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ORIGINAL PAPER

Toxoplasmosis in geese and detection of two new atypical *Toxoplasma gondii* strains from naturally infected Canada geese (*Branta canadensis*)

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Abstract Wild birds are important in the epidemiology of toxoplasmosis because they can serve as reservoir hosts, and vectors of zoonotic pathogens including *Toxoplasma gondii*. Canada goose (*Branta canadensis*) is the most widespread geese in North America. Little is known concerning *T. gondii* infection in both migratory, and local resident populations of Canada geese. Here, we evaluated the seroprevalence, isolation, and genetic characterization of viable *T. gondii* isolates from a migratory population of Canada geese. Antibodies against *T. gondii* were detected in 12 of 169 Canada geese using the modified agglutination test (MAT, cutoff 1:25). The hearts of 12 seropositive geese were bioassayed in mice for isolation of *T. gondii*. Viable parasites were isolated from eight. One isolate was obtained from a seropositive goose by both bioassays in mice, and in a cat; the cat fed infected heart excreted *T. gondii* oocysts. Additionally, one isolate was obtained from a pool of four seronegative (<1:25) geese by bioassay in a cat. The *T. gondii* isolates were further propagated in cell culture, and DNA extracted from cell culture-derived tachyzoites were characterized using 10 polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) genetic markers (SAG1, 5′ and 3′SAG2, alt.SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico). The results revealed five different genotypes. ToxoDB PCR-RFLP genotype #1 (type II) in one isolate, genotype #2 (type III) in four isolates, genotype #4 in two isolates, and two new genotypes (ToxoDB PCR-RFLP genotype #266 in one isolate and #267 in one isolate) were identified. These results indicate genetic diversity of *T. gondii* strains in the Canada geese, and this migratory bird might provide a mechanism of *T. gondii* transmission at great distances from where an infection was acquired.

Keywords Canada geese · *Toxoplasma gondii* · Bioassay · Genotype

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Introduction

The protozoan *Toxoplasma gondii* infects virtually all warm-blooded animals, including birds, humans, livestock, and marine mammals (Dubey 2010). The consumption of raw or undercooked meat infected with *T. gondii* is considered an important source of infection in humans (Dubey 2010). Canada goose (*Branta canadensis*), the most widespread goose in North America, is found in every contiguous US states and Canadian provinces at one time of the year or another. Their populations increased 4.5 folds from 1.26 million in 1970 to 5.69 million in 2012 (Dolbeer et al. 2014). Canada geese are wild, hunted for their meat for human consumption. Geese can serve as a reservoir host and vector host of *T. gondii* to infect the other hosts, and the new ecosystems along the flyway. Little is known of *T. gondii* infection in Canada geese. Here, we report the serology, isolation, and genetic characterization of *T. gondii* from Canada geese. Additionally, we

reviewed worldwide surveys of *T. gondii* infections in different species of geese.

Materials and methods

Sample collection and serology

The personnel of the US Department of Agriculture Farm Services, Beltsville, Maryland, hunted 169 Canada geese during September 2014 to March 2015 because of crop destruction. The geese belonged to a migrant population that typically summered in northern North America and flew south when cold weather arrived. These geese were submitted to the Animal Parasitic Diseases Laboratory, Beltsville, Maryland, for evaluation of protozoan infection. The heart and blood were collected from individual geese.

The sera were tested for antibodies against *T. gondii* by the modified agglutination test (MAT) in order to evaluate the *T. gondii* prevalence as described previously (Dubey and Desmonts 1987). Briefly, the sera were diluted two fold serially from 1:25 to 1:200. The titer of 1:25 or higher was considered as *T. gondii*-positive.

Isolation of *T. gondii* by bioassay in mice and cats

Bioassay in mice

After serological screening, the MAT positive geese were bioassayed in mice for isolation of *T. gondii*. The heart of individual goose (30 g) were homogenized, digested in acidic pepsin solution, and washed, and aliquots of homogenates were inoculated subcutaneously into 3–4 outbred Swiss Webster (SW) albino female mice and/or 1 gamma interferon gene knock out (KO) mouse (Dubey 2010). Mice were bled on day 45 post inoculation (p.i.), and a 1:25 dilution of serum was tested for *T. gondii* antibodies by MAT as described previously. Tissues imprints of lungs and brains of inoculated mice that died or killed on 46 days p.i. were examined for *T. gondii* tachyzoites or tissue cysts (Dubey 2010). The inoculated mice were considered infected with *T. gondii* when tachyzoites and/or tissue cysts were found in tissues, and/or antibodies to *T. gondii* were demonstrable in their sera.

Bioassay in cat

To detect a low level of *T. gondii* in goose heart, cats were used as bioassay because they can excrete millions of oocysts after ingesting even a few bradyzoites (Dubey 2010). The oocysts can be detected easily by fecal examination. Two *T. gondii*-free cats (#30, #39) from an indoor colony (Dubey 1995) were used, cat #30 was fed heart tissue of a seropositive (MAT ≥200) goose #92. Cat #39 was fed tissue of hearts pooled from four seronegative (<1:25) geese. Feces of cats were collected daily from day 4 to 14 after feeding goose hearts. Feces were floated in sucrose solution, and after microscopic examination, the floats were mixed with sulfuric acid, aerated, and stored at 4 °C as described previously (Dubey 2010). Sporulated oocysts were neutralized with 3.3% NaOH, diluted with PBS, and inoculated orally in to two SW mice. The recipient mice were examined for *T. gondii* infection as described above.

In vitro cultivation

African green monkey kidney fibroblast cells (CV-1 cell line) were utilized for in vitro cultivation of *T. gondii*. Lung or brain tissues of bioassayed mice that were found positive for *T. gondii* were homogenized in aqueous antibiotics (1000 units penicillin, 100 μg streptomycin/ml saline), and seeded into CV-1 cell culture flasks. Tachyzoites from successfully grown cultures were harvested from the medium for DNA isolation, and infected host cells were cryopreserved in liquid nitrogen for future studies as described previously (Dubey 2010).

Genetic characterization

T. gondii DNA was extracted from cell culture-derived tachyzoites using DNeasy® Blood and Tissue Kit (QIAGEN, Valencia, CA) according to manufacturer's instruction. DNA quantification and quality were determined by NanoDrop Lite Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). The multilocus polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) typing using 10 genetic markers: SAG1, SAG2 (5′-3′SAG2, and alt.SAG2), SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico, was done by following the procedures as described previously (Su et al. 2010). Appropriate positive and negative controls were included in all analyses.

Results

Antibodies to *T. gondii* were detected in 12 (7.1%) of 169 geese with MAT titers of 1:25 in eight, 1:50 in two, 1:100 in one, and ≥1:200 in one. Viable *T. gondii* was isolated by mouse bioassay of eight of 12 geese (Table 1). All SW mice inoculated with digest of geese hearts remained asymptomatic, and tissue cysts were found in the brains of all seropositive mice. The KO mice inoculated with hearts digests of five geese died of acute toxoplasmosis, and tachyzoites were found in their lungs (Table 1). Both cats fed goose hearts excreted *T. gondii* oocysts. One isolate was obtained by both bioassays in mice and in cat from a goose with MAT ≥200. Another one isolate was obtained by

Table 1 *Toxoplasma gondii* bioassay from Canada goose hunted in Maryland, USA

Original ID	MAT titer	Bioassay[a]		Isolate designation
		SW	KO	
Goose #24	25	0/3	1/1	TgGooseUS1
Goose #28	25	2/3	0/1	TgGooseUS2
Goose #29	25	3/3	0/0	TgGooseUS3
Goose #82	50	4/4	1/1	TgGooseUS4
Goose #88	25	0/4	1/1	TgGooseUS5
Goose #92 (cat# 30)	≥200	1/4	1/1	TgGooseUS6
Goose #108	50	4/4	1/1	TgGooseUS7
Goose #169	25	4/4	0/1	TgGooseUS8
Goose #131, 133–135 (cat #39)	<25	–	–	TgGooseUS9

SW Swiss Webster outbred albino mice, *KO* interferon gamma knockout mice

[a] No. of mice infected with *T. gondii*/no. of mice inoculated

cat bioassay from a pool of four seronegative (<1:25) geese (Table 1).

Lung homogenates from KO mice that died (or euthanized when ill) after inoculation with geese hearts were seeded directly onto cell cultures for propagating tachyzoites. Brain homogenates from SW mice inoculated with hearts of three geese were subinoculated into KO mice, and when the KO mice died, their infected lung homogenates were seeded onto cell cultures. Sporulated oocysts from two cats were fed to SW mice, and when the mice became ill, they were euthanized, and their mesenteric lymph nodes containing tachyzoites were seeded onto cell cultures. Thus, all nine isolates were successfully propagated in cell cultures, and they were designated TgGooseUS1 to 9 (Tables 1 and 2).

Multilocus PCR-RFLP genotyping of nine *T. gondii* isolates revealed three previously recognized ToxoDB PCR-RFLP genotypes; #1, #2, and #4, and two new genotypes; #266, and #267 (Table 2).

Discussion

Canada geese populations have increased 4.5-folds from 1.26 million in 1970 to 5.69 million in 2012 (Dolbeer et al. 2014). Goose meat is an important cuisine in North America, and

Table 2 PCR-RFLP genotyping of *T. gondii* isolates from Canada goose collected in Maryland, USA

Strain ID	ToxoDB PCR-RFLP genotype no.	Genetic markers										
		SAG1	(5′-3′) SAG2	alt. SAG2	SAG3	BTUB	GRA6	c22-8	c29-2	L358	PK1	Apico
GT-1	#10 (type I)	I	I	I	I	I	I	I	I	I	I	I
PTG	#1 (type II)	II or III	II	II	II	II	II	II	II	II	II	II
CTG	#2 (type III)	II or III	III	III	III	III	III	III	III	III	III	III
MAS	#17	u-1	I	II	III	III	III	u-1	I	I	III	I
TgCgCa1	#66	I	II	II	III	II	II	II	u-1	I	u-2	I
TgCtBr5	#19	I	III	III	III	III	III	I	I	I	u-1	I
TgCtBr64	#111	I	I	u-1	III	III	III	u-1	I	III	III	I
TgRsCr1	#52	u-1	I	II	III	I	III	u-2	I	I	III	I
Present study												
TgGooseUS1	#266 (new)	II or III	III	III	III	III	I	III	III	I	I	III
TgGooseUS2, 8	#4	II or III	II	II	II	II	II	II	II	I	II	I
TgGooseUS3, 5, 6, 7	#2	II or III	III	III	III	III	III	III	III	III	III	III
TgGooseUS4	#267 (new)	I	I	I	III	III	III	II	I	III	III	III
TgGooseUS9	#1	II or III	II	II	II	II	II	II	ND	II	II	II

ND no data available

Table 3 Worldwide surveys of *T. gondii* infection in various species of geese

Species	Location	Test	Total no.	Prevalence	References
Canada geese (*Branta canadensis*)	Pennsylvania, USA	MAT	2	100 %	(Dubey et al. 2014)
	Svalbard, Russia, the Netherlands, Denmark	MAT	79	7.9 % in 38 adults	(Sandström et al. 2013)
	Illinois, USA	MAT	1	100 %	(Dubey et al. 2007)
	Mississippi, USA	Bioassay	4	1/4	(Dubey et al. 2004)
Magpie geese (*Anseranas semipalmata*)	Texas, USA	MAT, H, IHC	13	18.18 %	(Dubey et al. 2001)
Nene geese (*Nesochen sandvicensis*)	Molokai, Hawaii	MAT	21	48 %	(Work et al. 2015)
	Maui, Hawaii	MAT	31	23 %	(Work et al. 2015)
	Kauai, Hawaii	MAT	42	21 %	(Work et al. 2015)
	Hawaii, USA	H, IHC	2	100 %	(Work et al. 2002)
Lesser snow geese (*Chen caerulescens*)	Karrak Lake, Nunavut (Central Canadian Arctic)	ELISA	234	28.3 %	(Elmore et al. 2015)
	Karrak Lake, Nunavut (Central Canadian Arctic)	IFAT, DAT	121	25 %	(Elmore et al. 2014)
Ross's geese (*Chen rossi*)	Karrak Lake, Nunavut (Central Canadian Arctic)	ELISA	233	32.4 %	(Elmore et al. 2015)
	Karrak Lake, Nunavut (Central Canadian Arctic)	IFAT, DAT	123	26 %	(Elmore et al. 2014)
Barnacle geese (*Branta leucopsis*)	Svalbard, Russia, the Netherlands, Denmark	MAT	1543	8.5–25 % in adults	(Sandström et al. 2013)
	Svalbard, Norway	DAT	149	7.00 %	(Prestrud et al. 2007)
Pink-footed geese (*Anser brachyrhynchus*)	Svalbard, Russia, the Netherlands, Denmark	MAT	787	6.5–11.9 % in adults	(Sandström et al. 2013)
Greylag geese (*Anser anser*)	Svalbard, Russia, the Netherlands, Denmark	MAT	266	8.1 % in 161 adults	(Sandström et al. 2013)
	Lower Saxony, Germany	ELISA	373	25.2%	(Maksimov et al. 2011)
Domestic geese (*Anser domestica*)	Strakonice, Bohemia, Czech Republic	DT	32	15.60 %	(Literák and Hejlicek 1993)
	Three provinces, China	IHA	360	17.78 %	(Yan et al. 2011)
	Nanjing City, China	IHA	128	25.00 %	(Yan et al. 2011)
	Changchun City, China	IHA	95	13.70 %	(Yan et al. 2011)
	Guangdong Province, southern China	MAT	191[a]	14.14 %	(Yan et al. 2011)
	Guangdong Province, southern China	MAT	83[b]	16.87 %	(Yan et al. 2011)
Greater white-fronted geese (*Anser albifrons*)	Miyajima-numa, Japan	ELISA	51	7/51	(Murao et al. 2008)
	Miyajima-numa, Japan	ELISA	72	11/72	(Murao et al. 2008)
	Chukotka, Russia	ELISA	39	9/39	(Murao et al. 2008)
Taiga bean goose (*Anser fabalis*)	Kamchatka, Russia	ELISA	35	5/35	(Murao et al. 2008)
Geese, unspecified	Hainan province, China	IHA	600 blood 150 tissues	17 %	(Rong et al. 2014)
	Shenyang, northeastern China	MAT	128	4.7 %	(Yang et al. 2012)
	Czech Republic	IFAT	178	43 %	(Bártová et al. 2009)
	Lublin, Poland	DAT	34	5.90 %	(Sroka 2001)

MAT modified agglutination test, *H* histology, *IHC* immunohistochemical staining, *ELISA* enzyme-linked immunospecific assay, *IFAT* indirect fluorescent antibody test, *DAT* direct agglutination test, *DT* Sabin-Feldman dye test, *IHA* indirect hemagglutination test, *ND* no data

[a] Farm-bred

[b] House-bred

humans can become infected by eating raw or undercooked goose meat if it is infected by *T. gondii*. Hunters can themselves become infected if they inadvertently ingest tissues or fluids containing infectious parasites while dressing the carcasses or when undercooked goose meat is consumed. Leftover carcasses can be eaten by carnivores. *T. gondii* can travel long distances with infected geese and can enter in a new biologically and physically isolated ecosystem if eaten by other resident predators including felids that can excrete millions of environmentally resistant oocysts. It has been suggested that *T. gondii* entered in Arctic and sub-Arctic ecosystems via migratory birds (Elmore et al. 2014; Sandström et al. 2013). Geese are ground feeding birds; therefore, it is expected that they become infected postnatally by ingestion of sporulated *T. gondii* oocysts with feed or water.

In the USA, most geese are wild/free living, whereas in some countries, they are farmed for hunting and for human consumption. Worldwide serologic prevalence in different species of geese is summarized in Table 3. The variations in prevalence may be because of different serologic tests used, samples from different regions, and the type of geese surveyed. Nothing is known of the validity of the different serologic tests for detection of *T. gondii* antibodies in geese. More is known of the diagnostic value of MAT that we used than other serologic tests. The MAT has been evaluated in several avian species experimentally infected with *T. gondii*, but not in geese (Dubey 2010). Recently, its diagnostic efficacy was tested in 2066 free-range chickens (*Gallus domesticus*) from 19 countries by comparing MAT titer, and bioassay of tissues in mice and cats (Dubey et al. 2016). Viable *T. gondii* was isolated from 16 (15.2%) of 105 chickens with MAT titer of 1:5, and the isolation efficacy increased with MAT titer. Additionally, 23 cats fed hearts pooled from 802 chickens with MAT <1:5 (seronegative) did not excrete oocysts, supporting the validated of MAT (Dubey et al. 2016). In the present study, viable *T. gondii* was isolated from five geese with MAT titer of 1:25 (Table 1). The isolation of *T. gondii* from a pool of four geese each with MAT of <1:25 may be due to either they had not yet seroconverted or the titer was lower than <1:25; on repeat testing, the geese were negative at 1:10 dilution. The 1:5 dilutions were not tested because of the quality of serum; the samples were collected from the cavity surrounding the heart. These results suggested that even a low MAT titer may be indicative of *T. gondii* infection in geese as in chickens.

Little is known of clinical toxoplasmosis in geese. The geese sampled in the present study were hunted and apparently healthy. We are not aware of any report of clinical toxoplasmosis in migratory geese, including *B. canadensis*. Two captive magpie geese (*Anseranas semipalmata*) in a zoo died of acute toxoplasmosis (Dubey et al. 2001). The Hawaiian goose (Nene) (*Branta sandvicensis*) is listed as endangered. Toxoplasmosis-associated mortality was reported in 11 of 300 Hawaiian geese in the wild (Work et al. 2002, 2015). DNA isolated from frozen tissues of four Hawaiian geese with acute toxoplasmosis was genotyped (Work et al. 2016), and two new genotypes were discovered (Table 4). It is unknown if the two new genotypes of *T. gondii* contributed to mortality.

Until recently, *T. gondii* was considered clonal and grouped into three types: I, II, and III (Sibley and Ajioka 2008). Recent studies indicate a higher genetic diversity, especially among isolates from South America (Shwab et al. 2014). There are only few reports of genotyping of *T. gondii* isolates from migratory birds; all of them from the geese in the USA (Table 4). The detection of five different genotypes among nine isolates including two new atypical genotypes provide evidences of more strain diversity of *T. gondii* than it was thought previously.

Table 4 Summary of previous reports of *Toxoplasma gondii* genotypes and isolations from geese from the USA

Species	State	No. of viable *T. gondii* isolates	ToxoDB PCR-RFLP genotype no. (*n*)	References
Nene goose (*Nesochen sandvicensis*)	Hawaii	0[a]	#261 (3) #262 (1)	(Work et al. 2016)
Domestic goose (*Anser anser*)	Illinois	1	#1 (1)	(Dubey et al., 2007)
Canada goose (*Branta canadensis*)	Pennsylvania	1	#143 (1)	(Dubey et al., 2014)
	Mississippi	1	Type III by SAG2 (1)	(Dubey et al., 2004)
	Maryland	9	#1 (1) #2 (4) #4 (2) #266 (1) #267 (1)	Present study
Total		Eight genotypes in 16 strains	#1 (2), #2 (4), #4 (2), #143 (1), #261 (3), #262 (1), #266 (1), #267 (1), Type III by SAG2 (1)	

n number of *T. gondii* isolates

[a] DNA was extracted directly from tissues of four geese that died of toxoplasmosis

In conclusion, this article documents the exposure of *T. gondii* among the Canada geese populations migrating in Maryland, USA. The proportion of infected geese with different strains may be a source of *T. gondii* infection for humans and felids.

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Compliance with ethical standards All investigations reported here were approved by the institutional animal care and use protocol committee of the US Department of Agriculture.

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