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Isolation and characterization of new genetic types of *Toxoplasma gondii* and prevalence of *Trichinella murrelli* from black bear (*Ursus americanus*)

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ABSTRACT

Black bears (*Ursus americanus*) are hosts for two important zoonotic parasites, *Toxoplasma gondii* and *Trichinella* spp. and bears are hunted for human consumption in the USA. Little is known of the genetic diversity of *T. gondii* circulating in wildlife. In the present study, antibodies to *T. gondii* were found in juice from tongues of 17 (25.7%) of 66 wild black bear from Maryland during the hunting season of 2010 and 2011. Antibodies to *T. gondii* were assessed by the modified agglutination test. Tongues of 17 seropositive bears were bioassayed in mice and viable *T. gondii* was isolated from three samples. These three *T. gondii* isolates (TgBbMd1-3) were further propagated in cell culture and DNA isolated from culture-derived tachyzoites was characterized using 11 PCR-RFLP markers (SAG1, 5′- and 3′-SAG2, alt.SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1 and Apico). Results revealed three genotypes. TgBbMd1 is a Type 12 strain (ToxoDB PCR-RFLP genotype #4) and TgBbMd2 is ToxoDB PCR-RFLP genotype #216, and TgBbMd3 is a Type II clonal strain (ToxoDB PCR-RFLP genotype #1). The isolate TgBbMd2 was highly virulent for outbred Swiss Webster mice; all infected mice died of acute toxoplasmosis. Results indicate that mouse virulent strains of *T. gondii* are circulating in wildlife in the USA. These 66 tongues in addition to tongues collected during hunts in previous years were further investigated for the presence of muscle larvae of *Trichinella* spp. Tongues from 40 bears in 2005, 41 in 2006, 51 in 2007, 56 in 2008, 68 in 2009, 67 in 2010, and 66 in 2011 were subjected to digestion with pepsin/HCl and microscopic examination. Two bears were infected with *Trichinella* spp.; one in 2008 and one in 2009. Genotyping of collected muscle larvae revealed that the infecting species in both cases was *Trichinella murrelli.*

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1. Introduction

The protozoan *Toxoplasma gondii* infects virtually all warm-blooded animals, including birds, humans, livestock, and marine mammals (Dubey, 2010). In the USA, various surveys have found that 10–50% of the adult human population has been exposed to this parasite (reviewed in Dubey

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and Jones, 2008). Humans become infected post-natally by ingesting tissue cysts from undercooked meat, or by consuming food or drink contaminated with oocysts. However, only a small percentage of exposed adult humans or other animals develop clinical signs of disease. It is unknown whether the severity of toxoplasmosis in immunocompetent hosts is due to the parasite strain, host variability, or to other factors. Recently, attention has been focused on the genetic variability among *T. gondii* isolates from apparently healthy and sick hosts (Grigg and Sundar, 2009). Severe cases of toxoplasmosis have been reported in immunocompetent patients in association with atypical *T. gondii* genotypes (Ajzenberg et al., 2004; Demar et al., 2007; Elbez-Rubinstein et al., 2009; Grigg and Sundar, 2009; Vaudaux et al., 2010; Wendte et al., 2010). Little is known of the association between genotype and clinical disease in animals and humans in the USA (Dubey, 2010). A variant of Type II (NE-II) was recently found associated with prematurity and severe disease at birth in congenitally infected children in the USA (McLeod et al., 2012). Type II strains are the most prevalent in Europe and cause congenital toxoplasmosis in children (see Su et al., 2012).

Historically, *T. gondii* was considered to be clonal with low genetic diversity and grouped into three subtypes designated I, II, III (Dardé et al., 1992; Howe and Sibley, 1995; Ajzenberg et al., 2002a,b, 2004; Aubert et al., 2010). However, recent studies have revealed a greater genetic diversity of *T. gondii*, particularly in isolates from Brazil (Khan et al., 2011; Su et al., 2012). Most intriguing are findings that some genotypes such as Type 12 are predominantly found in wildlife in the USA (Dubey et al., 2011b; Khan et al., 2011). Though Type 12 has also been identified from pigs and sheep in the USA, the frequency is low, and the dominant genotype in these domestic animals is the Type II (Dubey and Jones, 2008; Velmurugan et al., 2009; Dubey et al., 2011a). Also, it is not clear how specific genotypes become established in a particular host, because all strains are transmitted by oocysts shed by felids or by ingestion of infected tissues and studies involving *T. gondii* in wildlife are time consuming, expensive, and difficult. Additionally, permission is needed to collect tissues from certain wildlife, including bears. In the present study we had an opportunity to genetically characterize three isolates of *T. gondii* from hunted black bears (*Ursus americanus*) from Maryland as part of a broader program to assess parasites in Maryland wildlife that pose a health risk to consumers.

Nematodes in the genus *Trichinella* are some of the most commonly recognized agents of foodborne parasitic disease. Human trichinellosis has historically been linked to the consumption of raw or undercooked pork or game meats, including bear. *Trichinella spiralis* arrived in the Western hemisphere relatively recently, transported in the muscle of pigs brought to the New World by European settlers (Rosenthal et al., 2008). The geographic distribution of *T. spiralis* in sylvatic carnivores in North America has been reported proximal to past or current foci of infections originating from the synanthropic cycle of pig transmission (Pozio and Zarlenga, 2005; Burke et al., 2008). Whether some scavenging mammals maintain transmission cycles of *T. spiralis* in the absence of infected pigs is unknown; recent surveys have failed to demonstrate *T. spiralis* cycling independently in scavenging mammals in North America. Hill et al. (2010) characterized *T. spiralis* circulating in wildlife surrounding a poorly managed pig farm. In this instance, transmission of *T. spiralis* in wildlife ceased once all infected pigs were removed from the farm. While *T. spiralis* is uncommon in wildlife in the USA outside of recognized pig-related foci, *T. murrelli*, whose appearance in North America predates that of *T. spiralis*, is frequently found in carnivores and scavengers (Hill et al., 2008; Pozio and La Rosa, 2000) and is believed to be the predominant species circulating in North American wildlife (Zarlenga et al., 1991; Snyder et al., 1993). In this study, we tested bear tongues for the presence and genotype of *Trichinella* spp. muscle larvae (ML).

2. Materials and methods

2.1. Naturally infected bears

Since 2005, tongues have been collected from each bear harvested from in Garrett and Allegany Counties in Western Maryland during the October hunting seasons. Bear density in this area is approximately 1 bear per 4 square kilometers. Tongues were tested for the presence of *T. gondii* during the 2010 and 2011 hunting seasons; tongues have been analyzed for the presence of *Trichinella* spp. ML since 2005 (40 bears in 2005, 41 in 2006, 51 in 2007, 56 in 2008, 68 in 2009, 67 in 2010, and 66 in 2011). Tongues were stored at 4 °C after collection, and were submitted in individual ziplock bags to the Animal Parasitic Diseases Laboratory, United States Department of Agriculture, Beltsville, Maryland for examination.

2.2. T. gondii serology

Fluids from bags containing bear tongues were tested for antibodies to *T. gondii* by the MAT as described by Dubey and Desmonts (1987) because serum was not available. Undiluted fluid and 4 dilutions (25, 50, 100 and 200) from the fluid were tested for antibodies.

2.3. T. gondii bioassay

Tissue (50 g) from each of the 17 seropositive tongues were homogenized, digested in acidic pepsin and washed as described (Dubey, 2010). Aliquots of homogenates were inoculated subcutaneously into outbred SW mice and/or two KO mice (Table 1) (Dubey, 2010). Tissue imprints of lungs and brains of inoculated mice that died were examined for *T. gondii* tachyzoites or tissue cysts. Survivors were bled on day 45 days p.i. and a 1:25 dilution of serum was tested for *T. gondii* antibodies by MAT. Mice were killed 46 days p.i. and brains of all mice were examined for tissue cysts as described (Dubey, 2010). The inoculated mice were considered infected with *T. gondii* when tachyzoites or tissue cysts were found in tissues. Infected mouse tissues were seeded on to CV1 cell culture flasks and tachyzoites were harvested from the medium.

Table 1
Toxoplasma gondii isolates from black bears in Maryland.

Bear #, sex[a]	MAT	Bioassay[b]			*T. gondii* isolate designation	ToxoDB #
		SW	KO	Oocysts shed by cat #		
265, male	200	2/3	2/2	59	TgBbMd1	4
377, male	100	3/3 (11, 12, 12)[c]	No	55	TgBbMd2	216
413, female	200	3/3	No	63	TgBbMd3	1

[a] The bears were trapped 25–27 October, 2011 in Garrett County.
[b] SW = Swiss Webster, KO = knockout mice. No. of mice infected with *T. gondii*/no. of inoculated.
[c] Day of death of mice.

2.4. Pathogenicity of oocysts of T. gondii isolates from bears in mice

Pathogenicity of the oocysts of *T. gondii* isolates derived from bears was assessed in SW mice. For this, *T. gondii*-free cats were fed tissues of mice infected with the three bear isolates, and oocysts were collected from the cat feces (Dubey, 2010). Oocysts were sporulated in 2% sulfuric acid for a week on a shaker at room temperature, washed, counted, and diluted 10-fold from 10^{-1} to 10^{-7} to reach an end point of ≅ one oocyst (Table 2). Mortality was recorded, and after 2 months mice were tested for *T. gondii* infection as described above.

2.5. Genetic characterization of T. gondii

T. gondii DNA was extracted from cell-cultured tachyzoites. Isolate genotyping was performed using the genetic markers SAG1, 5′- and 3′-SAG2, alt.SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico as described previously (Su et al., 2010).

2.6. Trichinella analysis

Tongues collected from each bear were trimmed of connective tissue and chopped by blending in a small amount of tap water. Chopped meat was combined in a beaker with 10 times the volume of digestion fluid (1% pepsin [1:10 000 IU], 1% HCl) in 45 °C tap water. The beaker was covered and vigorously stirred for 30 min in an environmental chamber maintained at 45 °C.

After 30 min, the digestion fluid was poured through a #45 wire sieve into a round-bottomed Pilsner glass and allowed to stand for 30 min. The supernatant was poured off and the sediment was repeatedly washed with water and resettled until the supernatant was clear. The final sediment was poured into a larval counting chamber and examined for the presence of *Trichinella* larvae using a stereo microscope. Collected larvae were identified to species level by molecular genotyping using multiplex PCR. For multiplex PCR, ML collected by the digestion procedure described above were washed 3 times in Hanks Balanced Salt Solution, pelleted, and subjected to genomic DNA extraction utilizing a DNeasy Tissue Kit (Qiagen Inc., Valencia, CA, USA). Following RNase A/T1 treatment and repurification, DNA concentrations were determined spectrophotometrically. The multiplex PCR was carried out essentially as described by Zarlenga et al. (1999).

2.7. Ethics

All animal experimentations were performed in accordance with protocols approved by the U.S. Department of Agriculture.

3. Results

3.1. T. gondii

Antibodies to *T. gondii* were found in fluids from tongues of 17 (25.7%) of 66 bears, in titers of 25 (three samples), 50 (three samples), 100 (six samples), and 200 (three samples). Viable *T. gondii* was isolated from tongues of three of 17 seropositive samples that were bioassayed in mice (Table 1).

The pathogenicity of the three isolates of *T. gondii* from bears for SW mice varied with the isolate. All three SW mice inoculated with digest of tongue from bear #377 died

Table 2
Pathogencity of oocysts of *T. gondii* isolates derived from black bears to Swiss Webster mice.[a]

Dose[b]	TgBbMd1 (cat #59-bear #265)	TgBbMd2 (cat #55-bear #377)	TgBbMd3 (cat #63-bear #413)
1,000,000	Not done	5 (4, 4, 4, 4, 5)[c]	Not done
100,000	5 (5, 6, 6, 6, 7)	5 (5, 5, 5, 6, 6)	5 (6, 6, 6, 6, 6)
10,000	5 (7, 7, 7, 7, 8)	5 (5, 6, 6, 6, 7)	5 (7, 7, 7, 7, 9)
1000	5 (8, 8, 8, 9, 9)	5 (7, 7, 8, 9, 9)	5 (7, 7, 8, 8, 10)
100	5	5 (9, 9, 9, 9, 9)	5
10	5 (34)	5 (10, 11, 11, 12, 12)	5
1	3	2 (11, 12)	2
<1	0	0	0

[a] Five mice per group.
[b] Based on estimation that the last infective dilution has 1 infective organism.
[c] No. of mice infected out of five mice inoculated. Day of death of each mouse is in parenthesis. Tissue cysts or tachyzoites were found in all infected mice.

Table 3
Genetic characterization of *T. gondii* from black bears from Maryland.

Strain ID	ToxoDB genotype	Genetic markers										
		SAG1	(5′ + 3′) SAG2	alt. SAG2	SAG3	BTUB	GRA6	c22-8	c29-2	L358	PK1	Apico
GT-1	#10	I	I	I	I	I	I	I	I	I	I	I
PTG	#1	II	II	II	II	II	II	II	II	II	II	II
CTG	#2	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												
TgBbMd1	#4	II	II	II	II	II	II	II	II	I	II	I
TgBbMd2	#216	I	I	I	III	III	I	III	III	III	I	III
TgBbMd3	#1	II	II	II	II	II	II	II	II	II	II	II

of toxoplasmosis on 11, 12, and 12 days p.i.; tachyzoites were found in their lungs. All 12 SW mice inoculated with tachyzoites from lungs of mice also died between 12 and 18 days p.i.; cat #55 fed tissues of acutely infected mouse tissues (12 days p.i.) shed oocysts.

The SW mice inoculated with tongues of bears #265 and #413 became infected with *T. gondii* but remained asymptomatic (Table 1); cats #59 and #63 fed mouse tissues infected with these same two isolates, respectively shed oocysts (Table 2).

Pathogenicity of the oocysts derived from three bear *T. gondii* isolates to SW mice also varied (Table 2). All mice orally inoculated with oocysts from cat #55 (TgBbMd2) died of acute toxoplasmosis between 4 and 12 days p.i. and tachyzoites were found in their lungs, irrespective of the dose. Oocysts of *T. gondii* isolates derived from bear #265 (TgBbMd1, cat #59) and #413 (TgBbMd3, cat #63) were less pathogenic than oocysts from cat #55 (Table 2); their lethal dose was 1000 oocysts.

Genotyping of these three *T. gondii* isolates revealed three different genotypes (Table 3). TgBbMd1 is a Type 12 strain (ToxoDB PCR-RFLP genotype #4) and TgBbMd2 is a new genotype, designated as ToxoDB PCR-RFLP genotype #216, and TgBbMd3 is a Type II clonal strain (ToxoDB PCR-RFLP genotype #1).

3.2. *Trichinella*

A total of 389 bear tongues were analyzed between October, 2005 and October, 2011. Digestion of tongues revealed that two bears were infected with *Trichinella* spp., one 77 kg male in the 2008 cohort (1/56, 1.78%), and one 66 kg male in the 2009 cohort (1/68, 1.47%), both were from Garrett County, Maryland. Muscle larval density in both bears was less than two larvae/gram of tissue. Genotyping of the isolated larvae revealed that both bears were infected with *T. murrelli*.

4. Discussion

The black bear is the largest terrestrial mammal native to Maryland. They are territorial; their home range sizes vary based predominantly on age and sex. Adult females tend to have the smallest home ranges and sub-adult males have the largest. Annual home range size of black bears is considered to be approximately 10 square kilometers for adult female and 50 square kilometers for adult males. Loss of habitat and indiscriminate hunting resulted in elimination of black bears in many parts of the state by the early 1900s. Return of forest ecosystems and the protected status of black bears have once again generated a thriving population helped in part by a limited hunting season enacted in 2004. Hunters are issued permits, and are required to eviscerate the carcass in the field at the site of the kill prior to submitting it to a checking station.

Though black bears are omnivorous, their diet is comprised mostly of fruits, nuts, other vegetation, and insects. Small mammals, birds, fish, and carrion make up a smaller, but potentially important proportion of their diet with respect to *T. gondii* and *Trichinella* transmission (Wilson and Ruff, 1999). Due to their rooting and meat scavenging behavior, they are important sentinel hosts for both *T. gondii* and *Trichinella* in the environment. Since hunted bears are frequently eaten by people, they are a source of infection for humans for both parasites. Hunters can themselves become infected if they ingest tissues or fluids containing infectious parasites while dressing the carcasses. Adult bears can weigh as much as 275 kg. Consequently, one undercooked carcass can provide many infected meals which can increase the size of an outbreak since the meat is frequently shared with others. Additionally, state regulations require the hunter killed carcass to be field-dressed, and these discarded tissues can be a source of infection for carnivores that act as reservoirs for both parasites, and for felids that can spread *T. gondii* by shedding oocysts. Also, infected, hunted bear in North America are more often diagnosed with freeze resistant forms of *Trichinella* which eliminates freezing as a method that consumers can protect themselves against infection.

A very high prevalence of *T. gondii* antibodies has been reported in black bear in the USA (Table 4). In these surveys, seroprevalence varied from 15% to 84%. These differences may be related to geography, sample size, and serological tests used to assay antibodies. In all but two studies, LAT was used for diagnosis and in general this test is less sensitive than MAT (Dubey, 2010). Fortunately most of other surveys were based on MAT, and these samples were tested by one of the authors (O.C.K.) using a cut-off

Table 4
Prevalence of *T. gondii* in black bears from the USA.

State	Year sampled	Serological testing				Reference
		No	Test[a]	Cut-off	% positive	
Alaska	1988–1991	40	LAT	64	15.0	Chomel et al. (1995)
	1976–1996	143	MAT	25	43.3	Zarnke et al. (2000)
	2009	7[b]	MAT	25	14.2	Dubey et al. (2010)
Florida	1993–1995	66	LAT	64	56.1	Dunbar et al. (1998)
	2011	29	MAT	25	44.8	Chambers et al. (2012)
North Carolina	1996	143	MAT	25	83.9	Nutter et al. (1998)
Pennsylvania	1889–1992	665	MAT	25	80.4	Briscoe et al. (1993)
	1992	322	MAT	25	78.8	Dubey et al. (1994)
	1993	28[b]	MAT	25	78.6	Dubey et al. (1995)
	1998	80[b]	MAT	25	82.5	Dubey et al. (2004)

[a] LAT = latex agglutination test, MAT = modified agglutination test.
[b] *T. gondii* bioassay.

1:25. The seroprevalence of *T. gondii* has remained stable at 80%, especially in Pennsylvania; however, these surveys are 15–20 years old. In the present study from Maryland the true seroprevalence is unknown because serum was not available for testing and the results were based on the fluid from tongues.

Little is known of the persistence of *T. gondii* in different tissues of bears. Viable *T. gondii* was isolated from the hearts of 17 of 32 Pennsylvania bears with MAT antibody titers of 1:25 or higher but not from six seronegative bears (Dubey et al., 1995, 2004). Viable *T. gondii* was also isolated from the hearts of one of one seropositive black bear, and one of three brown bears from Alaska (Dubey et al., 2010, 2011b). In the present study, only tongues were available for bioassay and *T. gondii* was isolated from only three of 17 seropositive bears. Whether the tissues (heart versus tongue) or the quality of tissues affected this variability is unknown.

Limited data are available on genotyping of *T. gondii* from bears. Initial attempts on genotyping of 14 of 17 *T. gondii* isolates from black bears were based on RFLP typing and few markers (Howe and Sibley, 1995; Dubey et al., 2004). Recently, five of the 17 isolates from Pennsylvania bears were revived from cryopreserved samples and retyped with the 11 RFLP markers (Table 5); two were ToxoDB #1 (Type II clonal), two were ToxoDB #2 (Type III), one was ToxoDB #4 (Type 12). The isolate from black bear from Alaska was new genotype (ToxoDB #147), not recorded from any other host (Dubey et al., 2010, 2011a,b). Type 12 is most prevalent in wildlife in North America, and the Type II is also a major type in this region. Thus, of the nine isolates from black bears so far genotyped, three were ToxoDB #1 (Type II clonal), two were ToxoDB #2 (Type III), two were ToxoDB #4 (Type 12), one was ToxoDB #147, and one was ToxoDB #216. The former three genotypes are considered predominant clonal lineages in wildlife in North America. Thus, two of the nine isolates were not clonal. An isolate from a brown bear in Alaska was ToxoDB #5 (Type 12) (Dubey et al., 2011a,b). The ToxoDB #216, identified here was recently reported from mute swan (TgSwanUs3) in USA; the ToxoDB #216 is an atypical *T. gondii* strain that is highly virulent in outbred mice (Dubey et al., in press).

There is only one species of *Toxoplasma*, *T. gondii* that infects all warm-blooded hosts. Why some hosts become sick whereas others remain asymptomatic is unknown. Before the discovery of molecular genotyping methods, *T. gondii* was grouped into virulent and non-virulent strains based on pathogenicity in outbred mice; however, there has not yet been data to link the virulence of *T. gondii* in mice to virulence in higher animals including humans and sheep. Given the wide dissemination of *T. gondii*, such an association may come to light and become increasingly more important as studies expand on *T. gondii* infections in immunocompromised individuals. Nothing is known of

Table 5
Genotypes of *T. gondii* in bears in the USA.

Host	State[a]	No. of isolates typed	ToxoDB PCR-RFLP genotypes						Reference
			#1	#2	#4	#5	#147	#216	
Black bear (*Ursus americanus*)	PA	5	TgBbPa3, TgBbPa5	TgBbPa1, TgBbPa4	TgBbPa2				Dubey et al. (2011b)
	AK	1					TgBbUs1		Dubey et al. (2010)
	MD	3	TgBbMd3		TgBbMd1			TgBbMd2	Present study
Brown bear (*Ursus arctos horribis*)	AK	1				TgBbAk1			Dubey et al. (2011b)
Total		10	3	2	2	1	1	1	

[a] AK = Alaska, MD = Maryland, PA = Pennsylvania.

the genotypes of *T. gondii* in the normal human population; however, it is important to note that results of the present study clearly show that highly virulent mouse strains are prevalent in wildlife hosts that are part of our food chain. Two of the 10 strains (ToxoDB type #147 and #216) from bears have not been recorded in any other hosts (Su et al., 2012).

Trichinella spp. have been isolated from tissues of black bears in many locations in the USA (Jordan et al., 1975; Zimmermann, 1977; Rogers, 1975; Ruppanner et al., 1982; Dubey et al., 1994; Schad et al., 1986; Nutter et al., 1998; Pozio et al., 2001; Hill et al., 2005; Hall et al., 2012). Reports published before 1990 identified all isolates as *T. spiralis*; however, since the development of assays which allow speciation of *Trichinella*, *T. spiralis*, *T. nativa*, and *T. murrelli* have been identified from black bears (Pozio et al., 1992; Zarlenga et al., 1999; Pozio et al., 2001; CDC, 2003; Hill et al., 2005). In this study, both bears were infected with *T. murrelli*, the *Trichinella* species most commonly found in sylvatic carnivores in North America (Zarlenga et al., 1991; Snyder et al., 1993; Pozio and La Rosa, 2000; Hill et al., 2005). *T. murrelli* is infectious to humans, and has been implicated in a recent trichinellosis outbreak in the USA resulting from human consumption of black bear meat which sickened more than 30 people (Hall et al., 2012). It was also identified as the causative agent in an outbreak in France originating from horsemeat imported from the USA (Ancelle, 1998). The prevalence of *Trichinella* infection in our study (1.78% in 2008 and 1.47% in 2009) is similar to the prevalence assessed in black bears in the neighboring state of Pennsylvania in 1982 (1.8%; Schad et al., 1986), and 1992 (1.8%; Dubey et al., 1994), while none of 143 black bears from North Carolina were found to be seropositive for *Trichinella* (Nutter et al., 1998).

T. spiralis, commonly found in pigs, was not found in this study. A recent investigation of a poorly managed pig farm in a Maryland county near the harvest location of the bears revealed high levels of infection with *T. spiralis* in resident hogs and in small carnivores (raccoons and opossums) in the surrounding ecosystem. Once infected pigs were removed, *T. spiralis* transmission dropped to non-detectable levels in the surrounding small carnivore population (Hill et al., 2010). No *T. murrelli* infection was found in any of the animals.

Results presented herein suggest that there is a low but measurable prevalence of *T. murrelli* in black bears in Maryland, and that *T. spiralis* infection is rare or absent in black bears and other sylvatic carnivores in this region. Nonetheless meat from black bears and other game animals pose a risk for human infection if the meat is eaten in an undercooked state.

Conflict of interest

None.

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