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High Prevalence and Intensity of *Trichinella* Infection in Yukon American Black (*Ursus americanus*) and Grizzly (*Ursus arctos*) Bears

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ABSTRACT: *Trichinella* spp. nematodes are commonly found in bear species (Ursidae) and can pose severe health risks to humans when infective first-stage larvae are ingested in meat. Samples of tongue or masseter muscle from 22 male and 11 female American black bears (*Ursus americanus*; mean age 6.5 yr, range 1–16 yr) and 22 male, eight female, and one unknown sex grizzly bears (*Ursus arctos*; mean age 8.8 yr, range 2–28 yr), from Yukon, Canada, were tested to determine prevalence and intensity of *Trichinella* spp. infection. For black bears, prevalence was 20% and mean intensity was 401 larvae per gram of tissue (LPG), whereas for grizzly bears, prevalence was 71%, and mean infection intensity was 35 LPG. Isolates from all positive samples were identified as genotype *Trichinella*-T6 by multiplex PCR. For black bears, prevalence is the highest reported in Canada and infection intensity the highest recorded in North America. One black bear had a larval burden of 1,173 LPG, the second highest recorded in any host species. The prevalence in grizzly bears was the highest reported in Canada for this host. In total, 90% (27 of 30) of infected bears had infection burdens above the human food safety threshold of ≥1 LPG, reinforcing the importance of communicating the health risks to people consuming bear meat.

Key words: American black bear, grizzly bear, *Trichinella*, Ursidae, *Ursus americanus*, *Ursus arctos*, Yukon.

Surveys of *Trichinella* spp. in wild carnivores have been conducted in various jurisdictions to better understand the human health risks posed by consumption of wildlife meat containing infective first-stage larvae (L1; Rausch et al. 1956; Appleyard and Gajadhar 2000; Gajadhar and Forbes 2010). All *Trichinella* taxa are zoonotic, and one larva per gram (LPG) in tissue is considered the food safety threshold above which clinical disease in humans can occur (Gajadhar and Forbes 2010). From previous reports, most infected bears of both American black bears (*Ursus americanus*) and grizzly bears (*Ursus arctos*) harbor >1 LPG.

Choquette et al. (1969) conducted the only previously recorded survey of *Trichinella* spp. in bears in Yukon, Canada. We aimed to improve knowledge of the regional prevalence and intensity of infection of *Trichinella* spp. in black and grizzly bears in Yukon, to better inform decisions on public health risks.

Thirty black bears and 34 grizzly bears harvested by hunters across Yukon (Fig. 1) were submitted to the Department of Environment (Yukon Government) and sampled by Yukon Government staff for *Trichinella* spp. between May and November 2012 (n=47) and from May to October in 2013 (n=17). Hunters reported the game zone and subzone in which each bear was harvested. Samples were obtained from tongue (n=57) or masseter muscles (n=7), frozen at –20 C, and submitted to the Centre for Food-borne and Animal Parasitology, Canadian Food Inspection Agency, Saskatoon, Saskatchewan, Canada. For black bears, the mean sample weight as tested was 11.7±6.0 g and for grizzly bears 14.6±9.3 g. A first premolar tooth was removed and aged by Yukon Government staff by counting the cementum annuli from the root of the premolar (Matson 1981).

Trichinella L1 were recovered by the pepsin and hydrochloric acid magnetic stirrer artificial digestion assay by the double separatory funnel digestion method (Forbes and Gajadhar 1999). For *Trichinella* spp. genotype or species identification, DNA extracted from five samples of individual larvae, plus a sixth sample of 10 pooled larvae per positive animal, was analyzed by a standardized

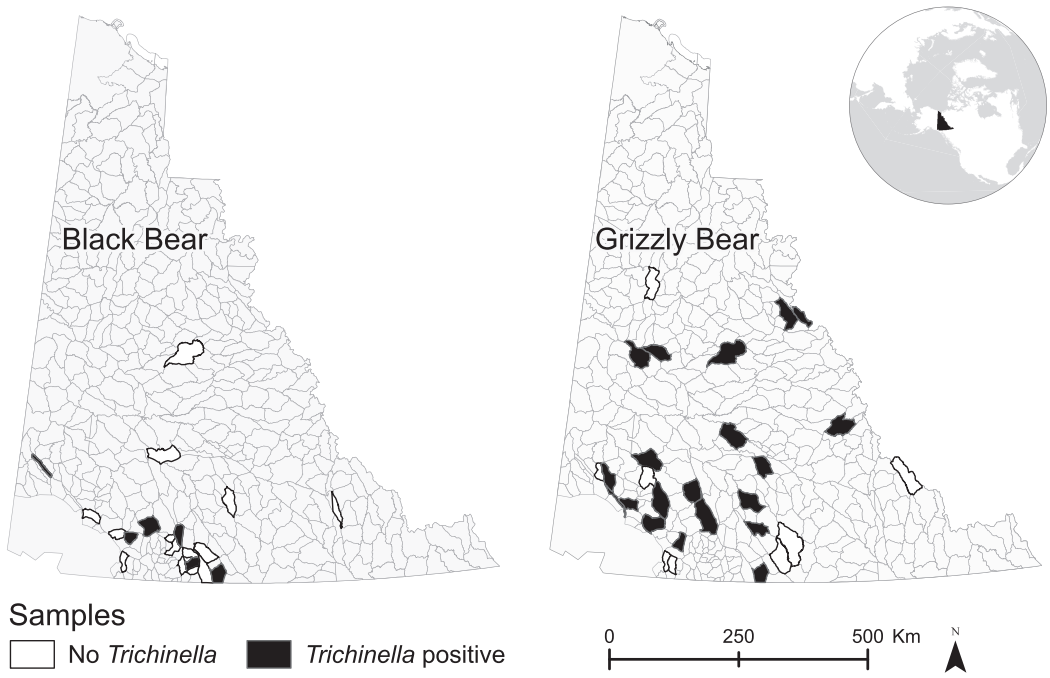

FIGURE 1. Maps of Yukon, Canada, showing the locations of *Trichinella* spp. positive and negative American black bears (*Ursus americanus*) and grizzly bears (*Ursus arctos*) sampled in this study.

multiplex PCR assay (mPCR; Zarlenga et al. 1999; Scandrett et al. 2018). Findings were analyzed by a two-tailed *t*-test with unequal variances.

In black bears, 20% (6/30) of samples tested positive, whereas grizzly bears had the highest prevalence of infection, with 71% (24/34) of samples positive (Table 1). All isolates yielded amplicons on mPCR identifying them as the *Trichinella* T6 genotype. For positive black bear samples, the mean LPG was 401.2±443.03 (range 0.09–1173), with 83% >1 LPG; for positive grizzly bears, the mean LPG was 35.2±42.24 (range 0.16–149), with 92% having >1 LPG. (Table 1). For both host species combined, 59% of positive animals yielded motile or tightly coiled L1, suggestive of larval viability and infectivity.

TABLE 1. Prevalence and intensity (larvae per gram; LPG) of infection with *Trichinella* spp. in tongue (*n*=57) or masseter (*n*=7) muscle of hunter-harvested American black bears (*Ursus americanus*) and grizzly bears (*Ursus arctos*) from Yukon, Canada, sampled May–November 2012 (*n*=47) and May–October 2013 (*n*=17).

					Intensity (LPG)	
Species	Sex	Negative	Positive	% Prevalence	Mean	Range[a]
Black bear	Male	17	5	23	247	0.09–612.9
	Female	7	1	13	1,173	NA
	Total	24	6	20	401.2	0.09–1173
Grizzly bear	Male	8	14	64	40.9	0.16–149
	Female	2	9	82	30.2	0.18–122.7
	Unknown	0	1	100	1.3	NA
	Total	10	24	71	35.2	0.16–149

[a] NA = not applicable.

More samples were obtained from males than females for both species: 73% (22/30) for black bears and 67% (22/33) for grizzly bears (Table 1). One sample from a grizzly bear was of unknown sex. In male black bears 22% (5/23) were *Trichinella*-positive, whereas in females, only 14% (1/7) were positive (Table 1). In contrast, in grizzly bears 82% (9/11) of females tested positive, and only 64% (14/22) of males were positive. However, sample sizes were small, and none of the differences observed between sexes were significant (black bear $P=0.58$; grizzly bear $P=0.55$). There were no significant differences between LPG in male and female grizzly bears (males 40 LPG, females 30 LPG; $P=0.6$).

The mean age of black bears was 6.5 yr (range 1–16 yr), and the mean age of grizzly bears was 8.8 yr (range 2–28 yr). In black bears, individuals had an 18% chance of being positive regardless of age. Grizzly bears <8.8 yr old had a 43% probability of testing positive, compared with 100% of grizzly bears 8.8 yr and older. No grizzly bears under the age of 6 yr old tested positive. There was no significant relationship between infection intensity and age in either species (black bears: $n=4$, $P=0.14$; grizzly bears: $n=24$, $P=0.15$), although younger bears tended to have higher larval burdens than older bears. For black bears, we excluded from the age comparison a sample with a very high LPG value from a yearling (LPG=1,173) and a positive sample from an unaged bear.

Black bear meat is a well recognized source of trichinellosis outbreaks in Canada (Schellenberg et al. 2003; Dalcin et al. 2017). However, the overall prevalence of *Trichinella* in black bears is <1.5% across most of Canada (Appleyard and Gajadhar 2000). Gajadhar and Forbes (2010) found a 7% prevalence ($n=193$) in black bears sampled 1998–2007 from several jurisdictions. The highest recorded prevalence is 12% in the Kootenay region of British Columbia (Schmitt et al. 1978). In the Northwest Territories, Larter et al. (2011) and Johnson et al. (2013) report a prevalence of 5.8%. Our 20% prevalence in black bears was the highest reported in Canada, although our sample size is small. An early survey in Alaska, US (Rausch et al. 1956) found a prevalence of 22% (5 of 23 sampled). In Northwest Territories (Johnson et al. 2013; Larter et al. 2016), a range of 0.06–177 LPG was reported, and the highest larval burden was 177 LPG. We detected a yearling black bear female with a burden of 1,173 LPG, to our knowledge the highest recorded for black bears. In the Alaskan study (Rausch et al. 1956) the larval burdens were lower (mean 81 LPG, range 0.5–400 LPG).

Grizzly bears typically have higher prevalence but lower larval burdens compared with black bears (Rausch et al. 1956; Gajadhar and Forbes 2010; Larter et al. 2016). Both Rausch et al. (1956) and Larter et al. (2016) reported 50% prevalence of *Trichinella* in grizzly bears in Alaska and Northwest Territories, respectively. Gajadhar and Forbes (2010) reported 29% prevalence for British Columbia and Nunavut. Schmitt et al. (1978) found 35% prevalence in three regions of British Columbia. In the mid 1960s, samples from Yukon had a prevalence of 88% (Choquette et al. 1969). Zarnke et al. (1997) reported a strong north to south trend for *Trichinella* prevalence in Alaskan grizzly bears, with 87% prevalence in arctic regions, 25% in interior regions and 5% in southern peninsular and island regions. Our estimate of 71% prevalence in grizzly bears is high compared with most studies and corroborates the findings of the earlier Yukon study (Choquette et al. 1969).

Our estimate of a mean 35 LPG for grizzly bears is also the highest of all reported studies in North America. We did not find a significant relationship between age and LPG in either species, similar to the results of Larter et al. (2011), although we did find a trend toward younger bears of both species having higher larval burdens than older bears. Younger animals in a population might have higher parasite burdens because of induction of less effective specific immunity during initial infection, compared with re-exposure or superinfection, or lower innate resistance to infection (Davies et al. 2008). Grizzly and black bears may consume conspecifics (Mattson et al. 1992) and other species that may be

infected with *Trichinella* spp. through scavenging or predation. Although data for other wildlife species in Yukon are limited, wolverines (*Gulo gulo*), for example, are known have relatively high burdens of *Trichinella* spp. (Sharma et al. 2018). Variation in prevalence between bear species and in different locations may reflect variation in food habits.

Yukon bear samples yielded only the cold-tolerant *Trichinella* T6 genotype (Gajadhar and Forbes 2010). Larter et al. (2011) reported both T6 and *Trichinella nativa* in both black and grizzly bears in Northwest Territories. Although *T. nativa* has been described as the most cold hardy *Trichinella* species, T6 may survive for years at between –6 C and –20 C. Yukon winter temperatures are often colder than –20 C, but temperatures under the snowpack normally fall within the temperature range enabling long-term survival of T6 L1 (Pozio 2016).

Our study contributes to a better understanding of *Trichinella* in northwestern North America and provides wildlife management and public health officials with additional data to inform the public of the risks posed by this zoonotic parasite. In Yukon, the licensed harvest of black and grizzly bears averages 89 and 76 animals per year, respectively (Milligan 2018). Culling for other purposes (e.g., bears in conflict with people) varies per year, as does the unreported indigenous harvest. Of the licensed bear harvest, black bears are a preferred source of food in Yukon, although no cases of human trichinosis in Yukon have been reported to our knowledge. Groups most likely to consume bear meat probably include local hunters and their families and First Nation harvesters. A Yukon-specific public health message, such as recommending cooking bear meat well, can now include data on *Trichinella* prevalence and infection intensity in both of these game species.

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