Clinical Infectious Diseases

MAJOR ARTICLE

Trichinella Nativa Outbreak With Rare Thrombotic Complications Associated With Meat From a Black Bear Hunted in Northern Ontario

Daniel Dalcin,[1] Dante S. Zarlenga,[2] Nicholas C. Larter,[3] Eric Hoberg,[2] Daniel A. Boucher,[1] Samuel Merrifield,[1] Rachel Lau,[4] Filip Ralevski,[4] Karamjit Cheema,[4] Kevin L. Schwartz,[4,5] and Andrea K. Boggild[4,6,7]

[1]Northern Ontario School of Medicine, Thunder Bay, Canada; [2]US Department of Agriculture, Animal Parasitic Diseases Laboratory, Beltsville, Maryland; [3]Government of Northwest Territories, Department of Environment and Natural Resources, Fort Simpson, and [4]Public Health Ontario, Toronto, [5]St Joseph's Health Sciences Centre, Toronto, [6]Tropical Disease Unit, Toronto General Hospital, and [7]Department of Medicine, University of Toronto, Ontario, Canada

Background. Although trichinellosis is known to cause thrombotic disease, serious thrombotic events are rare and have not been previously associated with *Trichinella nativa* infection.

Methods. Patient interviews and medical chart reviews were conducted on 10 men who became ill following consumption of a common source of black bear meat. *Trichinella* serology on patient sera as well as polymerase chain reaction (PCR) and larval identification of the meat samples was conducted.

Results. All 10 exposed individuals developed an acute illness clinically compatible with trichinellosis, characterized by fever, abdominal pain, and diarrhea, along with eosinophilia ranging from 0.9×10^9/L to 6.1×10^9/L. Within 2 weeks of the diarrheal illness, systemic symptoms developed in all exposed individuals characterized by fever, myalgia, periorbital edema, and fatigue. ST-elevation myocardial infarction and sinus venous tract thrombosis occurred as a complication of trichinellosis in 2 patients. Acute serology was nonreactive in all patients, though convalescent serology was reactive in 6 of 8 (75%) patients for whom sera was available. Multiplex PCR identified *T. nativa* from the bear meat, and was corroborated by microscopic larval identification.

Conclusions. We report a 100% attack rate of *T. nativa* from bear meat among those who were exposed, and demonstrate that this species can cause serious thrombotic complications of trichinellosis in humans. Education of hunters and the public regarding the importance of proper preparation of wild game prior to ingestion is warranted.

Keywords. black bear; eosinophilia; *Trichinella nativa*; trichinellosis; thrombotic sequelae.

Trichinellosis is a helminthiasis that causes significant morbidity and mortality worldwide. Of 9 species and 3 undefined genotypes of *Trichinella*, only *Trichinella nativa*, the *Trichinella* T6 genotype, and, rarely, *Trichinella spiralis* are enzootic in arctic and subarctic regions of North America and are transmitted by sylvatic carnivores [1] *Trichinella nativa* and the T6 genotype are freeze-resistant and consequently are the most prevalent in sylvatic hosts endemic above the –6°C isotherm. In Ontario, Canada, trichinellosis is rare with only 5 cases reported to Public Health Ontario between 2004 and 2015 [2].

In this case series, we describe the clinical and epidemiological features of an outbreak of human trichinellosis involving 10 adult men caused by *T. nativa* resulting from the ingestion of dried meat (jerky) from a black bear (*Ursus americanus*) hunted in Northern Ontario. This outbreak was accompanied by rare, life-threatening thrombotic complications in 2 patients that included ST-elevated myocardial infarction (STEMI) and acute sinus venous thrombosis (SVT) leading to cranial nerve (CN) VI palsy.

METHODS

Clinical and Epidemiological Investigation

Ten patients had their medical charts reviewed after they fulfilled the case definition for trichinellosis [3], including epidemiologic exposure to affected meat, clinically compatible symptoms, and eosinophilia, with or without positive serologic testing. The implicated meat was identified and additional meat from the same bear that had been ground and frozen but not processed into jerky was obtained for analysis.

Laboratory Testing

Frozen, ground black bear meat was sent for microscopy and molecular analysis to the Public Health Ontario Laboratory (PHOL), and for additional molecular analysis to the US Department of Agriculture (USDA) in Beltsville, Maryland.

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Correspondence: A. K. Boggild, 200 Elizabeth St, 13EN-218, Toronto, ON, Canada, M5G 2C4 (andrea.boggild@utoronto.ca).

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Squash preparations of thawed meat were made at PHOL and photomicrographs were obtained using an Olympus BX51 light microscope. Serologic testing was performed at PHOL by a routine in-house complement fixation (CF) assay on patient sera collected during acute illness and approximately 6 weeks after symptom onset. Additionally, sera were tested using an investigational Health Canada–approved commercial enzyme-linked immunosorbent assay (ELISA; Scimedx Inc, Denville, New Jersey) that detects *Trichinella* genus-specific antibodies to excretory/secretory antigens of *T. spiralis*.

The previously frozen ground meat was digested for 3 hours at 42°C in 1% pepsin:1% hydrochloric acid (HCl) then left to settle for 1 hour. Crude parasites were purified through successive washes in tap water followed by settling. Three separate digestions were performed to obtain the level of infection defined as larvae per gram (LPG) of tissue. Larvae were photographed using Brightfield light microscopy on a Zeiss Axiophot microscope.

USDA Molecular Testing

Remaining parasites were digested in proteinase K:sodium dodecyl sulfate (SDS) followed by organic extraction and ethanol precipitation [4]. Isolated DNA was subjected to multiplex polymerase chain reaction (PCR) as previously described [5] using ExTaq DNA polymerase, which obviated the need for nested PCR. Stock DNA from *T. spiralis* (ISS 4), *T. nativa* (ISS 45), and the *Trichinella* T6 genotype (ISS 34) were used as positive PCR controls; water was used as a negative control. Amplified products along with 100-bp DNA markers were separated on 3% NuSieve 3:1 agarose in the presence of GelRed stain (Phenix Research Products) and photographed.

PHOL Molecular Testing

DNA was extracted with QIAmp DNA Mini kit (Qiagen, Germantown, Maryland). In brief, frozen bear meat (25 mg) was cut into pieces and manually homogenized. The tissue was then lysed overnight with 180 µL of Buffer ATL and 20 µL of proteinase K at 56°C, and subsequent steps were followed according to the DNA Mini kit Tissue protocol, and DNA was eluted with 60 µL Buffer AE.

Trichinella genus–specific PCR targeting the mitochondrial large subunit ribosomal DNA was performed with Amplitaq Gold Fast PCR mix (Thermo Fisher Scientific) with 250 nM of each primer: Tfwd 5'-TGGCCGCGGTAACTTGACCG-3' and Trev 5'-CCAACCTGTCTTGCGACGGTT-3' [6].

Cycling conditions were 95°C for 10 minutes followed by 35 cycles of 96°C for 5 seconds, 60°C for 5 seconds, and 68°C for 5 seconds, with a final extension at 72°C for 10 seconds. PCR products were resolved on 1% agarose gel electrophoresis and further confirmed by sequencing with a BigDye version 3.1 cycle sequencing kit (Thermo Fisher Scientific) in ABI 3130xl analyzer and National Center for Biotechnology Information BLAST (Basic Local Alignment Search Tool) search for sequence identity.

RESULTS

Clinical and Epidemiological Investigation

In March 2016, 10 men (25–50 years old) became ill following consumption of dehydrated black bear meat (jerky) originating from a 150-kg male bear killed near Kapuskasing, Ontario, Canada, on 31 August 2015. The meat was immediately butchered, packaged, and stored in a freezer at –22°C as whole muscle sections until March 2016. The tissue was then thawed overnight, ground, seasoned, and placed in a meat dehydrator at 63°C for 5 hours. All 10 exposed individuals developed symptomatic disease.

Nine patients presented individually at a local emergency department in Northern Ontario within several days of each other. Each patient demonstrated nonbloody watery diarrhea, diffuse abdominal pain, fever, and nausea; 1 patient also had a diffuse maculopapular rash (Figure 1). Symptoms first appeared 4–7 days postconsumption and were thought to be symptoms of viral gastroenteritis. None of the patients reported sick contacts or volunteered information regarding the consumption of wild game. Approximately 2 weeks postconsumption, the patients returned for assessment with diffuse myalgia, periorbital edema, fever, and fatigue. Bloodwork revealed a leukocytosis with peripheral eosinophilia and elevated creatine kinase, aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase levels (Table 1). Given the known

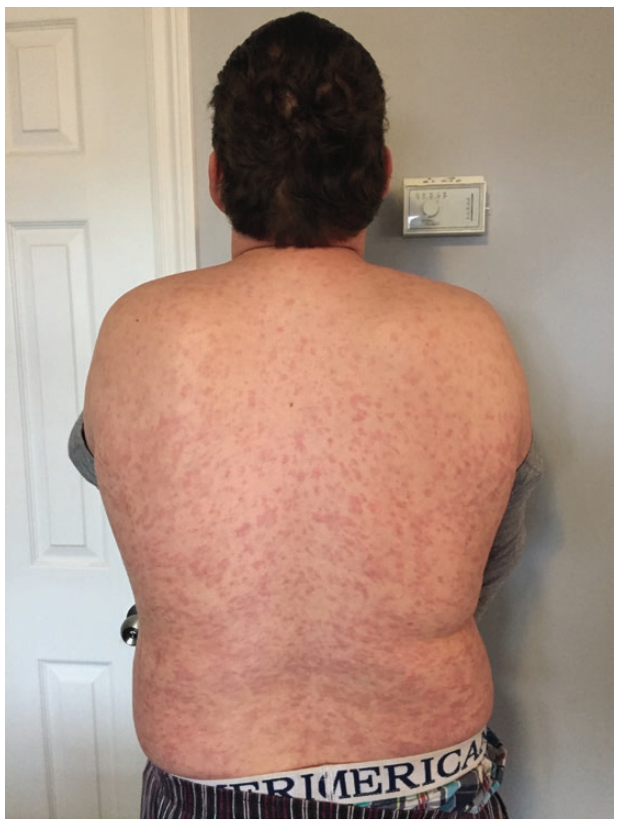

Figure 1. Diffuse maculopapular rash in a patient with trichinellosis due to *Trichinella nativa*.

Table 1. Clinical and Laboratory Data on Trichinellosis Patients During Acute Illness and Convalescence

Pattients With Complications	Pt	No. of 10 g Dehydrated Strips of Jerky Ingested[a]	Age, y	CK (55–177 U/L)		Eosinophils (0.04–0.40 × 10^9/L)		WBC (4–10 × 10^9/L)		LDH (313–618 U/L)		Complement Fixation Assay		AST (15–46 U/L)		ALT (13–69 U/L)	
				s	c	s	c	s	c	s	c	s	c	s	c	s	c
	1	45	25	3514	132	4.3	0.2	16.6	6.1	1914	427	Non-reactive	Reactive 1:8	209	21	380	12
	2	5	24	1477	163	5.3	0.3	17.5	6.8	1139	463	Non-reactive	Reactive 1:4	97	42	130	43
	3	1	26	286	49	1.8	0.2	15.4	7	585	359	Non-reactive	Reactive 1:16	26	24	37	15
	4	3	41	214	161	6.1	0.6	19.1	8.5	792	502	Non-reactive	Nonreactive	35	30	51	44
	5	1	49	349	182	1.7	0.4	9.3	7	520	380	Non-reactive	Not tested	37	35	47	27
	6	1.5	35	405	67	1.3	0.4	14	6.1	610	423	Non-reactive	Reactive 1:32	36	25	54	27
STEMI	7	10	35	296	69	6.01	0.4	19.1	9.2	683	375	Non-reactive	Reactive 1:64	44	20	82	12
SVT	8	2	41	262	127	1.5	0.1	9.8	6.4	857	261	Non-reactive	Nonreactive	41	22	51	127
	9	5–10	26	…	56	1.5	0.2	8.7	5.4	689	376	Non-reactive	Not tested	25	23	39	27
	10	1	50	361	96	0.9	0.4	7.6	5.1	492	422	Non-reactive	Reactive 1:16	35	24	47	39

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; c = convalescent (approximately 6 weeks from symptom onset); CK, creatine kinase; LDH, lactate dehydrogenase; Pt, patient; s, symptom onset; STEMI, ST-elevated myocardial infarction; SVT, sinus venous thrombosis; WBC, white blood cell count.

[a]Note that the 10 g mass of the jerky strips is an estimate derived from separately interviewing each of the 10 patients.

connection between these cases and the identification of a common source of black bear meat, as well as the classic biphasic illness in all 10 cases, trichinellosis was suspected. All patients were treated empirically with 30–50 mg of prednisone daily for 14 days and 400 mg mebendazole 3 times daily for 14 days.

Two patients developed serious, life-threatening complications; 1 patient developed a cerebral SVT while already on treatment and another patient who had not previously sought medical attention presented to the emergency department with STEMI.

All serum specimens collected during acute illness were nonreactive by CF, while 6 of 8 convalescent sera received were reactive by CF, with titers ranging from 1:4 to 1:64 (reactive ≥1:2). By commercial ELISA, 4 sera collected acutely were reactive with optical densities (ODs) ranging from 0.46 to 2.10 (reactive ≥0.3), and 7 of 8 convalescent sera were reactive with ODs ranging from 2.09 to 3.96.

Cerebral SVT and Cranial Nerve VI Palsy

A 41-year-old man being treated for trichinellosis (Table 1) with prednisone and mebendazole returned to hospital for reassessment 9 days into treatment reporting severe headache and blurred vision. Physical examination revealed CN deficits consistent with CN VI palsy, and funduscopy demonstrated bilateral papilledema. The patient was sent for an urgent computed tomographic (CT) scan of the head, which revealed acute SVT. An urgent magnetic resonance imaging/magnetic resonance angiogram scan confirmed the findings on the CT scan and additionally identified a partially occlusive thrombus in the superior sagittal sinus and complete occlusion into the left transverse sinus (Figure 2). The inferior sagittal sinus and straight sinuses were also found to have thrombi within them. An infectious disease physician was consulted, who recommended changing mebendazole to albendazole (due to increased tissue penetration) and increasing the prednisone from 30 mg daily to 60 mg daily. The patient was admitted to an intensive care unit and treated with intravenous heparin while being bridged to warfarin. A neuro-ophthalmology consultation determined the patient had bilateral CN VI palsy secondary to SVT. Four months later the CN VI palsy was resolved and the patient became asymptomatic.

ST-Elevation Myocardial Infarction

A 35-year-old man developed a STEMI 2 weeks after ingestion of jerky from bear meat. He presented to the emergency department with temperature of 39.5°C, chest pain with diffuse myalgia, and a 2-week history of nonbloody watery diarrhea, fever, and fatigue. No history of street drug use was elicited. An electrocardiogram demonstrated ST elevation in leads V2, V3, and V4 (with reciprocal inferior ST segment depression consistent with acute STEMI). Percutaneous coronary intervention was not immediately accessible and therefore thrombolytic therapy was administered. Chest pain and ST elevation resolved rapidly postthrombolysis. The patient was immediately transferred to a larger hospital by aircraft where a coronary angiogram demonstrated entirely normal coronary arteries, suggestive of transient thrombus occlusion of the left coronary system.

Parasite Load and Speciation

Digesting a total of 160 g of ground, previously frozen tissue in 3 separate experiments resulted in a mean LPG of 129. Given that this was pooled and ground meat tissue, the location on the carcass from which the tissue was obtained is unknown. Upon

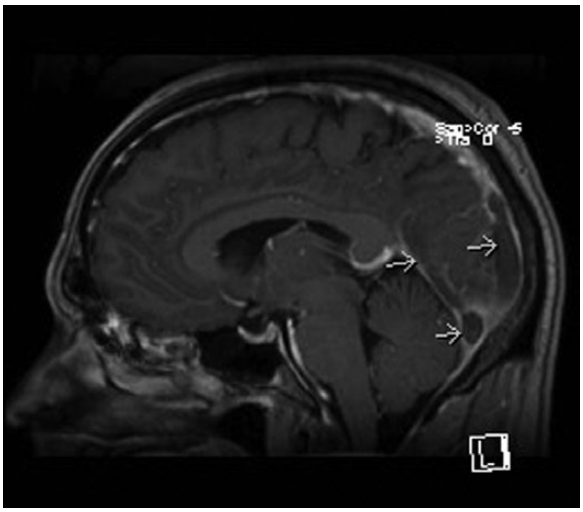

Figure 2. Brain magnetic resonance imaging demonstrating acute cerebral venous sinus thrombosis in a patient with trichinellosis due to *Trichinella nativa*.

thawing, nearly all recovered larvae were viable and tightly coiled in keeping with a freeze-resistant genotype. Larvae were readily visible by standard light microscopy of meat digests and squash preparations (Figures 3 and 4).

Results from multiplex PCR indicated that the genotype of the parasites obtained from pepsin:HCl digestion was *T. nativa,* as indicated by a single diagnostic band migrating at 127 bp and the absence of the band indicative of *Trichinella* T6 that migrates at 210 bp (Figure 5). These molecular data were corroborated by the isolation of viable larvae from tissue that had been frozen at –22°C for approximately 6.5 months and by the endpoint PCR assay followed by target sequencing through PHOL, which demonstrated 99%–100% sequence homology to *T. nativa*.

Figure 3. Photomicrograph of *Trichinella nativa* larva digested from dehydrated meat of a black bear.

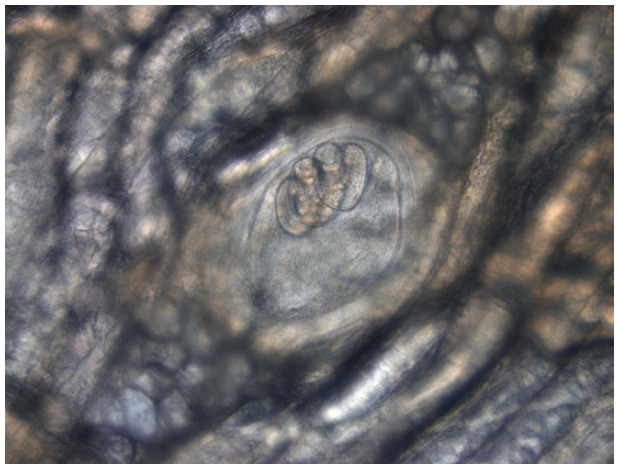

Figure 4. Photomicrograph of *Trichinella nativa* larva in squash preparation of dehydrated bear meat.

DISCUSSION

In Canada, the majority of reported cases of trichinellosis involve the Indigenous population of the Northwest Territories and Northern Quebec [7, 8]. Within Ontario, there have been only 5 cases of trichinellosis reported to Public Health Ontario from 1994 to 2015 [2]. However, it is likely that additional cases go undetected and unreported because often only those with moderate to severe symptoms seek medical attention and diagnosis. Moreover, many cases are likely misdiagnosed, as our patients initially were, due to nonspecific initial symptoms and rarity of disease.

Two patients in this outbreak developed thrombotic complications; however, such conditions associated with trichinellosis are rare and have not been previously been reported to be

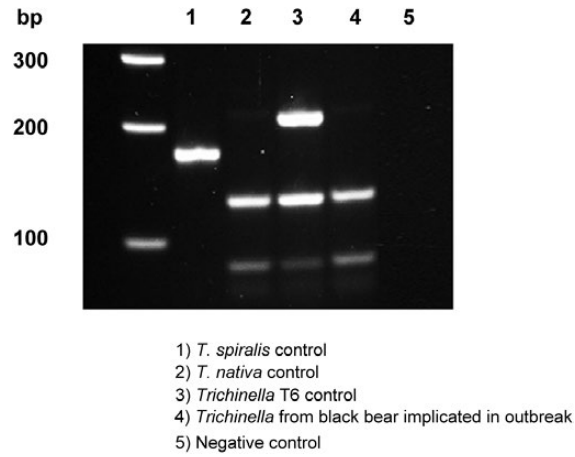

Figure 5. *Trichinella* species determination by multiplex polymerase chain reaction. Lane 1, *Trichinella spiralis* control. Lane 2, *Trichinella nativa* control. Lane 3, *Trichinella* T6 control. Lane 4, *Trichinella* from black bear implicated in outbreak. Lane 5, Negative control.

associated with *T. nativa* [9]. A case report originating from Canada describes STEMI occurring as a complication of trichinellosis in a 20-year-old man associated with the consumption of infected pork [10]. Both our patient and this case report demonstrate complete resolution of cardiac function following thrombolysis [10]. However, other reports describe fatal outcomes with myocardial infarction occurring with neurological dysfunction [11].

There are also few reports of SVT resulting from trichinellosis [12–14]. A case report originating from Canada describes a patient with cavernous SVT complicated by temporary CN VI palsy, similar to our patient [12]. Both our patient and the patient described by Barr et al [12] had complete resolution of symptoms following anticoagulation. Five other reports of SVT have been reported in the literature, 2 of which resulted in death [13]. It is not clear what causes thromboembolic disease to occur in only a subset of patients with trichinellosis and whether this is related to worm burden; however, hypereosinophilia appears to be common to all. Previous reports also note that histopathologically, it is multiple arteriolar fibrinocruoric thrombi, which are noninflammatory in nature and which could occur in the setting of disseminated intravascular coagulation, that are observed in the thrombotic complications of trichinellosis [11]. The 2 patients in this outbreak who developed thrombotic complications did not have risk factors for thrombotic disease, were relatively young, and consumed less jerky than others who did not develop complications.

Although there have been several reported cases of trichinellosis in Ontario, most cases/outbreaks did not involve genetic analysis and instead relied on either microscopy or serology for diagnosis, which cannot identify *Trichinella* to the species or genotype level. To our knowledge, this outbreak is the largest trichinellosis outbreak associated with the ingestion of black bear meat in Ontario and the first outbreak where the species was genetically confirmed to be *T. nativa.* The largest reported outbreak of *T. nativa* in Canada occurred in Northern Saskatchewan involving 78 individuals who consumed black bear meat and resulted in 31 confirmed cases of trichinellosis [15]. The meat implicated in the Saskatchewan outbreak had a higher parasite load (2 samples processed by Pepsin-HCl digestion found parasite loads of 257 and 310 LPG) compared with the meat implicated in our outbreak (mean LPG of 129). Despite having a higher parasitic load of *T. nativa*, the Saskatchewan outbreak reported no thrombotic complications and had a lower rate of observed patient symptomatology (40% [n = 78]) compared with our study (100% [n = 10]).

The meat analyzed by chemical digestion in our study was previously ground and frozen, prohibiting the identification of the specific skeletal muscles analyzed. Given this limitation, the LPG may not reflect the parasitic burden transmitted to the patients in this outbreak. Because *T. nativa* is known to exhibit predilection sites within the host (eg, diaphragm, masseter), our results are subject to sampling error. Furthermore, although we identified the parasite load in unprocessed tissues, it is unknown how the additives, curing, and dehydration affected live worm burden in consumed meat. Variations in the number of worms ingested could explain why patients who consumed only 10 g of dehydrated meat developed symptomatic disease (Table 1). It is also well documented that host responses can vary dramatically even within the same species [9]; these variables are not well understood and may have contributed to variability in disease progression among the patients. Analysis of the patients who developed symptomatic disease in the Saskatchewan outbreak found that individuals who consumed dried meat rather than boiled meat were more likely to develop symptomatic disease owing to deficiencies in the curing process. Only dehydrated meat was consumed in this outbreak, which may have also contributed to the 100% infection rate. While all exposed persons in this particular outbreak developed symptomatic disease essentially simultaneously after ingestion of bear meat, literature supports the early prophylactic use of a 5-day course of mebendazole for prevention of trichinellosis in those not yet symptomatic [16].

Prevalence of *Trichinella* species in black bears is generally low throughout North America, but variation by population, hunting habits, and the number and breadth of surveys conducted regionally is likely. In Canada no positive reports of *Trichinella* species in black bears have been documented from Alberta (n = 265), Manitoba (n = 1), the island of Newfoundland (n = 66), Nova Scotia (n = 51), or Prince Edward Island (n = 1) [7, 17]. Prevalence in Ontario, Quebec, New Brunswick, and Labrador were 2.7% (n = 73), 1% (n = 258), 0.4% (n = 569), and 1% (n = 96), respectively [7, 16], with 4.1% reported in the Northwest Territories [18]. The highest reported prevalence in Canada was 12% (n = 193) from black bears in the Kootenay region of British Columbia [19]. In the United States, Mortenson and colleagues reported no positives (n = 250) for black bears in Oregon using the muscle digestion technique, but 2 positives (n = 103) based on serology [20]. Prevalence as high as 13% has been reported in black bears from Idaho and California [21, 22]. Chomel and colleagues reported 27.5% prevalence from interior Alaska [23]. Schad and colleagues reported 1.8% prevalence in black bears harvested in Pennsylvania [24]. Most of these reports were published prior to the development of technologies that permit accurate genotyping and speciation of *Trichinella*. However, it is likely that the species of *Trichinella* identified in the United States and in more temperate localities of North America were *T. spiralis* or possibly *Trichinella murrelli*, the most prevalent *Trichinella* species circulating among US wildlife [25]. Those emanating from the higher

latitudes are likely freeze resistant and therefore either *T. nativa* or *Trichinella* T6.

A sylvatic cycle of *Trichinella* species is well recognized in black bears in North America, where black bears act as natural hosts. Black bear infections in Canada are caused by either *T. nativa* or *Trichinella* T6, with *T. nativa* being more common [26]. *Trichinella spiralis* infection in black bears is rare but has been reported in bears sampled in Pennsylvania [24]. Larter and colleagues found bears from the Dehcho region of the Northwest Territories infected with either *T. nativa* or the *Trichinella* T6 genotype. *Trichinella nativa* is cold-adapted and found in wild mammals from the arctic or subarctic zones of North America, Europe, and Asia and is more resistant to freezing temperatures. *Trichinella* T6, on the other hand, is the most common genotype observed in sylvatic infections of Canadian wildlife and has a wider documented host distribution in comparison to *T. nativa* [26]. To date, *Trichinella* T6 has only been found in North America. Of note, evidence has surfaced indicating hybrid forms consisting of crosses between *T. nativa* and the T6 genotypes, suggesting that microsatellites might be more appropriate for delineating hybrids [27–29].

Although prevalence may be low, the few black bears infected with *Trichinella* often have infection intensities >1 LPG, which is of concern for human consumption [26]; however, the number of reports that also examined infection intensity in bears testing positive for *Trichinella* is limited. Larter reported that 3 of 8 infected bears had >1 LPG, with 1 bear having an infestation of 177 LPG; however, it is uncommon to find intensities >100 LPG [26]. Schad and colleagues reported 37 positive cases (n = 2056), where 23 (1.1%) had intensities >1 LPG and 6 (0.3%) had intensities ≥300 LPG, where the highest worm burden was 912 LPG [24]. Black bears in Pennsylvania have access to local slaughtering plants and garbage dumps, which makes scavenging behavior likely. The black bear associated with this outbreak had an infection intensity of 127 LPG with a 100% human infection rate, even with consumption of 10 g of dehydrated meat. Although the bear was hunted close to city limits, it is unlikely that the high LPG resulted from scavenging among domestic food sources. As this bear was infected with *T. nativa*, not the more domestic *T. spiralis* [9], another sylvatic host and not swine was more likely to be involved.

Trichinellosis remains a rare infectious disease in Canada. This is the largest reported outbreak of *T. nativa* in Ontario associated with black bear meat and consisted of 10 affected individuals, 2 of whom developed life-threatening thrombotic complications. In arctic and subarctic North America, freezing, curing, drying, and/or salting wild game are insufficient methods to inactivate *T. nativa* and *Trichinella* T6 larvae. This report highlights the importance of appropriate preparation of game meats and demonstrates that *T. nativa* can cause serious thrombotic disease.

Notes

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Author contributions. D. D. conceived the series, and contributed to data collection and interpretation, and to primarily writing the manuscript. D. S. Z., N. C. L., E. H., D. A. B., S. M., R. L., F. R., K. C., K. L. S., and A. K. B. contributed to data collection, analysis, and interpretation, and to critical appraisal and revision of the manuscript.

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