Echinococcus multilocularis in Urban Coyotes, Alberta, Canada

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Echinococcus multilocularis is a zoonotic parasite in wild canids. We determined its frequency in urban coyotes (Canis latrans) in Alberta, Canada. We detected E. multilocularis in 23 of 91 coyotes in this region. This parasite is a public health concern throughout the Northern Hemisphere, partly because of increased urbanization of wild canids.

Echinococcus multilocularis is the causative agent of alveolar echinococcosis in humans. This disease is a serious problem because it requires costly long-term therapy, has high case-fatality rate, and is increasing in incidence in Europe (1). This parasitic cestode has a predominantly wild animal cycle involving foxes (Vulpes spp.) and other wild canids, including coyotes (Canis latrans), as definitive hosts. However, it can also establish an anthropicogenic life cycle in which dogs and cats are the final hosts. Rodents are the primary intermediate hosts in which the alveolar/multivesicular hydatid cysts grow and are often fatal. Humans are aberrant intermediate hosts for E. multilocularis (2).

In North America, E. multilocularis was believed to be restricted to the northern tundra zone of Alaska, USA, and Canada until it was reported in red foxes (Vulpes vulpes) from North Dakota, USA (3). This parasite has now been reported in the southern half of 3 provinces in Canada (Manitoba, Saskatchewan, and Alberta) and in 13 contiguous states in the United States (4).

Foxes are the traditional definitive hosts for E. multilocularis worldwide. However, in North America, coyotes may be prominent hosts, particularly when they are more abundant than foxes. E. multilocularis was reported in 7 (4.1%) of 171 coyotes in the northcentral United States in the late 1960s (3), and subsequently prevalences ranging from 19.0% to 35.0% have been reported in coyotes in the central United States (4).

In Canada, E. multilocularis was detected in 10 (23.0%) of 43 coyotes in Riding Mountain National Park, Manitoba (5). In Alberta, 1 case was recorded from the aspen parkland in 1973 (5) but it was not found in coyotes from forested regions and southern prairies (6,7). Nonetheless, E. multilocularis is generally considered enzootic to central and southern Alberta on the basis of its prevalence in rodent intermediate hosts. During the 1970s, sixty-three (22.3%) of 283 deer mice (Peromyscus maniculatus) trapped in periurban areas of Edmonton were positive for alveolar hydatid cysts (8), and E. multilocularis was also detected in 2 deer mice collected <1.8 km from Lethbridge in southern Alberta (9).

Because mice and voles (family Cricetidae, including Peromyscus spp.) have been reported as main prey (70.1%) of coyotes in Calgary (10), and coyotes are common in urban areas of Calgary and Edmonton, we suspected a role for this carnivore in the maintenance of the wild animal cycle of E. multilocularis in such urban settings. Thus, we aimed to ascertain the frequency of E. multilocularis in coyotes from metropolitan areas in Alberta, Canada.

The Study

Ninety-one hunted or road-killed coyotes were collected during October 2009–July 2011. Most (n = 83) of the carcasses were from the Calgary census metropolitan area (CMA) (Figure 1). The remainder (n = 8) were opportunistically collected from the Edmonton CMA. Of those from the Calgary CMA, the exact location of collection was known for 60 animals: 27 were from Calgary and 33 were from the rural fringe, including 2 near Strathmore. Of the carcasses from the Edmonton CMA, 7 were from Edmonton and 1 was from a periurban site. Sex and age of 90 of the coyotes were recorded.

Before necropsy, all carcasses were stored at −20°C. Gastrointestinal tracts collected at necropsy were refrozen at −80°C for 3–5 days to inactivate Echinococcus spp. eggs. Once thawed and dissected, intestinal contents were washed, cleared of debris, and passed through a sieve (500-μm pores), and the material in the sieve was examined for Echinococcus spp.

Adult tapeworms were counted and identified as E. multilocularis on the basis of morphologic features (Figure 2). To confirm morphologic identification, PCR was performed by using species-specific primers (11). Briefly, a representative adult worm from each positive animal was

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lysed in 50 μL of DNA extraction buffer (500 mmol/L KCl, 100 mmol/L Tris-HCl, pH 8.3, 15 mmol/L MgCl₂, 10 mol/L dithiothreitol, and 4.5% Tween 20) containing 2 μL of proteinase K. This lysate was further diluted (1:20 in double-distilled water), and 2 μL was used for PCR. Amplicons of an expected 395 bp confirmed infection with *E. multilocularis*.

*E. multilocularis* was identified in 23 (25.3%) of 91 coyotes by using morphologic and molecular identification. Among positive animals, 18 (20.5%) of 83 were from the Calgary CMA and 5 (62.5%) of 8 were from the Edmonton CMA. In the Calgary CMA, 4 (14.8%) of 27 positive animals were found in the city and 9 (27.3%) of 33 were found in the rural fringe (Figure 1). Five (21.7%) of 23 coyotes for which the location was not recorded were also positive.

*E. multilocularis* intensity (number of cestodes per host) ranged from 1 to 1,400 (median 20.5). The frequency of infection was significantly higher in male coyotes (n = 44, 34.19%) than in female coyotes (n = 46, 15.2%; χ² 4.337, df 1, *P* = 0.05) (Table). No difference was detected between 43 juvenile coyotes and 47 adult coyotes (Table).

**Conclusions**

We demonstrated that *E. multilocularis* is common in coyotes of metropolitan areas of Calgary and Edmonton, Alberta, Canada, including their urban cores. This finding might indicate an emerging phenomenon similar to that observed in Europe with infiltration of urban centers by *E. multilocularis* caused by an increase in red foxes in cities such as Copenhagen, Geneva, and Zurich (2). In Alberta,
public awareness about prevention and transmission risk are unfeasible (eradication from free-ranging domestic dogs and cats is effective, should be increased in North America. Although removal of this parasite from domestic dogs and cats may pose a zoonotic risk, as documented on Saint Lawrence Island, Alaska, and in China (2,12), a human case probably represent the main infection route for humans in North America (2,12). Genetic characterization of E. multilocularis and spatially explicit transmission models should also be developed to better assess risks of this emerging zoonosis in North America and worldwide.

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Table. Echinococcus multilocularis in coyotes carcasses collected in Calgary (n = 83) and Edmonton (n = 8) census metropolitan areas, Alberta, Canada, October 2009–July 2011*

| Characteristic | Total | No. (%) positive or median (range) | No. negative | IQ distance | $\chi^2$ (z) | df | $p_{\text{exact}}$ value† |
|----------------|-------|-----------------------------------|--------------|-------------|-------------|----|----------------------|
| Sex‡ |       |                                   |              |             |             |    |                       |
| M    | 44    | 15 (34.1)                         | 29           | NA          |             |    |                       |
| F    | 46    | 7 (15.2)                          | 39           | NA          |             |    |                       |
| Parasite intensity |       |                                   |              |             |             |    |                       |
| M    | NA    | 9 (1–1,400)                       | NA           | 83          |             |    | 0.19                  |
| F    | NA    | 59 (9–822)                        | NA           | 137         | (–1,406)    |    |                       |
| Age‡ |       |                                   |              |             |             |    |                       |
| Juvenile | 43 | 14 (33.3)                       | 29           | NA          |             |    |                       |
| Adult | 47    | 8 (17.0)                          | 39           | NA          | 1.661       | 1  | 0.226                |
| Parasite intensity |       |                                   |              |             |             |    |                       |
| Juvenile | NA | 9 (1–151)                       | NA           | 71          |             |    |                       |
| Adult  | NA    | 32 (1–1,400)                      | NA           | 520         | (–0.737)    |    | 0.518                |

*Values in **boldface** indicate a significant difference. Higher prevalence in male coyotes suggests a role for sex in parasite dispersion. Frequencies of cestodes in males vs. females and juveniles vs. adults were analyzed by using $\chi^2$ test. Parasite intensity (no. parasites per host) among sex and age classes was compared by using Mann-Whitney test for independent samples. IQ, interquartile distance; NA, not applicable.
†Probability of distribution was estimated by using the permutation approach ($p_{\text{exact}}$).
‡Sex and age of 1 coyote were not recorded.
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