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Trichinella nativa and *Trichinella* T6 in arctic foxes (*Vulpes lagopus)* from northern Canada

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ABSTRACT

Parasitic zoonotic nematodes of the genus *Trichinella* circulate in wildlife and domestic hosts worldwide through the ingestion of infected meat. Due to their role as scavengers and predators in terrestrial and marine arctic ecosystems, Arctic foxes (*Vulpes lagopus*) are ideal sentinels for the detection of *Trichinella* spp. In this study, we determined the prevalence, larval intensity, and species of *Trichinella* from 91 trapped Arctic foxes collected around the northern Canadian communities of Sachs Harbour (Ikaahuk) on Banks Island (n = 23), and Ulukhaktok and Cambridge Bay (Ikaluktutiak) on Victoria Island (n = 68). Using pepsin-HCl digestion, larvae of *Trichinella* spp. were recovered from the left forelimb muscle (*flexor carpi ulnaris*) in 19 of the 91 foxes (21% prevalence, 95% CI: 14–30%). For the first time in Arctic foxes in Canada, *Trichinella* species were identified using multiplex PCR that was followed up with PCR-RFLP to distinguish between *T. nativa* and *T. chanchalensis*. All infected foxes harbored *T. nativa,* and one fox was co-infected with *Trichinella* T6; the latter is a new host record. Age of the fox was significantly associated with *Trichinella* spp. infection and the odds of being infected were three times higher in foxes ≥2 years of age (p = 0.026), indicating cumulative exposure with age. While Arctic foxes are seldom harvested for human consumption, they serve as sentinel hosts of *Trichinella* spp., confirming the presence of the parasite in wildlife in the region.

1. Introduction

Trichinella spp. are zoonotic nematodes that complete their entire life cycle within a single vertebrate host (Jenkins et al., 2013). Female nematodes mate in the intestine and produce newborn larvae (NBL-1) that migrate through the circulatory system to various body tissues (Jenkins et al., 2013). To date, there are thirteen recognized species and genotypes that are classified into two groups, depending on the presence or absence of a capsule surrounding the larvae. The encapsulated group includes *T. spiralis* (T1), *T. nativa* (T2), *T. britovi* (T3), *T. murrelli* (T5), *T. nelsoni* (T7), *T. patagoniensis* (T12), *T. chanchalensis* (T13) and three unnamed genotypes: *Trichinella* T6, T8, and T9. The non-encapsulated group includes *T. pseudospiralis* (T4), *T. papuae* (T10) and *T. zimbabwensis* (T11) (Dick and Pozio, 2001; Sharma et al., 2020). In encapsulated taxa, larvae migrate to skeletal muscles, where they encyst inside muscle cells and form collagenous capsules (Jenkins et al., 2013). Transmission in animals and people occur by the consumption of first-stage larvae (L1) in striated muscle tissue from an infected animal. Larvae are subsequently released in the small intestine of the new host and develop into adult nematodes (Gottstein et al., 2009). Due to the nature of the life cycle, only carnivores and omnivores who consume meat are natural hosts for *Trichinella* spp.

Trichinella spp. exist in tropical, temperate, and polar ecosystems (Pozio and Zarlenga, 2013). In Canada, six species/genotypes have been reported in sylvatic and domestic animals (Gajadhar and Forbes, 2010; Jenkins et al., 2013; Sharma et al., 2020). *Trichinella spiralis*, the most common species in pigs, is considered eradicated in commercially raised swine herds in Canada (McIntyre et al., 2007; Dalcin et al., 2017) and rarely occurs in backyard pigs (Newman, 2014). More recently, human outbreaks of trichinellosis in Canada have been linked with the

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consumption of undercooked walrus and black bear meat (McIntyre et al., 2007). In northern Canada, *T. nativa* and *Trichinella* T6 are the most prevalent species and are freeze-resistant (Dick and Pozio, 2001; Gajadhar and Forbes, 2010; Jenkins et al., 2013; Pozio, 2016; Sharma et al., 2018). Once encysted in muscle tissue, these larvae can maintain infectivity despite multiple freeze-thaw events (Davidson et al., 2008; Pozio, 2016). The ability of larvae to survive in frozen muscle tissue has important consequences for the natural transmission cycle, as longer viability in the environment leads to a higher probability of ingestion by a scavenger (Pozio, 2016).

Scavengers and predators at the top trophic level are more likely to accumulate food-borne parasites. Thus, foxes *(Vulpes* spp.), polar bears (*Ursus maritimus*), and wolverines (*Gulo gulo*) can act as sentinels for *Trichinella* spp. in Arctic ecosystems (Bachand et al., 2018; Sharma et al., 2020; Gajadhar and Forbes, 2010). Foxes and wolverines are routinely trapped for traditional use and sale of fur, making carcasses accessible for research purposes. Two studies have reported the prevalence of *Trichinella* spp. in Arctic foxes from Canada (Smith and Snowden, 1988; Gajadhar and Forbes, 2010). However, neither of these studies identified larvae to species level. The recent discovery of *T. chanchalensis* in wolverines from Yukon (YT) and Northwest Territories (NT) (Sharma et al., 2019) has raised questions about the genetic diversity of *Trichinella* spp. and their distribution in Canadian wildlife. Therefore, we (1) determined *Trichinella* spp. prevalence and larval intensity, (2) evaluated whether age, sex, location, body condition, and weight of forelimb muscle processed were predictors of infectious status, and (3) identified species of *Trichinella* in harvested Arctic foxes from Banks Island and Victoria Island in the Canadian Arctic.

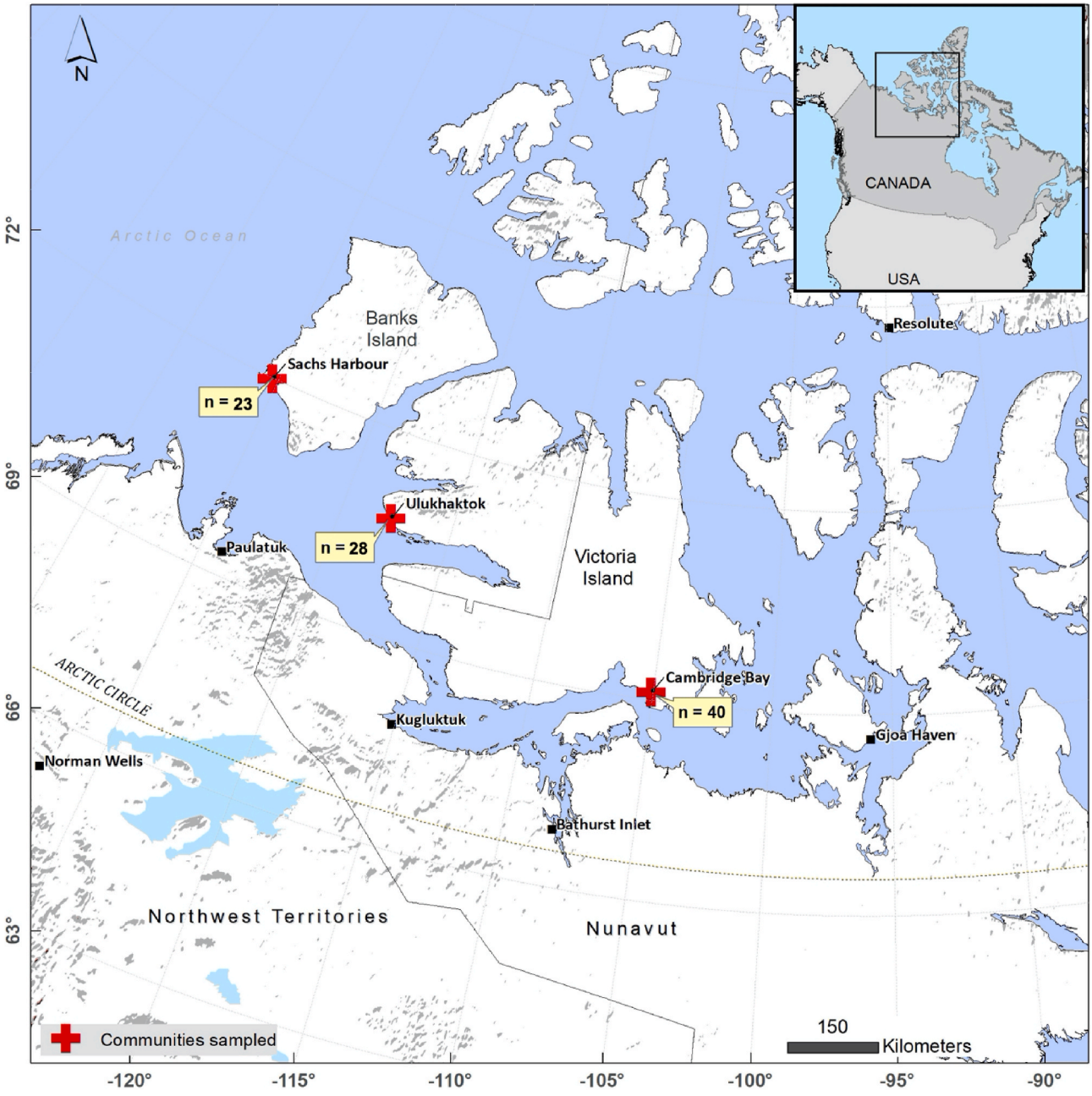

Fig. 1. Map of Nunavut and Northwest Territories showing the harvest locations of Arctic foxes (Vulpes lagopus) included in this study and their relative number by location for the sampling period 2018–2019.

2. Methodology

2.1. Study area

Fox carcasses were collected around the communities of Cambridge Bay, Nunavut (Ikaluktutiak; 69.1169° N, 105.0597° W), and Ulukhaktok (70.7368° N, 117.7704° W) and Sachs Harbour, Northwest Territories (Ikaahuk; 71.9851° N, 125.2465° W) (Fig. 1). The study areas are situated on traditional hunting and fishing grounds of the Nunavut Settlement Area and Inuvialuit Settlement Region (ISR), respectively. Inuit live in these areas characterized by a subsistence economy grounded in harvesting wildlife including the Arctic fox.

2.2. Collection of samples

This study includes 68 Arctic fox carcasses from Victoria Island [Cambridge Bay (n = 40) and Ulukhaktok (n = 28)] and 23 from Banks Island [Sachs Harbour] that were harvested for fur in 2018 and 2019 (Fig. 1). The skinned carcasses were then submitted either to the regional office of the Government of the Northwest Territories' Department of Environment and Natural Resources in Inuvik, NT or to the Canadian High Arctic Research Station (CHARS) in Cambridge Bay, where they were stored at −20 °C for 2–6 months. Gross necropsies and biometric measurements were performed on thawed carcasses at the Inuvik regional office and CHARS. Sex was recorded for each fox, body condition was visually assessed (Prestrud and Pond, 2003), and a tooth condition index was used to estimate age (Chevallier et al., 2017). Foxes were ranked from 1 to 5 based on fatness (Prestrud and Pond, 2003) and classified as young (≤1 yr) and adult (≥2 yr) based on tooth eruption and wear (Chevallier et al., 2017). The left *flexor ulnaris* muscle (forelimb muscle), a known predilection site for *Trichinella* spp. in foxes (Kapel et al., 1995), was collected, re-frozen, and transferred to the Zoonotic Parasite Research Unit (WCVM, Saskatoon, Canada). All samples were stored at −20 °C for 3–4 months until processing.

2.3. Recovery of larvae of Trichinella spp

Larvae of *Trichinella* spp. were recovered from the forelimb muscle by Pepsin–HCl digestion and sequential larval sedimentation. This enzymatic digestion method is the internationally accepted gold standard and has successfully been used to test for *Trichinella* spp. in wild and domestic mammalian species (Forbes and Gajadhar, 1999; Gajadhar and Forbes, 2010). Fat and connective tissue was removed from the muscle, and 5 g (minimum when available; if less, all available muscle sample) was cut into 1 cm × 1 cm pieces (Gamble et al., 2000). The remaining muscle was minced in a blender using 3–4 bursts of 10 s each. The tissue was digested in a 1% Pepsin-HCl solution for 1.5 h at 37 °C, followed by sequential sedimentation. Finally, 20 mL of the solution was collected in a Petri dish and examined under a dissecting microscope. After digestion, larvae were morphologically characterized as tightly coiled, lightly coiled, or C-shaped (Fig. 2). Larvae were counted and reported as larvae per gram (LPG) of muscle tissue. From each positive fox, 5 individual larvae and a pool of 10 larvae were collected in microcentrifuge tubes containing 1X PCR Buffer (Applied Biosystems 10X PCR buffer [Foster City, United States] diluted with ultrapure H_20; 10X PCR buffer composed of 100 mM Tris-HCl, pH 8.3; 500 mM KCl; 15 mM MgCl2; 0.01% gelatin) and stored at −20 °C until DNA extraction.

2.4. DNA extraction and multiplex PCR

DNA was extracted from larvae using the Proteinase K extraction method as per Scandrett et al. (2018). Multiplex PCR was performed as per Zarlenga et al. (1999). Larvae of six recognized species of *Trichinella* (*T. spiralis, T. nativa, T. britovi, T. pseudospiralis, T. murrelli* and *Trichinella* T6) were provided by the Canadian Food Inspection Agency (Saskatoon, Canada) as positive controls.

Fig. 2. Possible shapes of Trichinella spp. larvae after digestion showing C-shape (upper left), tightly coiled (middle), and lightly coiled (lower right). Scale bar as shown.

2.5. PCR-RFLP

In order to differentiate *T. nativa* from *T. chanchalensis* (T13), PCR-RFLP was performed on DNA of larvae that were identified as *T. nativa* on multiplex PCR as described previously (Sharma et al., 2019, 2020). On PCR-RFLP, ~920 bp amplified products of positive controls showed distinct band patterns after restriction digestion: three bands of approximately 407, 377 and 130 bp for *Trichinella* T6; two bands of approximately 537 and 377 bp for *T. nativa* (T2); two bands of approximately 507 and 407 bp for *T. chanchalensis* (T13). In each PCR-RFLP run, positive (T2, T13 and T6) and negative controls were included.

2.6. Statistical analysis

We used descriptive statistics to summarize the characteristics of the foxes included in this study. A fox was considered positive if one or more larvae of *Trichinella* spp. were recovered from the muscle tissue examined. Prevalence and 95% confidence intervals (CI) were calculated using EpiTools epidemiological calculators (Sergeant, 2019). Body condition index was ranked from 1 to 5, with 5 as obese, 4 as overweight, 3 as ideal weight, 2 as underweight, and 1 as emaciated. Possible associations between predictor variables (age, sex, location, body condition and weight of muscle tissue processed [< 5 g vs. ≥ 5 g]) and the outcome variable (infection status) were evaluated by binary logistic regression. A relaxed level of significance ($p \leq 0.20$) was initially used to identify variables on univariable logistic regression. Stepwise forward multivariable regression analysis was performed to include potential risk factors in the final model and only associated predictors at a significance level of $p < 0.05$ were retained in the model. Goodness of fit of the final model was evaluated by the Hosmer Lemeshow test. Odds ratios and their respective 95% confidence intervals were calculated to estimate the degree of the association between each significant predictor and infection status. Larval intensity was reported as LPG of muscle. All statistical analyses were performed using IBM SPSS (ver. 24; Armonk, New York, USA).

2.7. Ethical approval

As foxes were harvested for purposes other than research (lawfully harvested for fur), they are considered Category A and exempt from Animal Research ethics review at the University of Saskatchewan. We worked closely with the Governments of the Northwest Territories and Nunavut for wildlife research and export permits.

3. Results

3.1 Descriptive analysis, prevalence, and larval intensity The mean age class of the Arctic foxes examined (n = 90) was 1.52 years (SD 2.08, range 0–9). Age could not be estimated in one Arctic fox, as the head was not submitted with the carcass. A higher proportion of males than females [59% (54/91) vs. 41% (37/91)] were sampled. Processed muscle weight was ≥5 g in 55 of the animals (60%). *Trichinella* spp. larvae were recovered from 19 of 91 Arctic foxes, a prevalence of 21% (95% CI 14–30). Larvae were detected more frequently in adult foxes [34% (11/32), 95% CI 20–52) when compared to young foxes [14% (8/58), 95% CI 7–25]. A higher proportion of males were infected [24% (13/54), 95% CI 15–37) when compared to females [16% (6/37), 95% CI 8–31) (Table 1). Finally, a higher prevalence was observed in foxes from Banks Island [26% (6/23), 95% CI 13–47) when compared to those from Victoria Island [19% (13/68), 95% CI 12–30] (Table 1). Mean and median larval intensities were 192.81 LPG (SD 508.40; range 0.2–2224) and 16.76 LPG, respectively. Most recovered larvae were tightly coiled and still viable, and all recovered larvae were morphologically consistent with *Trichinella* spp.

3.2. Association of prevalence with risk factors

Univariable logistic regression revealed age (p = 0.026) as the only variable amongst the ones examined to be significantly associated with *Trichinella* spp. infection (Table 1). In this study, *Trichinella* spp. larvae were detected most frequently in adult foxes [34% (11/32), 95% CI 20–52] when compared to young foxes [14% (8/58), 95% CI 7–25] and the odds of being infected with *Trichinella* spp. were three times higher in adult than in young foxes. Although a higher proportion of males were infected with *Trichinella* spp. [24% (13/54), 95% CI 15–37] when compared to females [16% (6/37), 95% CI 8–31], sex did not appear to be a significantly associated with infection in our model (Table 1). The remaining variables considered – location, body condition index and muscle weight processed – did not appear to be associated with infection status (Table 1).

Table 1
Univariable analysis of variables associated with *Trichinella* spp. infection among Arctic foxes (*Vulpes lagopus*) from northern Canada.

Variable	P (%)	n	OR	95% CI	p value
Age					
Young[a](≤1 year)	8 (13.8)	58			
Adult (≥2 years)	11 (34.4)	32	3.27	(1.15–9.30)	0.026
Sex					
Female[a]	6 (16.2)	37			
Male	13 (24.1)	54	1.64	(0.56–4.80)	0.368
Location					
Banks Island[a]	6 (26.1)	23			
Victoria Island	13 (19.1)	68	0.67	(0.22–2.03)	0.48
BCI					0.30
BCI score 1 (Emaciated)[a]	3 (17.6)	17			
BCI score 2	1 (7.1)	14	0.13	(0.06–1.47)	0.29
BCI score 3	3 (13.6)	22	0.05	(0.01–1.02)	0.256
BCI score 4	6 (26.1)	23	0.05	(0.04–1.06)	0.892
BCI score 5 (Obese)	6 (42.9)	14	0.30	(0.12–1.93)	0.274
Weight processed					
Less than 5 g[a]	9 (25)	36			
≥5 g	10 (18.2)	55	0.67	(0.24–1.85)	0.44

n = number of Arctic foxes tested.
P (%) = number of positive animals (% age positive).
OR = Odd ratio.
95% CI = 95% Confidence Interval.
[a] = reference category.

3.2. Molecular characterization of larvae of Trichinella spp

Trichinella spp. larvae from 15 foxes were successfully speciated on multiplex PCR. The DNA of larvae retrieved from four foxes did not amplify, however larval morphology was consistent with *Trichinella* spp. Larvae retrieved from 14 of the speciated foxes were identified as *T. native* while larvae retrieved from the remaining tested fox from Ulukhaktok (Victoria Island) were identified as a co-infection with *T. nativa* and *Trichinella* T6. All larvae identified as *T. nativa* on the multiplex PCR were confirmed as such by the PCR-RFLP.

4. Discussion

The overall prevalence of *Trichinella* spp. in the Arctic foxes sampled in this study was 21%, which is higher than the previously reported 2–11% in Arctic foxes from Canada, Alaska, and Greenland (Jenkins et al., 2013). Variations in prevalence could be attributed to ecological differences in dietary habits, season, year, and geographical locations, in addition to sampling bias (male dominated in the current study) and laboratory techniques (Gajadhar and Forbes, 2010). Many foxes were trapped near communities and their diets may include scraps from hunting activities or human settlements. However, it is important to note that the pack ice that forms during the winter months can connect islands in the Arctic Ocean to the mainland. Thus, foxes harvested during the 2018 and 2019 winter may not have been restricted to Banks and Victoria Island, and/or could have consumed wildlife migrating from the mainland. Previous studies have suggested that small home ranges are characteristic for foxes in coastal habitats where prey abundance is concentrated and predictable, whereas larger home ranges are characteristic of inland habitats where prey abundance is more widely distributed (Anthony, 1997). Resource availability in coastal ranges may also support a larger population of Arctic foxes, producing greater home-range overlap between neighboring foxes (Eide et al., 2004). This may contribute to the higher prevalence of *Trichinella* infection through intensified scavenging behavior or cannibalism. Furthermore, foxes trapped near human settlements may provide increased opportunities for scavenging and territorial interactions. Differences in the sample size, amount of tissue available, and methodology can also affect the prevalence. We used leg muscles, which are considered predilection sites for *Trichinella* spp. in Arctic foxes. More than 60% of muscles processed weighed ≥5 g, which is the recommended sample amount for recovery of larvae of *Trichinella* spp. to detect infections as low as 1 LPG of tissue (Gamble et al., 2000).

The mean and median intensities of *Trichinella* spp. larvae in Arctic foxes reported in our study in the NT and NU were substantially higher than a previous study by Gajadhar and Forbes (2010) in NU and YT (mean 193 vs. 8, and median 17 vs. 2). More than or equal to 1 LPG is considered a significant risk for food safety (Gajadhar and Forbes, 2010), and 80% of the positive foxes in our study had ≥ 1 LPG. While foxes are not typically considered food animals, higher larval burden may indicate higher parasite biomass in sympatric wildlife. This suggests that further studies are required to identify *Trichinella* spp. in wild game animals that are consumed by people, such as walrus and bear. Domestic dogs co-habiting with humans potentially consume similar prey animals as foxes and could also serve as sentinels.

Our findings indicated that fox age was the only variable amongst the ones we considered that was significantly associated with *Trichinella* spp. infection. Larvae were most frequently detected in adult foxes, which is consistent with the nature of exposure to and bioaccumulation of the parasite over time. This could be attributed to the cumulative consumption of infected meat/carrion during a lifetime. For example,

predictability and availability of prey resources are important determinants for the dispersal range of Arctic foxes. Non-territorial juveniles often undergo long-range dispersal from natal areas with high prey abundance, while established monogamous pairs defend resource-rich territories (Eide et al., 2004). This provides optimal circumstances for foxes to accumulate the parasite while scavenging and hunting prey across wide geographic ranges over their lifetime. Similar findings have been reported in other wild animals, such as lynx, wolves, raccoon dogs, and red foxes (Frey et al., 2009; Kärssin et al., 2017; Oksanen et al., 1998; Zarnke et al., 1999).

A higher proportion of males were infected, which may be due to higher submission rates of male vs. female carcasses (54% vs. 46%). However, male biases in parasitism are common and may result from differences in exposure and/or immunological variance (Robinson et al., 2008). For example, a higher prevalence of *Trichinella* spp. in males has been previously reported in wolverines from NU, Canada (Reichard et al., 2008) and wolves and brown bears from Finland (Kojola et al., 2016). Sexual dimorphism exists between male and female Arctic foxes, as males had a heavier body weight (19%) and longer body length (4%). This is consistent with other canid species and suggests that energy requirements differ between sexes (Prestrud and Nilssen, 1995). The consumption of more prey by larger males, or larger dispersal distances, would increase the probability of *Trichinella* spp. infestation and could contribute to the observed male bias.

To our knowledge, this is the first report that identifies the species of *Trichinella* in Canadian Arctic foxes. All foxes with larvae that successfully amplified were infected with *T. nativa* (T2), a common sylvatic species in North America. We also detected a co-infection (*T. nativa* + *Trichinella* T6) in one female fox. Our findings are consistent with existing literature that documents dominance of *T. nativa* and in some cases T. *nativa* and T6 co-infections in wildlife from central northern regions of Canada (Nunavut) and eastern Canada (Québec) (Gajadhar et al., 2010, 2020; Bachand et al., 2019; Reichard et al., 2008). Both taxa of *Trichinella* are freeze-resistant and can survive in Arctic environments (Dick and Pozio, 2001; Gajadhar and Forbes, 2010; Jenkins et al., 2013; Pozio, 2016; Sharma et al., 2018). This is the first time that *Trichinella* T6, the most common genotype of *Trichinella* observed in a broad range of Canadian wildlife (Gajadhar and Forbes, 2010), has been found in an Arctic fox, and also the most northerly report globally, to the best of our knowledge. The co-infection may have resulted from exposure to multiple sources during the fox's lifetime or concurrently from a single source.

Our findings have important implications for broadening the known host and geographic ranges of *T. nativa* and *Trichinella* T6 in Arctic ecosystems. Future work is needed to characterize the genetic diversity and intensity of *Trichinella* spp. in Arctic foxes across their circumpolar range, and their primary sources of infection.

Declaration of competing interest

As Corresponding Author, I confirm that this manuscript has been read and approved by all named authors. There are no conflicts of interest to declare.

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