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Latitudinal variability in the seroprevalence of antibodies against *Toxoplasma gondii* in non-migrant and Arctic migratory geese

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Abstract

Toxoplasma gondii is an intracellular coccidian parasite found worldwide and is known to infect virtually all warm-blooded animals. It requires a cat (family Felidae) to complete its full life cycle. Despite the absence of wild felids on the Arctic archipelago of Svalbard, *T. gondii* has been found in resident predators such as the arctic fox and polar bear. It has therefore been suggested that *T. gondii* may enter this ecosystem via migratory birds.The objective of this study was to identify locations where goose populations may become infected with *T. gondii*, and to investigate the dynamics of *T. gondii* specific antibodies. Single blood samples of both adults and juveniles were collected from selected goose species (*Anser anser, A. brachyrhynchus, Branta canadensis, B. leucopsis*) at Arctic brood-rearing areas in Russia and on Svalbard, and temperate wintering grounds in the Netherlands and Denmark (migratory populations) as well as temperate brood-rearing grounds (the Netherlands, non-migratory populations). A modified agglutination test was used on serum, for detection of antibodies against *T. gondii*. Occasional repeated annual sampling of individual adults was performed to determine the antibody dynamics. Adults were found seropositive at all locations (Arctic and temperate, brood-rearing and wintering grounds) with low seroprevalence in brood-rearing birds on temperate grounds. As no juvenile geese were found seropositive at any brood-rearing location, but nine month old geese were found seropositive during spring migration we conclude that geese, irrespective of species and migration, encounter *T. gondii* infection in wintering areas. In re-sampled birds on Svalbard significant seroreversion was observed, with 42% of seropositive adults showing no detectable antibodies after 12 months, while the proportion of seroconversion was only 3%. Modelled variation of seroprevalence with field data on antibody longevity and parasite transmission suggests seroprevalence

of a population within a range of 5.2% to 19.9%, in line with measured values. The high occurrence of seroreversion compared to the low occurrence of seroconversion hampers analysis of species- or site-specific patterns, but explains the absence of an increase in seroprevalence with age and the observed variation in antibody titre. These findings imply that even though infection rate is low, adults introduce *T. gondii* to the high Arctic ecosystem following infection in temperate regions.

Keywords: *Toxoplasma gondii*; geese; Arctic; seroreversion; modified agglutination test; *Branta leucopsis; Anser brachyrhynchus*; *Anser anser*; *Branta canadensis*

Introduction

Infectious diseases represent a significant threat to both human and animal populations. As a consequence, it is of great relevance to understand the infection dynamics and distribution of important zoonotic pathogens (Altizer et al., 2011). *Toxoplasma gondii* is a globally distributed coccidian protozoan (Dubey, 2010). Infection with *T. gondii* is one of the most common parasitic infections of warm-blooded animals worldwide, including humans (Dubey and Beattie, 1988; Tenter et al., 2000). A wide range of mammals and birds can serve as intermediate hosts, where asexual reproduction and tissue cyst formation occur. Intermediate hosts can be infected by ingestion of oocysts or tissue cysts, and in some cases by placental transmission. Sexual reproduction can only happen in the intestines of the definitive host and results in infective oocysts being shed with their faeces. Oocysts are essential for the transmission to a non-carnivorous host and are only shed by domestic cats and other felines (Dubey, 2010). As the cat population has developed parallel to the human population, there is a strong potential for *T.gondii* transmission in rural settings (Amendoeira et al., 2003). Oocysts have been found both in water and in soil samples around human dwellings (Weigel et al., 1999; Dubey, 2010) and may enter the marine environment through freshwater runoff or via sewage systems (Lindsay et al., 2003; Conrad et al., 2005). Here, the oocysts can travel long distances via physical and biological processes, the latter including ingestion by marine mammals or accumulation in filter feeding fish and bivalves (Arktush et al., 2003; Fayer et al., 2004; Miller et al., 2008; Massie et al., 2010).

Polar regions are isolated both by their extreme environment and remote position. Nevertheless, both in Arctic (Prestrud et al., 2007; Oksanen et al., 2009; Jensen et al 2010) and sub-Antarctic (Afonso et al., 2007) regions

individuals seropositive with *T. gondii* have been found. Clearly, *T. gondii* is found in areas not inhabited by its definitive host. For example, in the high Arctic Svalbard archipelago (78–81°N, 10–30°E), including the main island Spitsbergen, no wild felines are present and domestic cats are prohibited. Yet, *T. gondii* infection has been observed in resident top predators such as Arctic foxes (*Vulpes lagopus*) and Polar bears (*Ursus maritimus*) (Prestrud et al., 2007). Whether the initial infection is a result of oocysts transported via ocean currents or tissue cysts from migratory animals is unknown.

Ecosystems are connected via seasonal migrations (reviewed in Altizer et al., 2011). Along the flyway migratory birds may transport infectious disease agents (Bradley et al., 2005; Altizer et al., 2011) and Prestrud et al. (2007; 2008a; 2008b) suggested that *T. gondii* is brought to the Arctic by migratory birds. In support of this notion, 7% of migratory barnacle geese (*Branta leucopsis*) on Svalbard were found seropositive, whereas no resident herbivores such as Svalbard reindeer (*Rangifer tarandus platyrhynchus*) (n=390) or sibling voles (*Microtus epiroticus*) (n=361) were found seropositive, suggesting that *T. gondii* oocysts in the terrestrial ecosystem are not an important mode of transmission on Svalbard (Prestrud et al., 2007). In the same study, foxes captured at sites devoid of goose colonies showed lower seroprevalence than foxes captured close to goose colonies (Prestrud et al., 2007). In addition, as the Svalbard goose populations have doubled over the last decades (Fox et al., 2010), so has the prevalence of antibodies to *T. gondii* in Svalbard's polar bears (Oksanen et al., 2009; Jensen et al., 2010).

The objective of this study was to determine *T. gondii* seroprevalence in goose populations at various locations in order to assess the role of migratory birds as vector of *T. gondii* to isolated Arctic ecosystems. As juveniles are infection naïve at birth and limited in their habitat exposure, they were

specifically targeted to determine the area of infection. To this end, we sampled adults and juveniles of two arctic migratory goose species; the barnacle goose (*Branta leucopsis*) and the pink-footed goose (*Anser brachyrhynchus*), at Arctic breeding and temperate wintering grounds. To expand the sampling of the temperate environment, resident Dutch populations of barnacle, Canada (*B. canadensis*) and greylag geese (*A. anser*) were included, sampled during the brood-rearing period.

Our main assumption was that the likelihood of infection with *T. gondii* is high in areas with suspected high densities of cats, and that infection results in increased specific antibody levels in the blood. Both adult and juvenile geese would consequently show higher seroprevalence at temperate, compared to Arctic, locations. Therefore, the following hypotheses were tested: *i*) in arctic areas only adults are seropositive; *ii*) in temperate areas both adults and juveniles are seropositive, and both show a higher titre of antibodies in the blood: *iii*) the proportion of seropositive individuals increases with age.

Materials and methods

Blood samples were collected between 2006 and 2010 at four locations: Svalbard (1), Nenets Autonomous Okrug NW Russia (2), Denmark (3) and the Netherlands (4) (Fig. 1). In the Arctic, birds were sampled on Spitsbergen, the western island of Svalbard (79°N/12°E) and in NW Russia at Tobseda (68°N/52°E) and Kolguev (69°N/49°E). In Denmark all birds were sampled during spring staging at Vest Stadil Fjord (58°N/8°E). In the Netherlands birds were sampled in the provinces of Groningen, Friesland, Gelderland, Noord and Zuid Holland (52°N/4°E - 53°N/6°E) during summer and in Friesland (53°N/6°E) during winter staging (Table 1).

In total four species of wild geese were investigated: barnacle goose (n = 1543), pink-footed goose (n = 787), greylag goose (n = 266) and Canada goose (n = 79) (Table 1). In the Arctic, barnacle geese were sampled from populations using two different flyways; those migrating from Arctic Russia to the Netherlands and those migrating from Svalbard to Scotland (Black et al., 2007). A second species sampled in the Arctic was the pink-footed goose migrating from Svalbard to Denmark-the Netherlands-Belgium (Madsen et al. 1999). The pink-footed goose is sharing habitat with both the earlier mentioned migratory barnacle goose populations. The migratory and non-migratory populations have overlapping winter habitats though do not fully mix during winter staging (van der Jeugd et al., 2001).

Both juvenile and adult birds were sampled at all locations (Table 1). Juvenile birds caught in Denmark were a maximum of 9 months old. From the population of barnacle geese on Svalbard age was known for 739 birds. The sample collection included 108 birds sampled more than once during the period 2006-2010, resulting in a total of 144 non-overlapping records (Table 2).

Flightless birds were captured during summer by being herded into a key-hole shaped net raised on land. Birds were captured on their winter and spring staging grounds by means of cannon netting. From each bird a blood sample of 0.2-2 ml was taken from the brachial vein, using non-heparinised equipment (syringe and needle). The blood was allowed to coagulate followed by centrifugation (10,000 rpm, 7 min) within 24 hours of sampling in order to separate red blood cells from serum. The serum was subsequently stored at -20 °C until analysis. All sampling was conducted according to national and international animal regulations, acts and laws.

The presence of antibodies against *T. gondii* in individual serum samples was tested at 1:40 dilutions, using a commercially available modified agglutination test (Toxo-Screen DA kit, bioMerieux S.A., Marcy-l'Etoile, France) following the manufacturer's instructions. Agglutinated samples at cut-off 1:40 were by eye defined seropositive and further analyzed at dilutions of 1:160, 1:640 and 1:2560 to assess antibody concentration. Incidental testing on lower titres proved hard to interpret (results not shown) which was confirmed by Prestrud (2008). Prestrud (2008) and Oksanen et al. (2009) compared results from titres of 1:10 to 1:80 and 1:25 to 1:40 (respectively) and found agreement between the results.

Using re-caught individuals the annual proportion of seroconverting individuals (prop. λ), and the proportion seroreverting individuals (prop. δ) were calculated (hereafter λ and δ respectively). These values were used in an iteration predicting seroprevalence (γ) in a population of 1000 individuals after 20 years. Together with random generated numbers it was decided if seroconversion has occurred, and if so, if seroreversion would occur the following year. Repeatedly sampled individuals from non-overlapping periods longer than one year gave additional values for λ and δ. Using these additional

values for λ and δ, the variation within the parameters resulted in a range of potential γ.

The differences in seroprevalence between various groups (site, age, species, and gender) were tested using a chi-square test. A Fisher's exact test was included when sample size in one of the observed groups was below 5. Non-parametric tests were used to compare the antibody concentration between groups, Mann-Whitney U-test comparing two groups and a Kruskal-Wallis ANOVA test comparing three or more groups. Binary logistic regressions were used to estimate the effect of age on the seroprevalence in the population. All statistical tests were performed using SPSS, version 16 (SPSS INC., Chicago, IL, USA).

Results

All juveniles sampled on brood-rearing grounds were found seronegative, (n=699) (Table 1). At the Arctic locations (Svalbard and Russia) 10.4% of all adults (n=1136) were seropositive. A similar seroprevalence was found during summer at temperate sites: 8.3% of all adults (n=302) were seropositive. The seroprevalence varied for different species and seasonal groups, ranging from 6.5% in pink-footed geese on Arctic breeding grounds to 25% in migratory barnacle geese on wintering grounds in the Netherlands (Table 1). On Svalbard the seroprevalence of pink-footed geese was significantly lower than of barnacle geese (X^2=8.170, p=0.006). Between the different populations of barnacle geese there was no significant difference in seroprevalence found for the various locations. When following goose populations from Arctic grounds to temperate wintering and spring grounds, the seroprevalence for pink-footed geese during spring staging was higher than during brood rearing (tested one-sided 6.5% to 11.9% X^2=3.617,p=0.036) while this increase was non-significant for adult barnacle geese (17.8% to 25.0% X^2=0.495) (Table 1). Of the 114 nine month old pink-footed goose juveniles sampled on spring staging grounds in Denmark, 11 were seropositive. Within the spring staging population, no difference was found comparing juveniles (9.6%) to adults (11.9%) (X^2=0.265, p=0.622) (Table 1).

Re-sampled individuals over a period of one to three years revealed the proportion of seroreversion (δ) and seroconversion (λ) (Table 2). For adults sampled negative at t=0 (n=64) λ was calculated to 3.1 % over one year while δ was more than ten times higher, at 41.7%. Other time intervals between repeatedly sampled individuals gave additional independent estimates for λ and δ (Table 2). In all cases, seroreversion was much higher than seroconversion. These findings were integrated in a simple model. This model

estimated combinations of λ and δ resulting in given values of seroprevalence (γ) (Fig. 2A) or in observed transitions (seroreversion or seroconversion) in γ over a defined time interval of one, two or three years (Fig. 2B). Both simulations combined suggest values of λ ranging from 2.5% to 5% and δ ranging from 20% to 45%, giving a span of γ in the population between 5.2% and 19.9%. We measured a γ range of 6.5% to 17.7% in the sampled population, with a highest value of 25% for a small sample size of 16.

The variance of antibody concentration within an individual showed that no seronegative individuals had antibody titres higher than 1:160 the following or previous year. The individuals with the highest measured concentration the first year (seropositive at dilution 1:640 at t=0) stayed seropositive the following year (1:160 at t=1). A seropositive individual (1:40 at t=0) showed the highest titre measured of 1:2560 the following year. No individuals maintained the minimum threshold level of 1:40 between the years.

To investigate the effect of age, individuals were grouped into four age classes. Number of positive individuals and total sample size were ($n_{positive}/n_{total\ sample}$): class <1, 0/158=0; class 1-5, 1/24=4.2%; class 6-10, 3/40=7.5%; class >10: 16/122=13.1%. There was a significant increase in seroprevalence over age (X^2= 25.276, p=0.000). This increase was not significant if only adults were considered. The number of seropositive individuals per age class was modelled by randomly assigning annual seroconversion (3.1%) to seronegative individuals and seroreversion (41.7%) to seropositive individuals in a population starting with 1000 naive individuals. The model showed that the number of individuals which have been seropositive over their life time increases with age while the number of seropositive individuals in a given age class stabilized for values of gamma (Fig. 3). At an age of 20 years, 466 individuals of 1000 have at least once been infected (Fig.3, dotted line),

nevertheless only 69 individuals were seropositive at the same age (Fig.3, full line). The average numbers of infections per once infected individual was found to increase from 1.00 to 1.33 times over a 20 year period (Fig. 3 bars). The results are based on 100 iterations.

Discussion

We hypothesized that both adult and juvenile geese would show higher seroprevalence at temperate, compared to Arctic, locations. We expected no positive juveniles in the Arctic. If only one Arctic juvenile had been confirmed seropositive, the marine infection pathway would have to be considered as relevant transport route of *T. gondii* to the high Arctic. Instead, the chance of sampling a seropositive juvenile on temperate breeding grounds was expected to be high due to felines shedding oocysts on the grasslands.

The absence of seropositive juveniles on temperate breeding grounds seemed counterintuitive. However, the exposure time for goslings to become infected before being sampled on the breeding grounds was on average only 35 days. With the observed annual proportion of seroconverting individuals of 3.1% and assuming a constant chance of infection over a year, the chance of sampling a seropositive gosling would be 1 out of 336 while our sample size of juveniles is only 312 individuals (see Table 1). In addition, the environment that flightless birds encounter is possibly less contaminated with oocysts than the environment visited by flying birds. Due to their exposure and vulnerability to predation, flightless birds inhabit areas with a lower predator encounter risk (Madsen and Mortensen, 1987; Kahlert et al., 1996).Such an area is often close to water, where cats are supposedly less likely to hunt. Systematic goose counts in the Netherlands during 2005-2011 showed that while only 1 cat was observed close to a water body, 142 cats were seen on grasslands (Voslamber, unpublished data). The observer counted all geese and mammals weekly in a predefined area of 1500 Ha, subdivided in fields of settlements, grasslands and shore land. During winter when the geese can fly, they forage on grasslands where they may be subjected to a higher infection risk.

Seroprevalence did not increase with age of tested individuals when juveniles were excluded. Rate of (re-)infection is seemingly low in relation to seroreversion. Our results suggest that the species investigated here undergo a more rapid seroreversion than previously known. The effect of seroreversion on immunity needs to be unravelled before definite statements can be made about the status of infection. As the exact relation between immunity and seroprevalence is unknown, in this study the status of infection is based on seropositive individuals.

Based on field values of infection and antibody dynamics a seroprevalence of 7% was calculated in the Arctic barnacle goose population. A value of 7% for seroprevalence is in the lower end found in our study, though it fits very well with previous work of Prestrud et al. (2007) in the same population. When integrating measured values from years with more than one winter between sample occasions the range of possible seroconversion and reversion increased and so did the variation of stable seroprevalence (from 5.2% to 19.9%), matching measured field values well.

Infection risk might be considerable higher than the 3.1% measured over a full year in barnacle geese. Juvenile pink-footed geese acquired seroprevalence of 9.6% over a lifetime of nine months. The observed values of seroprevalence in adult pink-footed geese (11.9%) would then rely on an almost twice as high species-specific seroreversion of 71%. However with the variation in sample sizes and potential species specific values of seroreversion, further interpretation would become speculative.

As the antibody titre in the blood is expected to increase when an individual is (re-)infected, we expected higher antibody titre on temperate grounds, especially during winter. However our data did not support this. On the other hand, within a migrating population a trend for higher antibody titres

on temperate grounds than on Arctic grounds was found both over the migration route within a species (summer vs. winter) as well as when comparing Arctic breeders to temperate breeders. Additionally, of all positive birds at temperate brood-rearing grounds 24% were positive at the highest titre (1:2560) compared to only 6% at arctic grounds. The largest fraction (35%) of birds at Arctic breeding grounds was positive at the lowest antibody titre (1:40). The different patterns observed in the concentration titres support our hypothesis that infection is occurring in temperate regions.

The average number of infection events per infected individual increases from 1.0 time for a one year old to a maximum of 1.3 times for an individual of 20 years. Re-infection would probably result in higher antibody concentrations with a slightly lower sero reversion rate. We have no significant evidence for this statement but that could result in a somewhat higher prevalence with age, which is hinted at in the non-significant highest value of seroprevalence in the age class older than 10 years.

Identification of the source of infection is vital for understanding how the parasite is infecting new ecosystems. The observed trend towards increased seroprevalence as well as antibody titre on wintering grounds suggested that the wintering grounds are a source of (re-)infection. However, geographical differences were not always significant, which can be explained by the big variation in sample numbers in combination with small differences in seroprevalence. For barnacle geese, we calculated the required sample size for the wintering population to obtain significant differences at 25% seroprevalence with the empiric results from the three summersampled sites. The required sample sizes were n=1108 for Arctic Russia on a seroprevalence of 17.8%, n=52 for Arctic Svalbard on a seroprevalence of 14.8% and n=20 for non-migratory geese in the Netherlands on a seroprevalence of 8.7%. This

clearly shows the sensitivity for sample size in combination with observed differences. Within the same flyway from Arctic Russia to the Netherlands, sample size for significant results would be extremely large. Nevertheless, if we expected a seasonal difference over the flyway, for pink-footed geese the seroprevalence in the Arctic was significantly lower than on winter and spring staging grounds. When considering variability within one season (brood-rearing) there was always a difference between Arctic and temperate regions. Surprisingly, the seroprevalence for Arctic breeding barnacle geese was significantly higher than the Dutch breeding equivalent. On the contrary, pink-footed geese breeding on Svalbard had the lowest seroprevalence measured which corroborate the hypothesis of northern regions carrying a lower disease risk (Piersma, 1997).

More evenly represented sampling populations would increase the rigidity to this study. The majority of the field campaigns were aimed on summer populations. However, an increased number of winter staging birds from both the Netherlands and Scotland would have contributed to the understanding for the seasonal variation of seroprevalence, antibody dynamics and the possible infection location in the light of the annual cycle.

In conclusion, infection with *T. gondii* is likely to happen on temperate grounds during the winter period when the birds are able to fly. Infected birds transport the parasite to Arctic breeding grounds, and if predated the parasite can enter the ecosystem. As no naive Arctic birds (juveniles) were found seropositive in the Arctic we have no support for an alternative transmission pathway of *T. gondii* to the high Arctic. We found the proportion of individuals seroreverting over a time interval of one year being >40%, while the proportion seroconverting was a magnitude lower. Using an iteration based on values from individuals sampled in multiple years we predicted the expected level of

seroprevalence in a population which corresponded well within the range of measured values.

This study advances our understanding of ecological drivers behind the occurrence of spatial and temporal variation of *T. gondii* within two naturally defined geographical areas. However, future studies should focus on achieving a full picture of the flyway to determine the antibody dynamics. In general, juveniles must be sampled in greater numbers to directly link site of infection with environment.

Conflict of interest statement

There are no known personal relationships with other people or organizations that could inappropriately influence this work. To the knowledge of the authors there are no conflicts of interest.

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Legends with figures caption

Figure 1

The four main sample locations with species and seasons sampled at each location. Location one and two fall within the Arctic Circle (66°N) while location three and four are located in the temperate zone. Resident (non-migratory) populations are indicated by a star (*). Species are abbreviated as follows: AnAn, *Anser anser*, greylag goose; AnBr, *Anser brachyrhynchus,* pink-footed goose; BrCa, *Branta canadensis,* Canada goose; BrLe, *Branta leucopsis*, barnacle goose.

Figure 2 A-B

Modelled combinations of delta (δ) and lambda (λ) resulting in: a set value of seroprevalence (γ) (panel A); an observed transition over a given time period of 1, 2, or 3 years, were each line shows conversion (a, b, c) or reversion (d, e, f) (panel B). In the range of values for λ: 0.025 to 0.050 and δ: 0.200 to 0.450, calculated values for γ range from 5.2% to 19.9%. Input-values are presented in Table 2.

Figure 3

Modelled numbers of infected individuals per age groups. Lines represent individuals in a population of 1000 individuals in a given age class (left y-axis). Bars show average numbers of infection per once infected individuals (right y-axis). Individuals were in each age class assigned to seroconversion and/or – reversion based on random numbers and measured population values. The iteration is run 100 times.

species	season	location	% infected adults (n)		% infected juveniles (n)	
B. leucopsis	summer	Arctic (SV)	14.8	(811)	0.0	(259)
	summer	Arctic (RU)	17.8	(157)	0.0	(28)
	summer	Temperate (NL)*	8.7	(103)	0.0	(166)
	winter	Temperate (NL)	25.0	(16)	0.0	(3)
A. brachyrhynchus	summer	Arctic (SV)	6.5	(168)	0.0	(100)
	spring	Temperate (DK)	11.9	(405)	9.6	(114)
A. anser	summer	Temperate (NL)*	8.1	(161)	0.0	(105)
B. canadensis	summer	Temperate (NL)*	7.9	(38)	0.0	(41)

Table 1. Percentage of individuals with detectable antibodies to *T. gondii* per species, season and sample location. Resident populations are marked with a star (*) and are only sampled during summer (June-July). Migratory populations are sampled in the Arctic during summer (July-August) and at temperate regions in winter (January) and spring (March). All birds caught during summer were moulting at brood-rearing grounds. Birds younger than one calendar year are referred to as juveniles. Locations are abbreviated as follows: SV, Svalbard; RU, Russia; NL, the Netherlands and DK, Denmark.

Transition negative -> positive	Time interval (year)	N samples negative at t=0	N samples positive at t=y	Calculated proportion converting (λ)
	0 -> 1	64	2	0.0313
	0 -> 2	39	3	0.0480
	0 -> 3	15	1	0.0335
	0 -> 4	2	0	n.a.
positive -> negative		positive at t=0	negative at t=y	reverting (δ)
	0-> 1	12	5	0.4167
	0 -> 2	10	5	0.2995
	0 -> 3	2	1	0.2160

TABLE 2. Conversion and reversion in individuals sampled at two different time intervals. In 144 cases an individual was re-sampled 1, 2, 3 or 4 years later (t=y). Only non-overlapping periods were considered.