High prevalence of *Echinococcus multilocularis* in urban red foxes (*Vulpes vulpes*) and voles (*Arvicola terrestris*) in the city of Zürich, Switzerland

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**SUMMARY**

Over a period of 26 months from January 1996 to February 1998, 388 foxes from the city of Zürich, Switzerland, were examined for intestinal infections with *Echinococcus multilocularis* and other helminths. The prevalence of *E. multilocularis* in foxes sampled during winter increased significantly from 47% in the urban to 67% in the adjacent recreational area, whereas prevalence rates of other helminths were similar in both areas. Seasonal differences in the prevalence of *E. multilocularis* were only found in urban subadult male foxes which were significantly less frequently infected in summer than in winter. The distribution of the *Echinococcus* biomass, as expressed by worm numbers per fox was overdispersed in 133 infected foxes randomly sampled in winter. Ten of these foxes (8%) were infected with more than 10000 specimens and carried 72% of the total biomass of *E. multilocularis* (398653 worms). Prevalences did not differ significantly in these foxes in regard to age and sex but worm burdens were significantly higher in subadult foxes as compared with adult foxes. In voles (*Arvicola terrestris*) trapped in a city park of Zürich, *E. multilocularis* metacestodes were identified by morphological examination and by PCR. The prevalence was 20%, among 60 rodents in 1997 and 9% among 75 rodents in 1998. Protoscolecates occurred in 2 of the cases from 1997. The possible risk for human infection is discussed with respect to the established urban *E. multilocularis* cycle.

Key words: Echinococcosis, *Echinococcus multilocularis*, *Arvicola terrestris*, *Vulpes vulpes*, urban, zoonosis.

**INTRODUCTION**

Human alveolar echinococcosis (AE), caused by larval stages of *Echinococcus multilocularis*, is one of the most lethal helminthic zoonoses (Amman & Eckert, 1995). In Europe, the natural cycle of *E. multilocularis* predominantly involves red foxes (*Vulpes vulpes*) as definitive hosts and several rodent species as intermediate hosts. Domestic dogs and cats have also been identified as definitive hosts, but their significance for zoonotic transmission needs further elucidation (Eckert & Deplazes, 1999).

In recent years, *E. multilocularis* prevalences in foxes of up to 60% have been reported from Central Europe, and this tapeworm was also reported from areas where it had not been described previously (Lucius & Bilger, 1995; Eckert & Deplazes, 1999). Furthermore, increasing fox densities were registered in several European countries (Chautan, Pontier & Artois, 1999; Breitenmoser et al. 1995) with this population increase being most noticeable in suburban and urban areas.

Although urban and suburban fox populations had been a well-known phenomenon in the UK since the 1940s (Macdonald & Newdick, 1982; Harris, 1977), it is only in approximately the last 15 years that high fox densities have also been reported from cities on the continent, e.g. from Berlin (Schöffel et al. 1991) and from Copenhagen (Willingham et al. 1996). In Switzerland, a considerable increase of the overall fox population was observed over the past 10 years (Breitenmoser et al. 1995), and foxes are now commonly seen in urban area with cubs being bred in public parks and in private gardens. The population of foxes permanently living in the municipality of Zürich is estimated to consist of 300–400 adult animals (Gloor, unpublished data).

The most important intermediate host species of *E. multilocularis* in Europe are *Microtus arvalis* and *Arvicola terrestris*. Few data are available about parasite prevalences in rodents, but in general they are low (<1–6%) as compared to those in foxes from the same area (20–60%). However, studies in France and Switzerland indicated that high-endemic
areas of rodent *E. multilocularis* infections do exist focally where prevalences of up to 39% were observed (Eckert *et al.* 2000a).

The epidemiological situation of alveolar echinococcosis in humans in Switzerland was stable over the last 36 years (Eckert & Deplazes, 1999). However, the invasion of urban areas by foxes raised new questions concerning potential public health risks caused by them with regard to zoonotic parasitic infections. The city of Zürich is surrounded by an endemic area where *E. multilocularis* was detected in 40% of foxes (Ewald *et al.* 1992). In order to investigate the potential contamination of public areas with *E. multilocularis* eggs and to assess whether an urban cycle of the parasite occurs, a survey of the intestinal helminths in foxes and metacestodes of *E. multilocularis* in voles was conducted in the city of Zürich.

**Materials and Methods**

**Study area**

The study area, the municipality of Zürich (92 km², 360000 inhabitants), was divided into an urban and an adjacent rural area consisting mainly of wood, parks, farmland and allotment gardens. By our definition, the urban area extends 250 m from the built-up area into the rural zone (Fig. 1).

**Foxes**

**Sampling.** A total of 388 red foxes (*Vulpes vulpes*) were collected by 3 game wardens of the city forest service between January 1996 and February 1998. In 297 cases (76.5%) the foxes were shot in the course of the official local population control programme. Deaths of another 91 foxes (23.5%) resulted from road or rail traffic accidents or from unknown causes. Carcasses were wrapped up in plastic bags and stored at −20 °C until necropsy. In winter (November–February) 123 foxes were sampled in the urban area and 129 foxes in the rural area. In the close season (spring, 1 March–15 June) shooting of foxes was performed with special permission in the urban area only. Therefore, only 39 foxes (19 shot and 20 killed in accidents) were sampled in spring, all originating from the urban area. In summer (July–October) 93 urban and 4 rural foxes were collected.

**Parasitological examination.** Necropsy and examination of the intestines were carried out following strict safety precautions as described by Deplazes & Eckert (1996) using 15 deep mucosal scrapings which were taken from equally distributed sites of the small intestine. The intestinal sedimentation and counting technique (SCT) was performed as described by Rausch, Fay & Williamson (1990) with modifications. Briefly, the small intestine was incised longitudinally and cut into 5 pieces of approximately the same length. These pieces were transferred to a glass bottle containing 1 litre of 0.9% NaCl solution. After shaking the bottle vigorously for a few seconds, the pieces of intestine were removed and the superficial mucosal layer stripped by means of pressure between thumb and forefinger to dislodge any attached helminths. After a sedimentation time of 15 min the supernatant was decanted and the bottle refilled with physiological saline solution. This procedure was repeated 2–6 times until the supernatant was clear. The sediment fraction was examined in small portions of about 5–10 ml in square Petri dishes (9 × 9 cm, Falcon®, Lincoln Park, NJ, USA) in transmission light under a stereomicroscope at a magnification of 120 ×. The whole sediment was checked if up to 100 worms were found; if higher numbers were present the total worm burden was calculated from the count of 1 subsample. The SCT was performed with 310 intestines, of which 170 had previously been examined by the IST. Seventy-eight intestines were examined by the IST only.

**Identification of helminths.** *E. multilocularis* was identified based on typical morphological characteristics. In cases where only juvenile stages were present, in particular scoleces, *E. multilocularis* was confirmed by PCR (Bretagne *et al.* 1993). The identification of *Taenia* spp. was based on length and shape of rostellar hooks (Verster, 1969). Specimens lacking hooks but with typical *Taenia* proglottids bearing taeniid eggs were recorded as *Taenia* sp.

**Age determination of foxes.** In line with the study of Wandeler (1976), cubs were assumed to be born on 1 April. Age determination of foxes collected after 1 July was done by measuring the relative width of the pulp cavity of a lower canine tooth by X-rays (Kappeler, 1985), allowing to discriminate adults (older than 12 months) from subadults. In addition, the age of 93 adult foxes randomly collected in winter was determined by counting annual incremental lines in the tooth cementum (Grue & Jensen, 1979).

**Rodents**

From October to December 1997 and from July to October 1998, 60 and 75, respectively, *Arvicola terrestris* were trapped with long traps in an urban public park (‘Irchelpark’) in the city of Zürich (Fig. 1). At necropsy, the liver in particular but also other
E. multilocularis in urban foxes and voles

Fig. 1. Distribution of the 388 foxes investigated in the municipality of Zürich. Foxes originating from a grid of 4 km² were taken together (no. of foxes infected with Echinococcus multilocularis/no. of foxes examined). White: rural area; light grey: urban area; dark grey: lake and rivers; black line: border of municipality; asterisk: Irchelpark (sampling site of Arvicola terrestris).

Table 1. Small intestinal helminths discovered in 388 foxes collected from January 1996 to February 1998 in the city of Zürich (urban and rural area)

<table>
<thead>
<tr>
<th>Helminth</th>
<th>No. of infected foxes</th>
<th>P (%)</th>
<th>CI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Echinococcus multilocularis</td>
<td>172</td>
<td>44.3</td>
<td>39.3–49.4</td>
</tr>
<tr>
<td>Taenia spp.*</td>
<td>64</td>
<td>16.5</td>
<td>13.0–20.7</td>
</tr>
<tr>
<td>Mesocestoides sp.†</td>
<td>17</td>
<td>4.4</td>
<td>2.7–7.1</td>
</tr>
<tr>
<td>Dipylidium sp.†</td>
<td>2</td>
<td>0.5</td>
<td>0.1–2.1</td>
</tr>
<tr>
<td>Uncinaria stenocephala</td>
<td>259</td>
<td>66.8</td>
<td>61.8–71.4</td>
</tr>
<tr>
<td>Toxocara canis</td>
<td>184</td>
<td>47.4</td>
<td>42.4–52.5</td>
</tr>
<tr>
<td>Alaria sp.†</td>
<td>8</td>
<td>2.1</td>
<td>1.0–4.2</td>
</tr>
</tbody>
</table>

* Taenia crassiceps 7.6%; T. polyacantha 0.5%; Taenia sp. 8.4%.
† No further species differentiation performed.

P, Prevalence; CI, upper and lower 95% confidence interval.

organs were examined for lesions. Metacestodes of E. multilocularis were identified directly on squashed metacestode material using the immunofluorescent labelled monoclonal antibody G11 (Deplazes & Gottstein, 1991) and by histological identification of typical structures in HE- and PAS-stain. Specimens giving doubtful results were examined by PCR (Bretagne et al. 1993) after proteinase K digestion of the cut-up material. Species determination of metacestodes of other cestodes was performed by gross morphology and by comparing hook morphology and length.

Statistics
Calculation of 95% confidence intervals (CI) of parasite prevalences was performed as described by Lorenz (1988). Prevalence differences were compared by the χ² test and differences in infection intensity were compared by the Mann–Whitney U-test. Differences were considered significant at P < 0.05.

Results
Helminths recovered
A total of 344 of the 388 foxes examined (88.7%) carried intestinal helminths (Table 1). The highest prevalence (66.8%) was recorded for Uncinaria stenocephala followed by Toxocara canis (47.4%) and E. multilocularis (44.3%). In 64 foxes (16.5%) infections with Taenia spp. were recorded but in
Table 2. Seasonal differences in the prevalences of *Echinococcus multilocularis* in urban and rural foxes collected from January 1996 to February 1998 in the city of Zürich

(Statistical comparisons were done (a) between age and sex groups, (b) between rural and urban foxes in winter, (c) between urban foxes in winter and summer. Statistically significant differences are indicated with a letter.)

<table>
<thead>
<tr>
<th>Season</th>
<th>Foxes</th>
<th>Urban foxes</th>
<th>Rural foxes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. investigated/no. infected*</td>
<td>No. investigated/no. infected*</td>
<td></td>
</tr>
<tr>
<td>Winter (Nov.–Feb.)</td>
<td>Adult females</td>
<td>39/16 (41%)</td>
<td>34/21 (62%)</td>
</tr>
<tr>
<td></td>
<td>Subadult† females</td>
<td>22/8 (36%)</td>
<td>20/14 (70%)</td>
</tr>
<tr>
<td></td>
<td>Adult males</td>
<td>29/13 (45%)</td>
<td>33/19 (58%)</td>
</tr>
<tr>
<td></td>
<td>Subadult males</td>
<td>39/24 (62%)</td>
<td>36/28 (78%)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>129/61 (47.3%)</td>
<td>123/82 (66.7%)</td>
</tr>
<tr>
<td>Summer (July–Oct.)</td>
<td>Adult females</td>
<td>22/5 (23%)</td>
<td>1/1 (N.A.)</td>
</tr>
<tr>
<td></td>
<td>Subadult females</td>
<td>31/5 (16%)</td>
<td>1/1 (N.A.)</td>
</tr>
<tr>
<td></td>
<td>Adult males</td>
<td>12/4 (33%)</td>
<td>1/1 (N.A.)</td>
</tr>
<tr>
<td></td>
<td>Subadult males</td>
<td>28/5 (18%)</td>
<td>1/0 (N.A.)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>93/19 (20.4%)</td>
<td>4/3 (N.A.)</td>
</tr>
</tbody>
</table>

* χ² test; **P < 0.01; ***P < 0.001; ****P < 0.001.
† Less than 12 months of age.
N.A., Not applicable.

approximately half of the cases the species could not be determined due to inappropriate conservation of the worms. *T. crassiceps* and *T. polyacantha* were found in 7.6% and 0.5% of the foxes, respectively.

**E. multilocularis infections**

*Comparison of 2 parasitological methods.* In 170 cases small intestines were investigated with both the intestinal scraping technique (IST) and the intestinal sedimentation and counting technique (SCT). *E. multilocularis* infections were detected in 87 cases (51.2%) with the SCT and in 68 cases (40.0%) with the IST. The sensitivity of the IST was 78.0% as compared with the results obtained with the SCT. None of the foxes diagnosed negative by the SCT turned out positive with the IST. In 12 of the 19 cases detected with the SCT only, less than 10 and, in 5 cases, 10–100 worms per fox were recovered. In the 2 remaining cases, infections with juvenile *E. multilocularis* stages were detected and confirmed by PCR (worm numbers of 432 and 11640, respectively).

*Prevalences in urban and rural foxes.* Sampled foxes were not homogeneously distributed within the study area (Fig. 1). Statistical comparison of urban and rural foxes could be performed with animals collected in winter (November–February) only. The *E. multilocularis* prevalences in urban (47.3%) and rural foxes (66.7%) were significantly different as assessed by the χ² test (P < 0.01) (Table 2). On the other hand, the prevalences of infections with the other helminths investigated did not differ significantly among these 2 fox populations (P > 0.1; χ² test; data not shown).

*Relation to fox sex and age.* Within both areas investigated no significant differences in the prevalences of *E. multilocularis* were found related to sex or to the age groups 'subadult' and 'adult' (Table 2). Furthermore, no significant differences in the prevalences were found between subadults and 93 adults whose age was determined more precisely. Hence, the prevalence was 63.0% in 117 subadults, 58.0% in 62 adult foxes aged 12–35 months, and 48.0% in 31 adult animals aged 36–70 months (P > 0.1, χ² test; data not shown).

*Animal and seasonal differences.* Prevalences in urban and rural foxes sampled during 2 subsequent winters showed no significant differences within both habitats (P > 0.1, χ² test; data not shown). Seasonal variations in the prevalence of *E. multilocularis* were investigated in the urban area only. Urban foxes collected in winter were significantly more frequently infected (47.3%) than those from summer (20.4%) (P < 0.001; χ² test) (Table 2). Interestingly, this significant difference was found in subadult male foxes only (P < 0.001; χ² test). Statistical analyses of 19 cubs (5.0% infected with *E. multilocularis*) and 20 adult foxes (30.0% infected with *E. multilocularis*) collected in spring revealed no significant differences considering age and sex (P > 0.05; χ² test; data not shown). This small group of animals was not used for further comparative analyses.

*Distribution of the E. multilocularis biomass.* The total *Echinococcus* biomass in 57 urban and 76 rural infected foxes randomly sampled during 2 winters was 398653 specimens. No significant difference in the worm burden was found between these urban
foxes, carrying 42%, and rural foxes, carrying 58% of the total biomass, respectively ($P > 0.1$, Mann–Whitney $U$-test). Therefore, further quantitative evaluations of the worm burden in foxes of both areas were not independently analysed. Fig. 2 shows the distribution of the biomass of *E. multilocularis* in the foxes investigated. In 64 (69%) of the foxes infections with less than 1000 *E. multilocularis* worms were found representing 3% of the total biomass. Infections with more than 1000 worms occurred in 41 foxes (31%) carrying 97% of the total biomass. As few as 10 foxes (8%) which were infected with more than 10000 specimens harboured 72% of the total biomass of *E. multilocularis*. The 2 heaviest infections (56970 and 45020 worms; 26% of the biomass) were detected in 2 subadult male foxes collected in February in the urban area. The worm burden revealed no significant differences related to sex but subadult infected foxes carried significantly higher worm burdens than adults (Table 3). Furthermore, the median number of *E. multilocularis* worms was more than twice as high in subadult as compared with adult foxes.

**Prevalence of metacestode infections in Arvicola terrestris**

Metacestodes of *E. multilocularis* were found in 19 (14%) of 135 *A. terrestris* trapped in the Irchelpark
in the city of Zürich (Fig. 1). The prevalence was 20% in 60 animals examined in 1997 and 9% in 75 animals examined in 1998. Protopoecoles occurred in 2 A. terrestris from 1997 only. In 27 animals (20%) an infection with the metacestodes of T. taeniaeformis (Strobilocercus fasciolaris) was found. Metacestodes of T. crassiceps were detected on 2 occasions in subcutaneous cysts and once in the pleural cavity.

**Discussion**

The E. multilocularis prevalence of 67% in foxes from recreational areas in the city of Zürich is comparable to the prevalences found in a previous study from adjacent areas (Ewald et al. 1992). Also, a high percentage of the urban dwelling foxes in the city of Zürich was infected with E. multilocularis (prevalence 47%). This decline in the E. multilocularis prevalences from the recreational to the urban area is significant and may be caused by a lower predation on rodents by urban foxes. Indeed, stomach content analyses of 229 foxes investigated in this study revealed a lower number of rodent items in the stomachs of urban foxes as compared with those from rural areas (Gloor, unpublished data).

The prevalences of Taenia species, however, which also are dependent on rodents as intermediate hosts did not differ significantly between these 2 fox populations most probably reflecting the higher biotic potential of Taenid-species as compared with Echinococcus.

Schelling et al. (1991) found significantly higher prevalences of E. multilocularis in foxes (age not determined) collected in winter as compared with those collected in summer. This overall difference could also be observed in our study in urban foxes but closer examination revealed that only young urban male foxes contributed to this fact. This might be explained by the findings of Tackmann et al. (1998) that young foxes’ diet contained lower proportions of rodents in June and July when they become less dependent on adults.

The influence of the foxes’ age on the prevalence of E. multilocularis, however, is not yet fully understood. Juvenile foxes were found to be significantly more frequently infected than adults (Ewald, 1993; Vos & Schneider, 1994), whereas in other studies no significant age-dependent differences were detected (for references see Tackmann et al. 1998). Interestingly, young foxes were significantly more frequently infected with E. multilocularis than adults under high-endemic conditions, whereas under low-endemic conditions prevalences tended to be higher in adult foxes (Tackmann et al. 1998).

However, in our study which was conducted in a high-endemic area, the prevalence rates of subadult and adult foxes collected in winter did not differ significantly as determined with the highly sensitive intestinal sedimentation and counting technique which even allows detection of infections with very low worm numbers. On the other hand, subadult foxes carried significantly higher worm burdens than adult foxes. This might be an indication for the acquisition of a partial immunity after repeated infections as had been shown in dogs experimentally infected with E. granulosus (Gemmell, Lawson & Roberts, 1986).

The high variation in the worm burdens of the individual foxes indicates that the parameter ‘prevalence’ might not be the most adequate one to characterize the epidemiological situation of E. multilocularis. A few highly infected foxes carrying thousands of fertile worms can be responsible for most of the egg contamination in a distinct area. A similar distribution of parasites has been observed in dingoes infected with E. granulosus in south-eastern Australia (Jenkins & Morris, 1991). Furthermore, subadult male foxes which carried the major part of the E. multilocularis biomass in our study could have a special role for spreading the parasite in the environment because they usually migrate further away than the age-matched females (Storm et al. 1976). The spectrum of the rodent fauna in the study area is not yet investigated. When analysing the stomachs of the foxes, the water vole Arvicola terrestris was the most frequently found potential intermediate host besides other species that also were present (Microtus arvalis, Clethrionomys glareolus and Mus domesticus; Gloor, unpublished data). According to many authors (reviewed by Weber & Aubrey, 1993) predation of foxes on A. terrestris occurs, but is generally considered to be light. However, Weber & Aubrey (1993) found that A. terrestris, when highly abundant, was the most frequent prey of foxes in a rural area of western Switzerland. Furthermore, in an endemic alveolar echinococcosis area in France, A. terrestris was the most abundant and the only infected rodent species (Laforge et al. 1992).

Low prevalences below 1% were found in geographically extensive studies (Eckert et al. 2000a) but E. multilocularis infections are not randomly distributed in A. terrestris populations in endemic areas. High-endemic foci were described with prevalences in A. terrestris of up to 39% (Pétavy & Deblock, 1983; Gottstein et al. 1996). Furthermore, seasonal differences were observed in France where the highest prevalence of 17.6% was found in January (74 A. terrestris examined), while no infections were detected in October (167 animals examined) (Pétavy & Deblock, 1983). Contrary to the previously mentioned study we found high prevalences in late summer and autumn when the vole density was high with prevalence rates being highest in October 1997 (one third of the 30 animals examined infected). Therefore, we suspect that prevalences could be higher in the park investigated.
at an earlier time of the year with more animals harbouring metacestodes that contain proscocoeles. The discovery of *E. multilocularis* infections in urban foxes and in *A. terrestris* originating from a high- endemic focus in a public park provides evidence for the existence of a parasite cycle within the urban area. Furthermore, a synanthropic cycle including rodent-catching domestic carnivores, as described in a village in France (Pétavy, Deblock & Wallbaum, 1991), seems to be possible in the area investigated. We detected urban *A. terrestris* that were infected with metacestodes of *T. taeniaeformis*, a cestode that is common in domestic cats, but was not found in our study in foxes. This indicates that an infection cycle between domestic cats and wild rodents indeed does exist. Although experimental studies with cats had shown that development of *E. multilocularis* was retarded and lower worm burdens were found as compared to dogs which are highly susceptible (Thompson & Eckert, 1983), cat ownership was identified as a risk factor for alveolar echinococcosis (AE) in a retrospective case-control study of patients in Austria (Kreidl et al. 1998). Low prevalences of *E. multilocularis* of 0.3% and 0.4%, respectively, were found in Switzerland (Deplazes et al. 1999) when investigating 660 randomly selected dogs and 263 cats, but higher prevalences of up to 12% have been reported in farm dogs with free access to rodents (Gottstein et al. 1997). Considering the high number of domestic dogs and cats in Central Europe the urbanization of the *E. multilocularis* cycle could increase the infection risk for domestic carnivores and consequently also for humans. *E. multilocularis*-infected foxes in urban areas pose novel epidemiological and infectiological questions. Recreational areas (e.g. public parks and outdoor swimming pool areas) within or adjacent to the city are frequently and intensively used by city-dwellers. However, it is not proven that an increased infection pressure results in a higher number of AE cases in humans. At present we have no evidence of an increase in the incidence of human AE in the study area. A major difficulty to unravel such epidemiological relations is the long incubation period (5–15 years) of AE in the human host (Amman & Eckert, 1995).

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