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Spatial heterogeneity and temporal variations in *Echinococcus multilocularis* infections in wild hosts in a North American urban setting

Stefano Liccioli [a,b,1], Susan J. Kutz [a,c], Kathreen E. Ruckstuhl [b], Alessandro Massolo [a,*,1]

[a] Department of Ecosystem and Public Health, Faculty of Veterinary Medicine, University of Calgary, 3280 Hospital Drive NW, Calgary, AB T2N 4Z6, Canada
[b] Department of Biological Sciences, University of Calgary, 2500 University Drive NW, Calgary, AB T2N 1N4, Canada
[c] Canadian Cooperative Wildlife Health Centre Alberta, 3280 Hospital Drive NW, Calgary, AB T2N4Z6, Canada

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ABSTRACT

Echinococcus multilocularis, the causative agent of human alveolar echinococcosis, has the potential to circulate in urban areas where wild host populations and humans coexist. The spatial and temporal distribution of infection in wild hosts locally affects the risk of transmission to humans. We investigated the spatial and temporal patterns of *E. multilocularis* infection in coyotes and rodent intermediate hosts within the city of Calgary, Canada, and the association between spatial variations in coyote infection and the relative composition of small mammal assemblages. Infection by *E. multilocularis* was examined in small mammals and coyote faeces collected monthly in five city parks from June 2012 to June 2013. Coyote faeces were analysed using a $ZnCl_2$ centrifugation and sedimentation protocol. Infection in intermediate hosts was assessed through lethal trapping and post-mortem analysis. Parasite eggs and metacestodes were morphologically identified and molecularly confirmed through species-specific PCR assays. Of 982 small mammals captured, infection was detected in 2/305 (0.66%) deer mice (*Peromyscus maniculatus*), 2/267 (0.75%) meadow voles (*Microtus pennsylvanicus*), and 1/71 (1.41%) southern red backed voles (*Myodes gapperi*). Overall faecal prevalence in coyotes was 21.42% ($n = 385$) and varied across sites, ranging from 5.34% to 61.48%. Differences in coyote faecal prevalence across sites were consistent with local variations in the relative abundance of intermediate hosts within the small mammal assemblages. Infections peaked in intermediate hosts during autumn (0.68%) and winter (3.33%), and in coyotes during spring (43.47%). Peaks of infections in coyote faeces up to 83.8% in autumn were detected in a hyper-endemic area. To the best of our knowledge, our findings represent the first evidence of a sylvatic life-cycle of *E. multilocularis* in a North American urban setting, and provide new insights into the complexity of the parasite transmission ecology.

1. Introduction

Echinococcus multilocularis is considered a globally emerging pathogen (Davidson et al., 2012). This cestode is the causative agent of human alveolar echinococcosis (HAE), which is among the most serious parasitic zoonoses of the northern hemisphere (Craig et al., 1996). As *E. multilocularis* mainly circulates among definitive and intermediate wild host species, HAE is not considered an eradicable disease (Ito et al., 2003); understanding the transmission ecology of the parasite is therefore crucial for disease prevention.

The geographic distribution of the parasite, as well as its prevalence in wild hosts, seems to be increasing as a direct or indirect consequence of human activities (Giraudoux et al., 2003; Davidson et al., 2012). During the last decade, coincident with growing urban populations of red foxes (*Vulpes vulpes*), *E. multilocularis* has been documented to circulate within numerous cities of Europe (Hofer et al., 1999; Deplazes et al., 2004) and Japan (Tsukada et al., 2000; Yimam et al., 2002). Given the close proximity to humans, such a phenomenon can potentially represent a public health emergency. This is well exemplified by the densely inhabited grasslands of the Tibetan plateau and China, where intense parasite transmission results in high incidences of HAE (Tiaoying et al., 2005; Giraudoux et al., 2006).

In North America, *E. multilocularis* was historically reported in the Northern Tundra Zone of Alaska and Canada (Eckert et al., 2000). Since the 1960s, the parasite has been reported in 13 US

* Corresponding author. Tel.: +1 403 210 6734.
E-mail address: amassolo@ucalgary.ca (A. Massolo).
[1] These authors contributed equally to the manuscript.

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states and four Canadian provinces (Alberta (AB), Saskatchewan (SK), Manitoba (MB), British Columbia (BC)) (Eckert et al., 2001; Jenkins et al., 2012), thus defining a second area of distribution, the North Central Region (NCR). In the NCR, definitive hosts are mainly represented by red foxes and coyotes (*Canis latrans*), whereas intermediate host species listed to date include deer mouse (*Peromyscus maniculatus*), meadow vole (*Microtus pennsylvanicus*), southern red-backed vole (*Myodes gapperi*), house mouse (*Mus musculus*) and bushy-tailed woodrat (*Neotoma cinerea*) (Hnatiuk, 1966; Leiby et al., 1970; Holmes et al., 1971; Kritsky et al., 1977; Liccioli et al., 2013). Recently, *E. multilocularis* was reported in coyotes within metropolitan areas of Alberta, Canada (Catalano et al., 2012), but no information was yet available regarding the transmission ecology of the parasite in this environment.

Recent evidence indicated that *E. multilocularis* transmission is spatially clustered, both at a regional (Viel et al., 1999; Said-Ali et al., 2013; Tolnai et al., 2013) and local spatial scale (Giraudoux et al., 2007). Although over large regions the main intermediate host species have been clearly identified (e.g., *Arvicola sherman* and *Microtus arvalis* in Europe, Deplazes et al., 2004), understanding *E. multilocularis* transmission dynamics still needs to be approached by looking at the entire complexity and composition of small mammal assemblages (Giraudoux et al., 2003). Unfortunately, to date research has often focused only on intermediate host population density as the main parameter regulating patterns of infection (Saitoh and Takahashi, 1998; Hegglin et al., 2007; Raoul et al., 2010).

Previous research also suggested that infections by *E. multilocularis* follow seasonal patterns, with higher prevalence recorded during winter for both definitive (Hofer et al., 2000) and intermediate hosts (Burlet et al., 2011). However, only a few studies have described the temporal patterns of infection throughout the year for both definitive and intermediate hosts within the same area (Stieger et al., 2002), and certainly no information is available for North American urban landscapes.

Herein, we aimed to: (i) investigate spatiotemporal patterns of *E. multilocularis* infections in coyotes and rodent intermediate hosts in an urban landscape; and (ii) assess the association between spatial variations in coyote infection and the relative abundance of intermediate host species within the small mammal assemblages.

Given the low overall prevalence (<1%) observed for intermediate hosts (Giraudoux et al., 2003) and the trophic linkage between intermediate and definitive hosts, we expected infection in coyotes to respond to spatial and temporal variations of prevalence in intermediate hosts. We also expected *E. multilocularis* faecal prevalence in coyotes to be higher in areas where the relative abundance of intermediate host species within the small mammal assemblage is higher, and to observe a time lag (3–4 months) between the peak of infection in rodents and coyotes, consistent with the prepatent period in the canid host (Eckert et al., 2001; Jones and Pybus, 2008).

2. Materials and methods

2.1. Study area and sample collection

The study occurred in five urban parks and natural areas of the city of Calgary (51°5′N, 114°5′W), AB, Canada: Nose Hill Park (NHP; 1,127.9 hectares (ha)), Bowmont (BM; 63.5 ha), Weaselhead (WSH; 208.7 ha), Southland lowlands (SL;15.0 ha) and Fish Creek Provincial Park (FCPP; 3,400.0 ha) (Fig. 1A).

From June 2012 to June 2013, in each area coyote faeces were collected along standardised trails and paths (Liccioli et al., 2012a), as well as opportunistically in areas known to be used by the animals. Faeces were identified as from coyotes and aged as described in Liccioli et al. (2012a).

In the same parks, small mammals were trapped, mostly using Woodstream© Museum Special Traps (7 × 14 cm) baited with a mixture of oatmeal and peanut butter. To reduce the risk of trap misfire, at specific points with low vegetation and in proximity of human trails, snap traps were replaced by Longworth© small mammal live traps (14 × 6.5 × 8.5 cm), always representing ⩽10% of the total number of traps set.

Small mammal capture sites constituted rectangular grids of 200 traps, set at regular intervals (7–10 m, depending on the shape and size of the site). Traps were checked and re-set every morning for 3 days (Millar et al., 1991), for a maximum total of 600 trap-nightsper capture session (not controlling for misfires). Capture sites were selected in order to be representative of the main habitat types available in each park (e.g., grassland, shrubs, forest). Each park had three different capture sites, with the exception of SL which had only two due to its small area. Even in the smallest park (SL), the shortest distance between capture sites was >300 m (and up to 1 km in larger parks), bigger than the average dispersal distance recorded for *Peromyscus* and vole species (King, 1968; Boonstra et al., 1987; Andreassen and Ims, 2001). Every month we sampled all the parks, rotating among different capture sites (Fig. 1B).

For each capture day, we recorded the number of traps that were active throughout the night (Village and Myhill, 1990), considering as misfired any trap found sprung, missing, with no bait, or not triggered. Small mammals that were caught alive (in live traps or not killed in snap-traps) were immediately euthanised through cervical dislocation by trained personnel.

The animal use protocol was approved by the Animal Care Committee of the University of Calgary (AC12-0037), Canada.

2.2. Echinococcus multilocularis in intermediate hosts

Small mammals were necropsied by trained personnel under level 2 conditions in a biosafety cabinet, in order to protect operators from potential Hantavirus exposure. Animals that could not be necropsied on the day of collection were frozen at −20 °C until analysis. At postmortem examination, small mammals were classified as adults or juveniles, based on combined information on body weight, body length and gonad development (Henttonen et al., 2001). Animals were morphologically inspected for *E. multilocularis* alveolar hydatid cysts (Liccioli et al., 2013). Any suspected lesion or mass in the abdomen was collected, stored in 95% ethanol and tested molecularly. Extraction of DNA was performed using the Qiagen DNeasy Blood & Tissue kit, with a final elute of 300 μL (Liccioli et al., 2013). Parasite identity was then confirmed through species-specific PCR as described by Catalano et al. (2012).

2.3. Small mammal availability and assemblage composition

The relative availability of small mammals was assumed to be reflected in their different capture rate (Calhoun, 1956; Woodman et al., 1996). For each trapping session, the effective capture rate of small mammals was calculated by dividing the number of animals caught by the number of active traps and multiplying it by 100 (%) (Village and Myhill, 1990). For this estimate, capture nights during which snowfall resulted in complete coverage of the traps were excluded from the analysis.

For each season and park, the proportion of intermediate hosts (IH) out of the total of small mammals captured was calculated as IHs/(IHs + NIHs), where NIHs are individuals of species not previously reported as intermediate hosts (Hnatiuk, 1966; Lee, 1969; Leiby et al., 1970; Holmes et al., 1971; Liccioli et al., 2013). As older animals are more likely to have been infected by *E. multilocularis*

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