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MUSKRATS (*ONDATRA ZIBETHICUS*) ARE COMPETENT INTERMEDIATE HOSTS OF *ECHINOCOCCUS MULTILOCULARIS* IN NORTH AMERICA

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ABSTRACT: The tapeworm *Echinococcus multilocularis* is an emerging pathogen of significance to human and animal health in Canada, yet little is known about key rodent intermediate hosts in local urban ecosystems. In Europe, invasive muskrats (*Ondatra zibethicus*) are an important indicator intermediate host species; however, the role of this semiaquatic rodent in the ecology of *E. multilocularis* is undetermined in the North American context. We examined 93 muskrats that were livetrapped in the spring of 2017 within Calgary, Alberta, Canada, for the presence of *E. multilocularis* infection. The objectives of this study were to 1) diagnose alveolar echinococcosis using macroscopic assessment, histopathology, and molecular analyses; 2) quantify infection severity; and 3) assess host demographic risk factors for infection. Macroscopic cysts consistent with alveolar echinococcosis were present in 24% of muskrats (22/93). Most individuals had hepatic cysts; however, cysts were also occasionally detected in the mesentery, reproductive organs, omentum, peritoneum, spleen, diaphragm, lung, or kidney. The mean number of cysts per liver was 2.1 (range, 1–4). We examined hepatic cysts from 18 individuals using histology; all had lesions compatible with alveolar echinococcosis. Protoscoleces, indicative of patent infections, were present in 14/18 (78%). No demographic risk factors (sex, body condition, body mass) were significantly associated with infection. Muskrats in the North American context are competent intermediate hosts with high infection prevalence and may play an important role in the ecology of this emerging parasite.

Key words: Disease, *Echinococcus multilocularis*, muskrats, *Ondatra zibethicus*, parasites, pathology, rodents, wildlife.

INTRODUCTION

Climate change and international movements of both people and animals have resulted in changing infectious disease epidemiology. Emerging infectious diseases of wildlife origin, including rodents, are a global public health concern (McFarlane et al. 2012; Han et al. 2015). Enormous diversity among rodents, representing 40% of all mammalian species; environmental adaptability of some species to altered environments such as urbanization (Bordes and Morand 2011; McFarlane et al. 2012); and short lifespans with high reproductive rates have positioned rodents as a prominent source of established and emerging zoonotic diseases (Han et al. 2015). Accelerating anthropogenic global processes that include land use change, climate change, and biodiversity loss (especially declines in megafauna populations), are leading to dramatic rodent population increases (the rodentization phenomenon; Mills and Childs 1998; Comer et al. 2001; Pongsiri et al. 2009; Lau et al. 2010). These changes may greatly alter rodent population dynamics, with profound consequences for infectious diseases (Mills and Childs 1998; Pongsiri et al. 2009).

Among emerging rodent-borne zoonoses in North America is the parasite *Echinococcus multilocularis*. This tapeworm causes disease in domestic dogs and humans, probably because of the recent introduction of a European strain (Peregrine et al. 2012; Gesy et al. 2013; Skelding et al. 2014; Massolo et al. 2019; Santa et al. 2021). Human and canine infections with this parasite are apparently increasing in Canada and may be an important indicator of endemicity.

Adult *E. multilocularis* live in canid definitive hosts such as coyotes (*Canis latrans*), red foxes (*Vulpes vulpes*), wolves (*Canis lupus*), and domestic dogs (*Canis lupus familiaris*), and produce eggs that are shed in feces (Deplazes and Eckert 2001). Intermediate rodent hosts ingest eggs from the environment. These develop into the metacestode stage, creating invasive liver cysts that spread throughout the abdomen of the intermediate host in a disease process called alveolar echinococcosis (AE; Deplazes and Eckert 2001). Canids consume infected rodents, completing the life cycle. Domestic dogs and people that incidentally consume eggs may develop AE, which is a severe, often fatal condition (Deplazes and Eckert 2001). Between 2013 and 2020 there have been 17 human cases in Alberta, Canada (Houston et al. 2021) and 27 known dog AE cases since 2009 (Kolapo et al. 2023), supporting that the parasite is highly endemic and probably emerging in Alberta.

Successful predation of intermediate hosts is necessary for infection to establish in definitive hosts and therefore impacts the risk to public health (Otero-Abad and Torgerson 2013). Although infection of North American canids has been explored (e.g., Massolo et al. 2014), there are still substantial knowledge gaps regarding intermediate hosts in this setting (Liccioli et al. 2014). In North America, meadow voles (*Microtus pennsylvanicus*), deer mice (*Peromyscus maniculatus*), and house mice (*Mus musculus*) are known intermediate hosts, yet infection prevalence is typically $<10\%$ and infected individuals may be highly clustered in small geographical areas (Massolo et al. 2014).

The muskrat (*Ondatra zibethicus*) is a potential intermediate host of importance. Muskrats are abundant, semiaquatic, medium-sized rodents that occupy wetlands in urban, rural, and natural environments (Erb and Perry 2003). Previous studies of invasive muskrats in Europe have established their role as hosts of zoonotic pathogens, including *E. multilocularis* (Gottstein et al. 2015; Umhang et al. 2013; Oksanen et al. 2016). Further studies are necessary to determine their role as hosts of these pathogens in North America and to determine host risk factors associated with infection.

The objective of our study was to investigate muskrats as potential competent intermediate hosts for *E. multilocularis* in Calgary, Alberta, Canada. Specifically, we aimed to 1) diagnose the presence of *E. multilocularis* infection using macroscopic assessment, histopathology, and molecular identification; 2) quantify infection severity in affected individuals; and 3) assess demographic risk factors associated with infection.

MATERIALS AND METHODS

In spring 2017, an experienced trapper collected muskrats adjacent to ponds within the city of Calgary, Alberta, Canada, for their fur. He frequently opened the abdomen during the skinning process and he noted the presence of abnormal liver and abdominal structures. He submitted skinned frozen carcasses of both grossly normal and abnormal individuals to the Canadian Wildlife Health Cooperative, Alberta Region, for diagnostic investigation. An anatomic veterinary pathologist with technician support completed a necropsy on each animal and collected the following data: lesion location, sex, body mass, and body condition score (subjective five-point scale based on internal and subcutaneous fat scores). We fixed samples of lesions and major organs (brain, esophagus, heart, kidney, liver, ovary or testis, skeletal muscle, spleen, tongue, thyroid glands, and trachea) in 10% neutral-buffered formalin for 48 h, then processed tissues and slides by routine methods. Veterinary pathologists examined 4-µm-thick sections of paraffin-embedded tissues stained with H&E. The University of Calgary Animal Care Committee approved this diagnostic investigation (approval number AC22-0179).

We froze additional samples of lesions and subsequently submitted lesions from three randomly selected individuals for which there were consistent gross and histologic liver lesions for confirmatory *E. multilocularis* PCR (NAD1 gene) at the Animal Health Laboratory, University of Guelph, Guelph, Ontario, Canada. Briefly, approximately 25 mg of tissue was homogenized in an RNA isolation reagent (TRI Reagent, Invitrogen, Waltham, Massachusetts, USA) using two stainless steel beads, in a high-energy cell disrupter (Mini-Beadbeater, BioSpec Products, Bartlesville, Oklahoma, USA). Nucleic acid was extracted from 100 µL of the homogenate with a magnetic particle processor (MagMAX Express-96 instrument, Life Technologies, Waltham, Massachusetts, USA) using a purification kit (MagMAX™ Pathogen RNA/DNA Kit, Life Technologies, Burlington, Ontario, Canada), and the manufacturer's low cell content protocol. The DNA extracts were tested using gel-based PCR assays specific for *E. multilocularis*, *Echinococcus granulosus* sensu lato (s.l.) and *Taenia* spp. (Trachsel et al. 2007). The PCR products were purified and sequenced using the primers Cest-1 and Cest-2 with an Applied Biosystems 3730 DNA Analyzer (Life Technologies, Burlington, Ontario, Canada). The sequences were assembled into a consensus sequence using a bioinformatic program (Geneious® 11.1.2, Biomatters, Auckland, New Zealand). After the primers were trimmed, the sequence was used to perform a Blast search in GenBank (NCBI 2022). We uploaded the sequence to GenBank (no. BankIt2710887 NAD1 OR098881).

We subsequently reexamined formalin-fixed organs to quantify cysts and the extent of the infection. We collected the following data: liver mass, number of cysts present, and the diameter of the superficial liver cysts. Based on the observation that superficial cysts were roughly spherical, we calculated the surface area ($A=4\pi r^2$) of the largest hepatic cyst in each individual. For this formula, we calculated r by taking the mean of the length and width of the cyst measurement and dividing by 2.

We performed statistical analyses using R (R Core Team 2023). We fitted univariable logistic regression models with AE gross lesion status (positive or negative) as the outcome, and demographic characteristics (sex, body mass, body condition score) as explanatory variables. To compare the mass of infected vs. noninfected livers, we used Welch's *t*-test. To compare the accuracy of the trapper's detection of lesions versus laboratory diagnosis, we calculated the Cohen's kappa statistic. We considered $\alpha=0.05$ significant. We calculated prevalence and 95% confidence intervals (CIs) using the Wilson method in Epitools (Sergeant 2018).

RESULTS

This study included 93 adult muskrats, 37 females (40%) and 55 males (60%); sex was not recorded for one individual. Among females, the mean skinned body mass was 745 g (interquartile range [IQR]=687–810 g) and mean body condition score was poor (2.0/5). Among males, the mean skinned body mass was 763 g (IQR=677–841 g) and body condition score was slightly higher than females (2.6/5).

Macroscopic cysts were present in 22 individuals (24%; 95% CI, 16.2–33.2%). Most cases had liver cysts (21/93; 23%); one animal presented with a single peritoneal cyst and no liver cysts. Cysts were also present in the following extrahepatic locations: mesentery (7/93; 8%), reproductive organs (5/93; 5%); omentum (4/93; 4%), peritoneum (3/93; 3%), spleen (3/93; 3%), diaphragm (1/93; 1%), lung (1/93; 1%) and kidney (1/93; 1%). Cysts expanded from the surface of the liver and invaded the liver parenchyma. When sectioned, cysts were well demarcated, multiloculated, opaque, and spongy and contained friable green-tan material and flocculent fluid (Fig. 1). Considering only individuals with liver cysts, there was a mean of 2.1 cysts per liver (range, 1–4 cysts per liver). The mean cyst surface area of the largest hepatic cysts in each individual was 1,510 mm^2 (range, 13–4,300 mm^2). Considering the sum of the four largest hepatic cysts, the mean total surface area was 2,470 mm^2 (range, 13–6,960 mm^2). Muskrats with hepatic cysts had significantly heavier livers compared with those without cysts (mean=38 g vs. 31 g; $P=0.03$). Using univariable logistical regression models, there were no significant demographic risk factors associated with the presence of gross cysts ($P>0.78$ for sex, body mass, and body condition score).

Histologically, hydatid cysts were characterized by multiloculated cystic structures that expanded from and partially effaced hepatic parenchyma (Fig. 2). The cysts were composed of a thick hyaline eosinophilic outer membrane, occasionally lined by a thin, lacy inner germinal epithelial layer that frequently contained calcareous corpuscles. Within brood capsules that occasionally budded from the germinal epithelium, and free within the lumen of the cyst, were frequent protoscoleces that ranged in size from 75 to 100 μm and contained birefringent, light brown

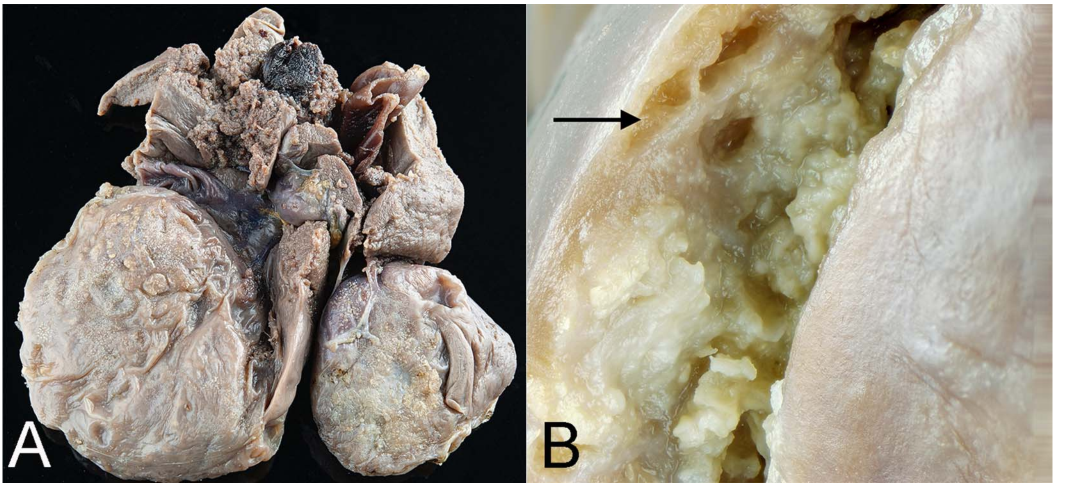

FIGURE 1. Macroscopic lesions of *Echinococcus multilocularis* alveolar cysts in a wild muskrat (*Ondatra zibethicus*) from Calgary, Alberta, Canada (formalin-fixed specimens). A. Cysts are present in multiple liver lobes. B. The inner cystic structures are multiloculated (arrow) and contain tan, flocculent debris.

hooklets. Edges of the cystic invasion contained variable numbers of lymphocytes, plasma cells, macrophages, and rare multinucleated giant cells. Variably thick bands of fibroblasts and fibrous connective tissue occasionally surrounded cysts.

Protoscoleces, indicative of patent infections, were present in 14/18 (78%; 95% CI, 54.8–91.0%) individuals for which histology of lesions was available for examination. Not all cysts were available for histopathology examination (missed

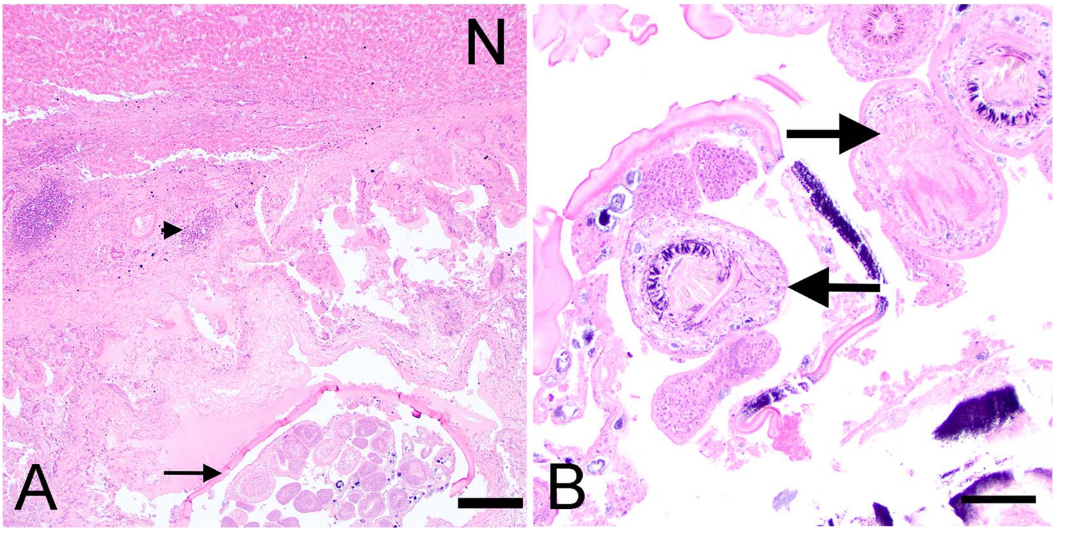

FIGURE 2. Microscopic lesions of an *Echinococcus multilocularis* alveolar cyst in the liver of a wild muskrat (*Ondatra zibethicus*) from Calgary, Alberta, Canada (H&E). A. A thick hyaline eosinophilic membrane surrounds a brood capsule containing numerous protoscoleces (arrow). This is surrounded by variably thick bands of fibroblasts and fibrous connective tissue that contain macrophages and multinucleated giant cells. Small clusters of lymphocytes and plasma cells are present at the edges of the cystic invasion (arrowhead), which abruptly transitions to normal liver (N); scale=200 µm; 4×. B. Protoscoleces (arrows) contain birefringent, light brown hooklets. The outer eosinophilic hyaline membrane is lined by a germinal epithelial layer that contains basophilic calcareous corpuscles (scale=50 µm; 20×).

during trimming because of discoloration with fixation and/or small size).

Among the 24 animals identified as abnormal by the trapper, 21 had macroscopic lesions consistent with AE identified during necropsy examination. Among the 69 remaining animals, we identified one additional case with macroscopic cysts during necropsy examination. There was near-perfect agreement between the laboratory analyses and the trapper's observations of abnormalities (Cohen's kappa=0.88).

Samples from the three individuals with hepatic cysts tested by PCR were positive for *E. multilocularis* using primers Cest-1 and Cest-2, whereas the *E. granulosus* s.l. PCR and *Taenia* spp. PCR tests were negative. Subsequent sequencing of the NAD1 gene revealed 100% (344/344 bp) identity to published sequences for the *E. multilocularis* NADH dehydrogenase subunit 1 gene (e.g., GenBank ID MN444749-MN444805, MH986749-MH986751, KX384668–KX384671).

DISCUSSION

Our results show that wild muskrats are a competent intermediate host of *E. multilocularis* with high infection prevalence in a North American setting. Historically, experimental infection studies have successfully used muskrats to maintain laboratory strains of this parasite (Lubinsky 1960) and to study cyst development (Rausch and Schiller 1956; Webster and Cameron 1961). Natural infection in wild muskrats in North America have been only rarely described. For instance, Rauch and Richards examined 12,142 muskrats from North Dakota, US, between 1966 and 1969; none had gross lesions compatible with *E. multilocularis* infection (Rausch and Richards 1971). Based on those results, the prevalence in that population was $<0.02\%$ (95% confidence; Hanley and Lippman-Hand 1983), supporting the rarity of infections. Two studies in southwestern Montana, US (approximately 800 km from Calgary, Canada), conducted between 1977 and 1981, identified 4/849 (0.5%) muskrats with fertile infections (Eastman and Worley 1979; Feigley and Worley 1988).

In Europe, where muskrats are an invasive species and *E. multilocularis* is endemic, prevalence among muskrats is decidedly variable. Prevalence in previous European studies of muskrats have included 0.1% in the Netherlands (Borgsteede et al. 2003), 1% in France (Umhang et al. 2013), and 4% in Germany (Baumeister et al. 1997); these are all considered low endemic areas. In contrast, prevalences were much higher in areas deemed highly endemic for the parasite, such as 32% in a particular area of Belgium (Mathy et al. 2009). A meta-analysis estimated the pooled prevalence of European muskrat infections at 4% and concluded that muskrats were important intermediate hosts on that continent based on their relatively high infection prevalence compared with other intermediate hosts (Oksanen et al. 2016). Some researchers have gone so far as to propose that muskrats are an ideal bioindicator for the geographical distribution of the parasite on the European continent (Hanosset et al. 2008; Mathy et al. 2009). Based on the prevalence of infection in Calgary, our results add further support to the evidence that this area is highly endemic for *E. multilocularis* (Houston et al. 2021).

Because the samples in our study originated from a single urban pond system, and given that infection in intermediate hosts is prone to geographical clustering (Otero-Abad and Torgerson 2013), it is possible that we identified a geographical area with abnormally high infection prevalence. All muskrats were trapped in the spring; thus, we cannot assess seasonality of muskrat infection. However, the apparent high prevalence of infection during spring is consistent with other intermediate hosts and infection in definitive hosts in this area and elsewhere (Otero-Abad and Torgerson 2013; Liccioli et al. 2014). Based on the size and presence of protoscoleces, indicative of a fertile infection, most of the lesions observed in these muskrats were probably several weeks to months old (Webster and Cameron 1961). This suggests that infection occurred during the fall and winter and supports the accumulation of infection in longer-lived rodent species such as muskrats.

We found no association between demographic risk factors (sex, body condition, and body mass) and infection status. Similarly, sex was not found to be a significant risk factor in studies of muskrats from Belgium (Hanosset et al. 2008; Mathy et al. 2009; Cartuyvels et al. 2022). However, age (adult vs. subadult) appears to be a significant risk factor for infection in European muskrat populations (Hanosset et al. 2008; Cartuyvels et al. 2022; Martini et al. 2022). Because our study included only adults, we could not assess the effect of age on infection status. In other wild rodents, such as wild Norway rats (*Rattus norvegicus*), parasitic infections tend to be more common in individuals with heavier body mass and those that are sexually mature (Rothenburger et al. 2019).

Fertile infections in our study were present in 78% of muskrats. The high rate of fertile infections is also common in studies of European muskrat populations. For instance, Mathy et al. described fertile infections in 99% of muskrats for which they examined liver lesions histologically for protoscoleces (Mathy et al. 2009). Martini et al. found a mean of approximately 300,000 protoscoleces per infected muskrat from Luxembourg (Martini et al. 2022), which was higher than that in other typical intermediate hosts such as voles, in European ecosystems (Deplazes and Eckert 2001). We did not quantify protoscoleces in our study because we examined lesions by histopathology, although this could be explored in future studies. The prevalence of fertile infections is probably an underestimate, because histopathology involves examination of a small area (4 μm) of the entire cyst. To increase accuracy, we ideally would have examined the negative cases further using multiple sections of different cysts and deeper recut sections; resource limitations precluded this. It is also possible that our sample included early-stage infections in which protoscoleces had not yet developed (Webster and Cameron 1961). Additionally, not all cysts were available for histology examination because small cysts were difficult or impossible to identify after formalin fixation. This could be improved by more careful sampling of small gross cysts at the time of necropsy.

Infection prevalence in these muskrats was markedly higher than in other rodent species that act as intermediate hosts in this geographic area, such as meadow voles (*Microtus pennsylvanicus*), deer mice (*Peromyscus maniculatus*), and southern red-backed voles (*Myodes gapperi*), in which the prevalence has been estimated at ≤1% (Liccioli et al. 2014); that study had a methodologic focus on terrestrial small mammals that precluded the inclusion of muskrats. The higher prevalence that we found in muskrats compared with other intermediate hosts in Calgary is consistent with the situation in Europe (Martini et al. 2022).

The main definitive host in Calgary is the coyote (*C. latrans*), which commonly inhabits this expansive urban area (Liccioli et al. 2014; Santa et al. 2021). Overall prevalence in coyotes in the city of Calgary and the surrounding area has been estimated at 21% but was as high as 84% in one particular area of the city during one season (Liccioli et al. 2014). Kotwa et al. identified a similar prevalence of 24% among coyotes and red foxes (*V. vulpes*) in southern Ontario, Canada, a densely populated area that was previously free of this parasite (Kotwa et al. 2019). A study in Europe identified a correlation between infection prevalence in muskrats and red foxes (Hanosset et al. 2008). In North American urban ecosystems, the relationship between definitive hosts and muskrat infection prevalence is unknown. However, the identification of this parasite in muskrats is important, given that Calgary is the first North American city known to contain a sylvatic life cycle of this parasite (Liccioli et al. 2014) and muskrats probably play a key role in its epidemiology elsewhere.

Fecal analysis of coyote scat from other areas of the city indicates that muskrats are rare in the diet of urban coyotes (<5%)

compared with small mammals such as meadow voles (*Microtus pennsylvanicus*; 34%; Liccioli et al. 2014). Nevertheless, given the high rate of fertile infections and the potential for extraordinarily high numbers of protoscoleces per individual (Martini et al. 2022), consumption of an infected muskrat may establish a heavy infection burden in a given coyote or other definitive host. This may lead to production of large quantities of eggs to contaminate the environment (Martini et al. 2022). Thus, muskrats may disproportionately affect transmission rates. This is also concerning from a One Health perspective, because domestic dogs can also act as definitive hosts; a dog with a particularly high infection burden from consuming an infected muskrat may represent a high risk for spreading the parasite to in-contact people (Deplazes and Eckert 2001). In some areas of Europe, muskrat carcasses are skinned and left on the land at the trapping location, making them available for red foxes (*V. vulpes*) to consume and complete the life cycle (Hanosset et al. 2008). Some have speculated that this practice has contributed to the high infection prevalence among red foxes in Europe. In western Canada, trappers typically use skinned muskrat carcasses as bait to trap carnivores or leave the carcasses on the land for scavengers (G. Styler and T. Rothenburger, pers. comm.). This practice might impact parasite transmission dynamics by making infected carcasses available to definitive hosts in the Calgary area.

The impact of AE on the fitness of individual muskrats is unknown, but these infections generally have a negative effect on survival. These muskrats were livetrapped rather than found dead, and there was no association between infection and poor body condition, which would be indicative of a direct effect on host fitness. Furthermore, there was no apparent degradation of fur quality based on fur sale price compared with previous years (G. Styler, pers. comm.). Therefore, these were not yet fatal infections, and if there were effects on health, these were probably subclinical. Through quantification of liver lesions, we identified a range of infection severity; however, given the tremendous regenerative capacity of the liver, these cysts were unlikely to have a significant direct effect on liver function. Additionally, there were no findings indicative of liver dysfunction, such as icterus. With chronicity, the invasive nature of *E. multilocularis* vesicular lesions may also directly contribute to mortality (Deplazes and Eckert 2001). However, recovery of muskrat carcasses in aquatic ecosystems is challenging and mortality events related to *E. multilocularis* are likely to go undetected.

There are also potential indirect effects of infection on host fitness. In other systems, macro- and microparasites may directly modify host behavior to increase the chance of pathogen survival, reproduction, and transmission (Webster 2001; Ezenwa et al. 2016). This phenomenon is evident in the life cycles of many parasites in which the parasite causes behavior changes in the infected prey animal that increases susceptibility to predation (e.g., *Toxoplasma gondii*; Webster 2001). It is unknown if *E. multilocularis* has similar behavior effects on muskrat intermediate hosts. Intrahost factors related to illness may impact pathogen ecology through sickness behaviors and by proinflammatory cytokine–mediated effects on the immune system, which may subsequently alter foraging, social interactions, and dispersals, thereby influencing pathogen dynamics (Tizard 2008; Mathy et al. 2009; Bouwman and Hawley 2010). Such potential associations need to be assessed.

Muskrats are long-lived species compared with other intermediate hosts. This longevity and a slower clinical course of infection may contribute to a bridge effect in which the parasite survives in long-lived intermediate hosts during times of year that the environment is generally less favorable for environmental egg survival (Massolo et al. 2022). This contrasts with other small rodent intermediate hosts with shorter lifespans and potentially more virulent infections. Although Calgary is in the northern hemisphere, and therefore within the typical range of *E. multilocularis*, summers are typically hot and dry, which would impede egg survival (Otero-Abad and Torgerson 2013).

The predominant strain of *E. multilocularis* in definitive, intermediate, and incidental hosts in western Canada is of European origin (Gesy et al. 2013; Massolo et al. 2019; Santa et al. 2021). It is possible that the European strain may have particular infectivity and virulence in muskrats compared with North American strains, given the high infection prevalence detected in this species on both continents. Future genetic work needs to investigate the similarities among *E. multilocularis* from muskrats and other hosts in this area.

Although researchers had previously acknowledged the possible role of muskrats in the Calgary urban *E. multilocularis* system (Liccioli et al. 2014), study of these rodents had not been specifically undertaken when the hepatic cysts were identified by the trapper. As in our study, knowledge gaps resulting from limitations of resources and study design may possibly be overcome by the opportunistic use of harvester knowledge. Experiential knowledge of hunters and trappers is an important channel for identifying new, emerging, and ongoing threats to wildlife health (Rothenburger et al. 2016). Individuals who harvest wildlife are a practical and knowledgeable information source and should be considered for wildlife disease surveillance activities (Kutz and Tomaselli 2019).

As with other urban wildlife, the potential for sustained and frequent contact between muskrats, people, domestic animals, and their environment in cities increases the risk of pathogen transmission (Bradley and Altizer 2007; Bordes and Morand 2011; Rothenburger et al. 2017). Knowledge of relevant hosts, including the potential importance of muskrats in this emerging endemic zone of *E. multilocularis*, provides a scientific basis for disease control that will have relevance for wildlife biology and veterinary and public health in the face of further anthropogenic climate and land use changes. At a time when humans are drastically altering our environment in unprecedented ways, and rodents are poised to be the uninhibited beneficiary of these changes, the need to understand rodent-borne diseases and the factors that contribute to their maintenance and spread is particularly urgent.

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