese from the Karrak Lake ecosystem in Nunavut, Canada. After harvesting geese by shotgun, we collected blood on filter paper strips and tested the eluate for *T. gondii* antibodies by indirect fluorescent antibody test (IFAT) and direct agglutination test (DAT). We estimated seroprevalence using a multi-state occupancy model, which reduced bias by accounting for imperfect detection, and compared these estimates to a naïve estimator. Ross's Geese had a 0.39 probability of seropositivity, while for Lesser Snow Geese the probability of positive for *T. gondii* antibodies was 0.36. IFAT had a higher antibody detection probability than DAT, but IFAT also had a higher probability of yielding ambiguous or unclassifiable results. The results of this study indicate that Ross's Geese and Lesser Snow Geese migrating to the Karrak Lake region of Nunavut are routinely exposed to *T. gondii* at some point in their lives and that they are likely intermediate hosts of the parasite. Also, we were able to enhance our estimation of *T. gondii* seroprevalence by using an occupancy approach that accounted for both false-negative and false-positive detections and by using multiple diagnostic tests in the absence of a gold standard serological assay for wild geese.

1. Introduction

The zoonotic parasite, *Toxoplasma gondii* has a worldwide distribution and a cosmopolitan suite of hosts; evidence of exposure was even recently detected in pinnipeds from Antarctica (Jensen et al., 2012). Oocyst-derived infections are the result of environmental contamination by felids, the definitive hosts of *T. gondii* (Dubey et al., 1970). In arctic tundra regions, felids are rare to absent and, while the complete transmission routes in such regions have yet to be fully elucidated, trophic routes and transmission from mother to offspring (vertical transmission) are likely to be important (McDonald et al., 1990; Messier et al., 2009).

Wild birds are common intermediate hosts of *T. gondii* (Dubey, 2002). The most common infective forms of *T. gondii* for herbivorous birds, such as geese, are sporulated oocysts, which can be found in contaminated water bodies or soil (Dubey, 2009) to which these birds may be exposed. When high densities of waterfowl congregate in a contaminated environment, oral transmission is likely to occur. If the birds become intermediate hosts of the parasite, they will eventually develop cysts in their organs and musculature. The population-level significance of infection in wild birds is unclear, but avian mortality has been reported in heavily infected birds (Dubey, 2001; Work et al., 2002). Arctic-nesting geese are probable vectors of the parasite from temperate latitudes

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to the arctic region of Svalbard (Prestrud et al., 2007) and likely along other migratory routes as well.

In North America, Ross's Geese (*Chen rossii*) and Lesser Snow Geese (*Chen caerulescens*) are two common arctic-nesting geese that overwinter in the southern United States and migrate through the midcontinent of North America to breeding areas in arctic Canada and Alaska (Alisauskas et al., 2011). Thus, these two populations of arctic-nesting geese are sympatric with both domestic and wild felids for at least 8 months of the year (September to April), but are thought to be largely allopatric to felids for about 4 months (May to August), when in the Arctic. Felid and goose ranges may overlap in the southern portions of the breeding range where about 10% of subarctic geese nest at known colonies near Hudson or James Bay, but 90% nest well above treeline, such as near Queen Maud Gulf, Southampton Island and Baffin Island (Alisauskas et al., 2011); these regions are thought to be largely or completely absent of felid populations. These geese are considered overabundant (Leafloor et al., 2012) because of demonstrated impacts on arctic vegetation (Abraham et al., 2012) from overgrazing (Alisauskas et al., 2012). Such high goose densities across an expanding range represent an increasing potential for seasonal *T. gondii* introduction to wildlife predators in ecosystems of both arctic and temperate latitudes. However, no estimates exist for the seroprevalence of *T. gondii* in these goose populations. Potential predators of geese in the Karrak Lake ecosystem include arctic foxes (*Alopex lagopus*), wolverines (*Gulo gulo*), wolves (*Canis lupus*), and barren-ground grizzly bears (*Ursus arctos horribilis*), and it is possible that infected geese could transmit *T. gondii* to these animals (Bantle and Alisauskas, 1998; Wiebe et al., 2009).

Most evidence for the occurrence of *T. gondii* in wildlife is obtained through serological tests, which, while providing limited information on current infection status, can be useful tools in determining exposure within a population. Filter paper blood collection is a technique that is increasingly used for post-mortem antibody detection in wildlife (Jakubek et al., 2012; Aston et al., 2014). The technique is especially useful in remote areas where sera cannot be refrigerated or frozen, and is commonly used in wild waterfowl (Maksimov et al., 2011). The direct agglutination test (DAT; equivalent to modified agglutination test (MAT)), is a widely used serological test for wildlife exposure to *T. gondii* because it is flexible for use in multiple species and can also be used with eluate from blood stored on filter paper (Jakubek et al., 2012). Although often described as sensitive and specific in wildlife serological applications (Hollings et al., 2013), the DAT has not been formally validated for wildlife and performance can vary among different species (Macrí et al., 2009). Indirect fluorescent antibody tests (IFATs) are also used with wildlife sera (Miller et al., 2002; Dabritz et al., 2008), but their use has been limited to animals for which a taxon-specific secondary antibody has been produced. The use of IFAT with eluate from blood-soaked filter paper is not often reported in *T. gondii* diagnostics, but is commonly used for other types of antibody detection in waterfowl (Maksimov et al., 2011). Both assays have subjective cut-off values based on visual inspection, which suggests the potential exists for misclassification and biased reporting of seroprevalence. In a comparison between IFAT and MAT, Macrí et al. (2009) reported 97.8% sensitivity in cat serum by MAT (with IFAT as the comparative test), but only 73.4% sensitivity in dog serum by MAT. In this case, the IFAT was considered the "gold standard" for comparison by the MAT.

The risk of *T. gondii* transmission from geese to other wildlife populations and people emphasizes the need for reliable parameter estimates from serosurvey data (McClintock et al., 2010). Observation error due to non-detection is not commonly accounted for in prevalence estimates from wildlife disease studies, although the increasing use of occupancy modeling approaches shows more attention to this concern (e.g., Gómez-Diaz et al., 2010; McClintock et al., 2010, Lachish et al., 2011, Eads et al., 2013). Occupancy approaches are analogous to mark-recapture analyses from wildlife biology and were originally used to estimate the probability of occurrence of cryptic or rare species within habitats where they may be detected imperfectly (MacKenzie et al., 2006). These approaches are useful in wildlife disease ecology because they acknowledge that detection is imperfect and account for this uncertainty in parameter estimates of disease prevalence (McClintock et al., 2010, Lachish et al., 2011). Because most wildlife serological assays are not formally validated and thus have no information on test sensitivity and specificity, occupancy approaches provide a method for quantifying some of the uncertainty in the diagnostic system.

Under a typical occupancy framework, multiple randomly selected 'sites' are repeatedly surveyed within a time frame where the occupancy state (species present or species absent) does not change. Surveys, or replicates, can be conducted at multiple times or at the same time by multiple independent observers or different detection methods. These replicates at each site enable estimation of two parameters: occupancy, defined as the probability that a site is occupied by the species of interest, and detection probability, the probability that the species is detected during a given survey (replicate), given the site is occupied (MacKenzie et al., 2006). In our application, diagnostic samples are 'sites' (i.e., an eluate produced from blood-soaked filter paper taken from each goose) and the species of interest are antibodies against *T. gondii*, and the replicates are repeated assays (DAT or IFAT) performed on each sample.

In more complex occupancy models, such as those handling multiple occupied states or multiple scales, additional parameters can be estimated. Traditional static occupancy models, in a diagnostic context, insulate prevalence estimates against false-negatives but these models assume that false-positive results do not occur. Yet, in all serological assays, and especially with samples from wildlife species, there is a risk of cross-reactivity with unknown non-target antibodies, which could lead to ambiguous test results. Results from the IFAT and DAT are subject to observer experience and opinion, which might cause ambiguous test results to be misclassified, leading to false-positive results. In this paper we utilized a generic multi-state occupancy approach (Nichols et al., 2007) and interpret model parameters in a disease ecology context. A similar approach was also proposed by Miller et al. (2011) and both approaches account for both false-positive and false-negative observational errors.

We hypothesized that Ross's and Lesser Snow Geese are routinely exposed to *T. gondii* because they overwinter in and migrate to areas where *T. gondii* oocysts are likely to be present in the environment. Our main objectives in this study were to: (1) estimate seroprevalence in hunted Ross's and Lesser Snow Geese using a general static multi-state occupancy approach to account for both false-positive and false-negative observational errors, and (2) compare seroprevalence, estimated with occupancy models, to naïve estimates of seroprevalence that assume detection probability is complete and diagnosis is error-free. An additional objective was to evaluate whether species and/or sex had an effect on the probability of seropositivity in a given individual, suggesting apparent differences exist between the species in foraging behavior (Jonsson et al., 2013) or that androgens might suppress immune function, thus leading to increased parasite susceptibility in males (Owen-Ashley et al., 2004). We propose that using different serological assays with multiple replicates and modeling techniques that account for imperfect detection in wildlife samples will reduce bias in estimates of *T. gondii* seroprevalence.