

Spatial and temporal aspects of urban transmission of *Echinococcus multilocularis*

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(Received 29 October 2001; revised 17 January 2002; accepted 17 January 2002)

SUMMARY

High prevalences of *Echinococcus multilocularis* have been reported from foxes of the city of Zurich, Switzerland. In order to characterize transmission in urban areas, a coproantigen ELISA was evaluated for diagnosing the infection in fox faecal samples collected in the environment. In addition, trapped rodents were investigated for the presence of metacestodes. Faecal samples could reliably be classified as being of fox origin by assessing physical properties as shown by the different parasite spectra of putative fox and dog faecal specimens. From the total of 604 tested putative fox faecal samples 156 (25.8%) were positive in the ELISA with a distinct increase in the proportion of positive samples from the urban to the periurban zone. Furthermore, samples collected in the border zone had significantly more coproantigen-positive results during winter. Prevalence of *E. multilocularis* in rodents was 9.1% (81/889) for *Arvicola terrestris* (with 3.5% of the animals harbouring between 14 and 244 400 protoscoleces) and 2.4% (2/83) for *Clethrionomys glareolus*. *E. multilocularis*-infected *A. terrestris* were found in 9 of 10 trapping sites in the border zone. The high infection pressure in the periphery of urban areas might pose a risk for infection with *E. multilocularis* for both domestic carnivores as well as for urban inhabitants. Interventions into the cycle aiming at reducing the infection pressure should therefore focus on these areas.

Key words: *Echinococcus multilocularis*, diagnosis, fox, rodents, urban area, infection pressure.

INTRODUCTION

Alveolar echinococcosis (AE) in humans is caused by the metacestode stage of the fox tapeworm, *Echinococcus multilocularis*, which grows in a tumour-like manner, predominantly in the liver. This helminthic zoonosis of the northern hemisphere is mostly a lethal infection if left untreated (Amman & Eckert, 1996).

In central Europe the sylvatic cycle of *E. multilocularis* is perpetuated by red foxes (*Vulpes vulpes*) as definitive hosts and voles (particularly *Arvicolidae*) as intermediate hosts (Eckert *et al.* 2001a). In endemic areas, prevalences of *E. multilocularis* in fox populations may reach over 60% (Eckert & Deplazes, 1999; Romig *et al.* 1999; Eckert *et al.* 2001a). Although foxes harbour the main parasite burden, domestic dogs and cats can be infected with intestinal stages but prevalences are generally low (Deplazes *et al.* 1999; Eckert & Deplazes, 1999). Relatively little is known about prevalences of *E. multilocularis* in *Arvicolidae*, the most suitable intermediate hosts, but they are low (1–6%) compared with those of foxes (Houin *et al.* 1982; Pétavy & Deblock, 1983; Bonnin *et al.* 1986; Eckert *et al.*

2001a). However, high prevalences (10–39%) have been found focally indicating the presence of so-called ‘hot spots’ in rural (Gottstein *et al.* 2001) but also in urban environments (Hofer *et al.* 2000).

In spite of high prevalences of *E. multilocularis* in foxes in Europe, the annual incidence rates of human infections (0.02–1.4/100 000 inhabitants) are low (Eckert & Deplazes, 1999; Romig *et al.* 1999). The significance of different ways of infection and infection risks still is unclear. It was suggested that there is a higher infection risk in rural areas, and a considerable proportion of patients with AE in Austria (50%; Auer & Aspöck, 1991) and France (34%; Vuitton *et al.* 1990) reported farming activities. In Switzerland, the incidence rates of human AE did not change greatly between 1956 and 1992 despite considerable fluctuations in the fox populations due to the occurrence of rabies and its control (Eckert & Deplazes, 1999). Whether domestic dogs and cats play a role in the transmission of *E. multilocularis* in central Europe needs further clarification (Deplazes & Eckert, 2001; Pétavy *et al.* 2000). Gottstein *et al.* (2001) recently reported that the prevalence of human AE in a Swiss rural area with extraordinary high prevalences, not only in foxes and voles but also in dogs and cats, was not higher than in the average national population.

Since the early 1990s a marked increase of fox densities was reported from several European

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countries (Artois, 1997). Coincidentally, suburban and urban areas were invaded by fox populations (Gloor *et al.* 2001) a phenomenon well known in Great Britain since the 1930s (Harris & Rayner, 1986). In Switzerland, fox breeding dens were reported in 20 of the 30 largest cities (Gloor *et al.* 2001). The urban fox population in the city of Zurich is currently estimated to consist of approximately 500 animals with densities of up to 10 adult foxes/km² (Deplazes *et al.* 2002). Only recently we have described the existence of an urban cycle of *E. multilocularis* in the city of Zurich (Hofer *et al.* 2000). In that study the prevalence of *E. multilocularis* was found to be 44.3% in urban foxes and up to 66.7% in foxes from the outskirts of the city. Hence, the increasing fox densities in urban dwellings, and the high prevalence of *E. multilocularis* in foxes, raise the question whether a much larger human population may be exposed to a higher infection risk. The main issue of this study was to characterize spatial and temporal aspects of urban transmission of *E. multilocularis* by investigating fox faecal samples collected in the environment and different rodent species at necropsy.

MATERIALS AND METHODS

Study area

The survey was conducted in the municipality of Zurich, Switzerland, referred to in this study as 'the city of Zurich' with a population of 390 000. The study area (87 km²) was divided into an urban, a border and a periurban zone comprising 34, 36, and 17 km², respectively (Fig. 1). The urban zone is mainly a residential one with little green space. The periurban zone consists of forests, fields, pastures and meadows, which are intensively used for recreational activities. The border zone, which divides the urban and the periurban zone and which was defined as extending 250 m from the border of the built-up area into the residential area of the city and into the periurban surrounding, includes mostly residential areas, allotments, cemeteries, sports fields and public places.

Sampling and identification of fox faecal samples

Between December 1998 and October 2000, a total of 604 putative fox faecal samples were collected from the urban zone (203 specimens), the border zone (344 specimens) and the periurban zone (57 specimens).

The year was divided into 3 periods: spring (March–June, to coincide with fox birth and lactation; 146 faeces); summer/autumn (July–October, to coincide with growth and independence of cubs; 201 faeces); winter (November–February, to coincide with the dispersal period and mating; 257 faeces).

For safety precautions, all samples were stored at –80 °C for at least 5 days before being further examined (Deplazes *et al.* 1999). The samples were identified as being of fox origin by examining their size, shape, smell and the presence of food remnants such as hair, fruit and feathers. Parameters like relative age, intensity of typical fox smell and homogeneity of faeces were partially recorded in the field with 4 graduations for each parameter. Relative age was judged by shape, humidity (considering weather conditions) and signs of decomposition.

Because of a large dog population in the city, this classification strategy was evaluated by comparing the parasite spectra of 3 groups of faecal samples from a public park. Faecal samples were judged as being of dog ($n = 25$) or fox ($n = 40$) origin by the criteria mentioned above. Another 31 faecal samples were obtained from deposit boxes into which dog owners dispose of their animals' faeces collected in plastic bags. Parasitological examination was performed with a combined sedimentation/flotation technique with zinc chloride solution (density 1.4) and microscopical identification of the helminth eggs.

Sampling of rodents

From August 1999 to November 2000, a total of 1155 rodents were caught. Tong traps were used to catch 889 *Arvicola terrestris* and 27 *Microtus* sp., whereas 83 *Clethrionomys glareolus*, 154 *Apodemus* sp. and 2 *Microtus* sp. were trapped with live traps (Longworth traps, Penlon Ltd, Oxon, GB). Other accidentally trapped animals e.g. shrews, moles and rats, were excluded from the study. Animals in live traps were anaesthetized with Metofane® (Pitman Moore, Mundelein, IL, USA) and subsequently killed by neck dislocation.

Most animals were trapped in the border and in the periurban zone. In order to estimate prevalences of *E. multilocularis* in different areas we regularly (1–3 month intervals) trapped *A. terrestris* at 10 trapping sites resulting in catching between 33 and 113 individuals per site throughout the investigation period. Rodents were stored at –20 °C if immediate dissection was not possible.

Age determination of rodents

The rodents were classified as being adults or subadults/juveniles based on body weight, body length (as measured from the tip of the nose to the first vertebra of the tail) and development of sexual organs. From 521 female *A. terrestris*, 163 showed signs of reproduction (placental scars, lactating mamma or different stages of gravidity). The body weight of all these females exceeded, with one exception, 60 g (62.2–135 g; mean 89 g; median 88 g) and their body length ranged between 122 mm

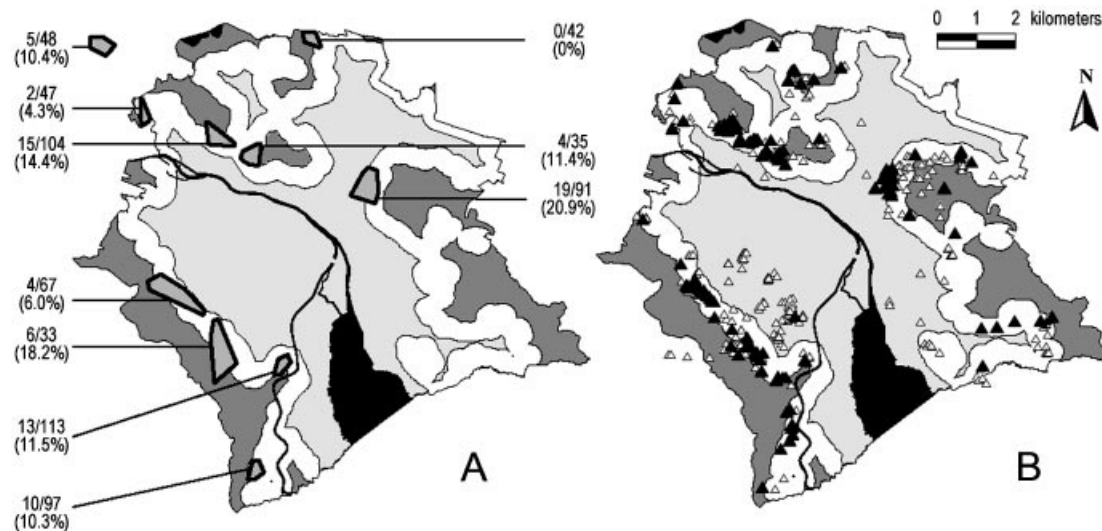


Fig. 1. Spatial distribution of trapped *Arvicola terrestris* and sampled fox faeces in the city of Zurich, Switzerland. (A) Polygons of trapping sites with more than 30 trapped *Arvicola terrestris*, number of infected and number of trapped animals per trapping site (prevalence of *Echinococcus multilocularis* in parentheses); (B) 156 coproantigen-positive fox faeces (black triangles) and 448 coproantigen negative fox faeces (white triangles). Black: lakes and rivers; light grey: urban zone (34 km²); white: border zone (36 km²); dark grey: periurban zone (17 km²).

and 165 mm. Therefore, *A. terrestris* with body weights above 60 g and body length above 120 mm were classified as adults.

Diagnosis of *E. multilocularis* in faecal samples collected in the environment

Detection of *E. multilocularis* coproantigens with a validated sandwich-ELISA (EM-ELISA) was performed exactly as described by Deplazes *et al.* (1999). Results were expressed as corrected A_{405nm} (value of specific reaction minus value of control reaction). Samples with high absorbancies in both specific and control reactions were repeated after being diluted 1:2 with a negative control. Samples with persisting high values in both reactions were excluded from the study.

A cluster analysis (SYSTAT®, SPSS Science, Chicago, IL, USA) was carried out with the 604 samples identified as being of fox origin to determine an intrinsic cut-off value (Greiner *et al.* 1994). After performing the cluster analysis 6 times (2-, 3-, 4-, 5-, 6-, 7-cluster separation) 27 clusters were obtained which were arbitrarily classified as 'high-' or 'low-responders'. Calculation of the mean value plus 3 standard deviations of all clusters classified as 'low-responders' yielded a cut-off value (A_{405nm}) of 0.21, which was slightly higher than that previously determined with fox intestinal contents (A_{405nm} 0.15; Deplazes *et al.* 1999).

Results from ELISAs were further evaluated by isolating *Taeniid* eggs from 40 randomly selected faecal samples of foxes followed by *E. multilocularis*-specific PCR as described previously (Mathis, Deplazes & Eckert, 1996).

Detection of metacestodes in rodents

All dissected rodents were carefully examined macroscopically for lesions in livers and other organs. Morphological identification of metacestodes of *Taenia* sp. included analyses of hook number and morphology. *E. multilocularis* metacestode tissue was identified either morphologically or by detection of *E. multilocularis* DNA using a modified PCR (Dinkel *et al.* 1998) with a single primer pair (EM-H15 [5'-CCATATTACAACAATATTCCTATC-3']; EM-H17 [5'-GTGAGTGATTCTTGTAGGGGAA-G-3']).

Lesions exceeding 2 mm in diameter were investigated microscopically. *E. multilocularis* metacestodes containing protoscoleces were cut into small pieces, squashed, washed with PBS through a sieve (1 mm mesh size) and the number of protoscoleces in the whole flow-through fraction was counted with an inverted microscope if few protoscoleces (< 100) were present or their total number was calculated from the counting of 3 subsamples of 100 μ l.

Statistics

Differences in the proportion of coproantigen-positive faeces and in prevalences of *E. multilocularis* in rodents were compared by χ^2 tests. If the minimum entry in the table of expectation was less than 5, *P* values were calculated with the program Actus (Estabrook & Estabrook, 1989) which performs randomized contingency tables and gives probabilities for deviations from expected values. *P* values less than 0.05 were considered significant if consistent with Bonferoni corrections. Confidence intervals (95%; CI) were calculated with GraphPad

Table 1. Helminthic infections determined by detection of *Echinococcus multilocularis*-specific coproantigens by ELISA and by microscopical identification of eggs in faecal samples collected in the field and classified as fox faeces (A), dog faeces (B) or dog faeces from deposit boxes (C) in a public park in Zurich, Switzerland

Sample type (n)	<i>E. multilocularis</i> coproantigen*	Taeniid eggs*	<i>Capillaria</i> eggs*	<i>Toxocara</i> eggs	<i>Trichuris</i> eggs
A (40)	18 (45%)	16 (40%)	22 (55%)	8 (20%)	3 (7.5%)
B (25)	0	0	0	1 (4%)	1 (4%)
C (31)	0	0	1 (3.2%)	1 (3.2%)	0

* Significant differences between (A) and (B, C): $P < 0.001$ (Actus randomization test).

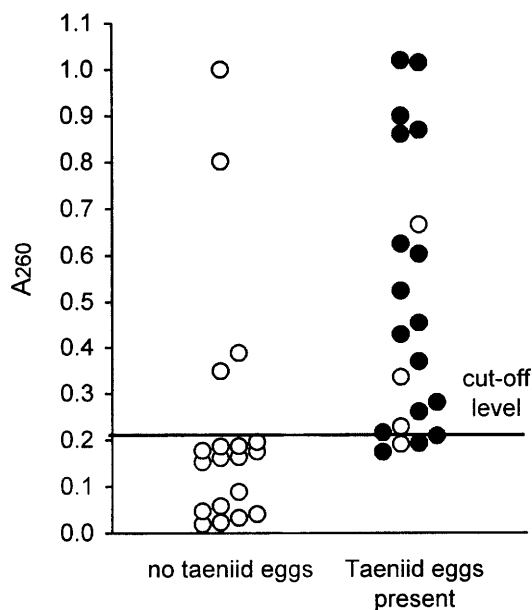


Fig. 2. Results of the *Echinococcus multilocularis* coproantigen ELISA (Deplazes *et al.* 1999), microscopical identification of Taeniid eggs and the *E. multilocularis*-specific PCR (Mathis *et al.* 1996) from 40 field samples classified as fox faeces in the city of Zurich, Switzerland. (○) PCR negative; (●) PCR positive. The cut-off value (A_{405nm}) for the ELISA was determined by a cluster analysis (Greiner *et al.* 1994) with 604 field faecal samples.

StatMate™ (GraphPad Software Inc., San Diego, CA, USA).

The degree of overdispersion of the protozoecoc burden in rodents was calculated using the maximum likelihood estimate of k , the parameter of the negative distribution which tends towards 0 as parasite aggregation increases (Wilson & Grenfell, 1997).

RESULTS

Identification of faecal samples collected in the environment

The sampling strategy for faecal samples from the environment was tested by examining the parasite

spectra of 2 groups considered to be of fox or dog origin and of dog samples derived from deposit boxes (Table 1). Parasitological examination revealed the presence of helminth eggs in 27 of the 40 (67.5%) environmental faecal samples, which were presumed to be fox faeces, in 2 of the 25 samples judged as dog faeces and in 2 of the 31 dog samples from deposit boxes. The 40 presumed fox faeces contained significantly more often Taeniid and *Capillaria* eggs, and in 45% of the samples coproantigens of *E. multilocularis* were detected by EM-ELISA (Table 1). Neither Taeniid eggs nor specific coproantigens of *E. multilocularis* could be detected in the 56 putative dog faeces (collected in the environment and from deposit boxes). No significant differences in the distribution of helminth eggs were found between these 2 groups of dog faeces.

Evaluation of the *E. multilocularis* coproantigen ELISA (EM-ELISA) with faecal samples collected in the environment

Comparison of *E. multilocularis* coproantigen detection, Taeniid egg isolation and *E. multilocularis*-specific PCR with 40 randomly selected samples identified as fox faeces are shown in Fig. 2. Taeniid eggs were isolated from 21 (53%) of the faecal samples of which 17 (43%) were positive in the *E. multilocularis*-specific PCR. The sensitivity of the EM-ELISA for patent *E. multilocularis* infections, as determined by PCR, was 88%. All 19 samples free of Taeniid eggs were negative by PCR, but 4 of these samples were positive in the coproantigen ELISA.

With the aim to determine antigen stability, coproantigen results of 381 environmental faecal samples of putative fox origin were related to parameters recorded in the field such as relative age, intensity of fox smell and homogeneity. No significant differences in the distribution of coproantigen positive samples were found related to the graduations of these parameters (Table 2).

Table 2. Detection of *Echinococcus multilocularis* coproantigens in 381 faecal samples collected in the field and classified as fox faeces in relation to increasing graduations of relative age (estimated by morphology and humidity), intensity of fox smell and homogeneity as judged in the field

Parameters	Increasing graduation			
	+	++	+++	++++
	No. of samples (% <i>E. multilocularis</i> coproantigen positive)			
Relative age*	21 (28.6%)	113 (33.6%)	222 (33.3%)	25 (32.0%)
Smell intensity†	131 (32.8%)	113 (33.6%)	107 (33.6%)	30 (30.0%)
Homogeneity‡	39 (35.9%)	135 (39.3%)	171 (28.6%)	36 (27.8%)

* Estimated age: + less than 2 days; ++++: older than 1 week.

† + No typical fox smell; ++++ intense fox smell.

‡ + Most components macroscopically identifiable (fruit remnants, feathers, hair, etc.); ++++ homogenous faeces without identifiable components.

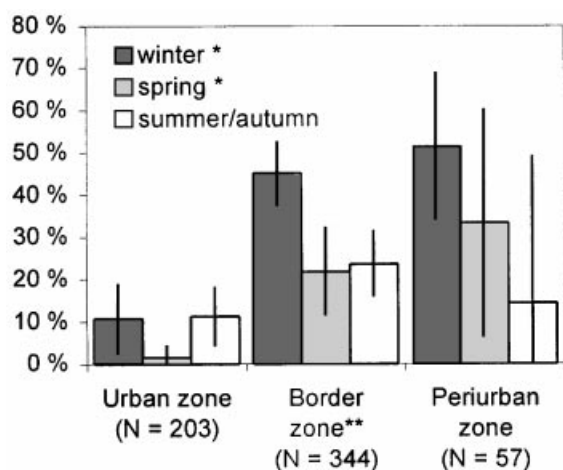


Fig. 3. Spatial and seasonal differences in the incidence of *Echinococcus multilocularis* coproantigens (Deplazes *et al.* 1999) in 604 field samples classified as fox faeces collected from December 1998 to October 2000 in 3 zones of the city of Zurich, Switzerland (% coproantigen positive samples and 95% CI). Significant spatial (*) and seasonal (winter: November–February, spring: March–June; summer/autumn: July–October) (**) differences are marked with asterisks (P values after Bonferroni corrections: $P < 0.01$, χ^2 test).

Coproantigens in fox faecal samples collected in the environment

Spatial and seasonal differences. Of the 604 tested fox faecal samples 156 (25.8%) were positive in the EM-ELISA. The proportion of coproantigen-positive faeces was higher in the border and periurban zone than in the urban zone. These spatial differences were highly significant for faeces sampled during winter and spring (Fig. 3). Samples from the border zone collected during the winter months (November–February) had significantly more coproantigen-positive results (45.2%) than samples of other seasons (21.9–23.7%). The same tendency was observed for the periurban zone. Coproantigen-

positive faeces of the urban zone were mainly found in the periphery of this zone whereas in more central parts nearly no coproantigen-positive faeces could be found (Fig. 1B).

E. multilocularis and *Taenia spp.* in rodents

At necropsy, liver lesions were observed in 277 of 889 dissected *A. terrestris* and in 3 *C. glareolus*, 1 *Microtus* sp. and 1 *Apodemus* sp. (Table 3). Prevalence of *E. multilocularis* (protoscoleces and/or PCR positive) was 9.1% (81/889) for *A. terrestris*. The prevalence in 83 trapped *C. glareolus* was significantly lower (2.4%; $P < 0.05$, χ^2 test) than in *A. terrestris*. Liver lesions of *Apodemus* sp. (1/154) and *Microtus* sp. (1/29) were negative in the *E. multilocularis*-specific PCR.

Metacestodes of *E. multilocularis* containing protoscoleces were found in 26 *A. terrestris* with numbers ranging between 14 and 244400 resulting in a total biomass of 926239 protoscoleces (Fig. 4). The maximum likelihood estimate of $k = 0.28$ indicates that the protoscoleces burden in *A. terrestris* is heavily overdispersed. Protoscoleces numbers below 1000 were found in 8 (31%) *A. terrestris* which harboured 0.2% (1779 protoscoleces) of the total biomass. Three animals (12%), with metacestodes containing more than 100000 protoscoleces (120000; 240000; 244400), carried 69% of the total biomass. In 83 *C. glareolus* investigated, 1 animal harboured *E. multilocularis* metacestode tissue containing 108000 protoscoleces, whereas the small lesion in the second animal was identified by PCR only. All 27 lesions with protoscoleces showed clear positive PCR results.

Prevalence of *Taenia spp.*

The prevalence of *T. crassiceps* and *T. taeniaeformis* in *A. terrestris* was 2.0% (18/889) and 12.1%

Table 3. Macroscopically visible liver lesions and detection of *Taenia taeniaeformis* and *Echinococcus multilocularis* metacestodes in 1155 rodents (*Arvicola terrestris*, *Clethrionomys glareolus*, *Microtus* sp. and *Apodemus* sp.) trapped in the city of Zurich, Switzerland

(Unidentifiable lesions were examined with an *E. multilocularis*-specific PCR.)

Rodent species (no. investigated)	No. of liver lesions	<i>T. taeniaeformis</i> strobilocerci	<i>E. multilocularis</i> protoscoleces	Lesions* investigated by EM-PCR	
				Positive	Negative
<i>A. terrestris</i> (889)	277†	108 (12.1%)	26 (2.9%)	55 (6.2%)	106
<i>C. glareolus</i> (83)	3	0	1 (1.2%)	1 (1.2%)	1
<i>Microtus</i> sp. (29)	1	0	0	0	1
<i>Apodemus</i> sp. (154)	1	0	0	0	1

* Morphologically unidentifiable lesions tested with EM-PCR modified according to Dinkel *et al.* (1998).

† 18 animals with 2 types of lesions.

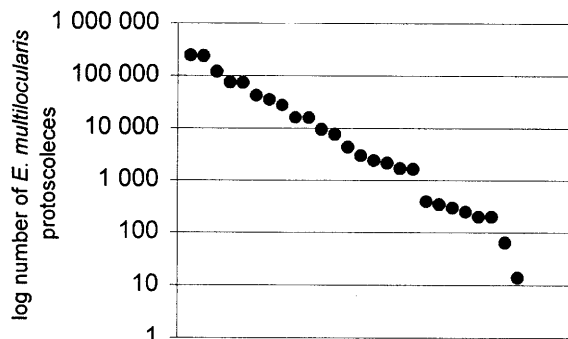


Fig. 4. Numbers of *Echinococcus multilocularis* protoscoleces (total number: 926 239) in 26 infected *Arvicola terrestris* trapped in the city of Zurich, Switzerland.

(108/889), respectively (Table 3). Metacestodes of *T. taeniaeformis* were found in liver tissue only, whereas *T. crassiceps* metacestodes were detected in pleural and peritoneal cavities and subcutaneous cysts. More than 1 metacestode species was found in 15 *A. terrestris* of which 8 carried *E. multilocularis* and *T. taeniaeformis* metacestodes and 1 animal carried metacestodes of all 3 species.

Prevalence of *E. multilocularis* in *A. terrestris* in relation to sex and age

In 734 adult *A. terrestris*, significantly higher prevalences of *E. multilocularis* (10.7%; CI [95%]: 8.6–13.2) and *T. taeniaeformis* (14%; CI [95%]: 11.6–16.7) were detected as compared to 155 subadults/juveniles (1.3% and 3.2%, respectively; χ^2 test: $P < 0.001$ and $P < 0.0001$). Differences in the prevalence of both cestodes related to sex were neither found in adult nor in subadult/juvenile *A. terrestris* (χ^2 -test: $P > 0.05$).

Prevalence of *E. multilocularis* in *A. terrestris*, spatial and seasonal differences

E. multilocularis-infected *A. terrestris* were detected in 9 of 10 trapping sites with prevalences ranging from 4.3% up to 20.9% (Fig. 1). The Actus

randomization test revealed significant differences in the prevalence of *E. multilocularis* between the trapping sites ($P = 0.019$). Animals harbouring *T. taeniaeformis* metacestodes were caught in all 10 areas (data not shown). The prevalence of *E. multilocularis* in 734 adult *A. terrestris* did not vary between the different seasons (Table 4). The same was observed for animals with protoscoleces. Prevalence of fully developed *T. taeniaeformis* strobilocerci was higher from November to February than in other months. However, due to Bonferoni corrections, this difference was not significant ($P > 0.05$).

DISCUSSION

The existence of an urban cycle of *E. multilocularis* in the city of Zurich has recently been described with a significantly lower prevalence of the parasite in foxes from urban areas as compared to those from the surrounding zone (Hofer *et al.* 2000). In the present study we observed a similar geographical distribution of the infection pressure as was obvious from the investigation of 604 faecal samples collected in the environment. Different factors may contribute to this difference of the parasite distribution. Preliminary results of radiotracking of foxes in our study area document small homerange sizes between 30 ha and 42 ha (Deplazes *et al.* 2002), which are in the range of those observed in urban areas in England (Harris & Rayner, 1986). Therefore, foxes living within the built-up area in a certain distance to the border zone do not roam these latter habitats, where good conditions for the development of vole populations exist. This assumption is supported by the fact that coproantigen-positive faeces from the urban zone were found mainly in the periphery of this area. Significant seasonal differences in the detection of *E. multilocularis* in faecal samples were only found in the border zone of the city, where the percentage of positive faecal samples was higher during winter months. Correspondingly, foxes from the same study area had also higher prevalences in winter (Hofer *et*

Table 4. Seasonal differences in the prevalence of *Taenia taeniaeformis*, *T. crassiceps* and *Echinococcus multilocularis* in 734 adult *Arvicola terrestris* trapped between August 1999 and November 2000

Period*	No. of animals	<i>T. taeniaeformis</i> positive	<i>T. crassiceps</i> positive	<i>E. multilocularis</i> positive	
				Total	With protoscoleces
Winter	146	20.3 %	3.4 %	12.3 %	3.4 %
Spring	141	14.2 %	4.3 %	9.9 %	3.5 %
Summer/autumn	447	12.3 %	1.6 %	10.5 %	3.6 %

* Winter: November–February; spring: March–June; summer/autumn: July–October.

al. 2000). In contrast to this study, Morishima *et al.* (1999) did not find significant seasonal differences in the percentage of *E. multilocularis* coproantigen-positive faeces. As red foxes are known to be opportunistic feeders, predation on rodents may differ seasonally. In suburban foxes in London (Great Britain), stomachs revealed higher percentages of mammals in autumn and winter (Harris, 1981).

Investigations on potential intermediate hosts of *E. multilocularis* are important to localize the cycle because voles are known to have small home ranges (Saucy & Schneiter, 1997). Therefore, infected voles represent a biological marker for the contamination of the ground in a distinct area. *A. terrestris* was the rodent species with the highest prevalence of *E. multilocularis* in our study. The only other infected species was *C. glareolus* with a significantly lower prevalence. *Apodemus* spp. have not yet been shown to be suitable intermediate hosts for *E. multilocularis* in central Europe (Rausch, 1995). This is confirmed by our results, as none of the 154 *Apodemus* sp., which were mostly trapped near sites with high prevalences of *E. multilocularis* in *A. terrestris*, were infected with the parasite. *Microtus* spp. are significantly involved in the transmission of *E. multilocularis* in Europe (Bonnin *et al.* 1986; Delattre, Giraudoux & Quere, 1990; Gottstein *et al.* 2001). In our study area, however, none of our trapping sites revealed a high activity of *Microtus* species suggesting that *Microtus* sp. might not be as common as *A. terrestris* in the city of Zurich. However, the sample size ($N = 29$) for this species was too small for definitive conclusions concerning the involvement of this species in the local cycle.

The high prevalence of *A. terrestris* suggests a major role in the perpetuation of the *E. multilocularis* cycle in the area investigated, which is supported by gut analysis of urban foxes (Gloor, unpublished data) revealing *A. terrestris* as the most frequent rodent in fox stomachs from Zurich. Furthermore, 47 fox faecal samples were discovered directly on vole ground systems of *A. terrestris* where signs of predation activities of carnivores were observed. Hence, the marking behaviour of red foxes seems to play an important role in the transmission of *E. multilocularis*.

The prevalences of *E. multilocularis* in *A. terrestris* varied significantly between the different trapping sites but were generally high (up to 20.9%) compared to other studies (Eckert *et al.* 2001a). *E. multilocularis* infected *A. terrestris* were found in 9 of 10 trapping sites in the border zone, which was highly contaminated with *E. multilocularis* coproantigen-positive faecal samples. We therefore assume that the transmission of *E. multilocularis* is not concentrated in so-called 'hot-spots' but occurs in extended areas along the border of the built-up area of the city.

In this area, free-ranging dogs and cats are common which could acquire infections with *E. multilocularis*. The presence of *T. taeniaeformis* in *A. terrestris* populations in all trapping areas along the border zone, and the fact that *T. taeniaeformis* was not found in foxes in this area (Hofer *et al.* 2000), indicates a considerable predation pressure of domestic cats on *A. terrestris*.

There is only scarce information about seasonal variation in prevalence of *E. multilocularis* in intermediate hosts. In our study, no differences in adult rodents were observed which is in agreement with findings from France (Massif Central, Auvergne) for *A. terrestris* (Pétavy & Deblock, 1983).

Protoscoleces were found in 32% of the *E. multilocularis* infected *A. terrestris* resulting in an overall prevalence of 3.5%, which is within the range described in earlier surveys (0.2–8.3%; Gottstein *et al.* 1996; Hofer *et al.* 2000; Pétavy & Deblock, 1983). Again, no seasonal differences of prevalences of fertile cysts were observed. To the best of our knowledge, no studies have so far been published on the numbers of protoscoleces of *E. multilocularis* in wild rodents. In the present study, there was a huge overdispersion of protoscoleces with a range from few (14) up to 244400 per rodent host. From the 26 *Arvicola*, 69% harboured more than 1000 protoscoleces per animal.

Until recently, monitoring of the infection pressure of *E. multilocularis* in endemic areas was performed by parasite identification in definitive hosts at necropsy. However, data collection was strongly influenced by hunting regulations (e.g. close seasons, protected areas) and especially difficult in periurban and urban settings. As faecal samples of

foxes can easily be collected in the environment, the identification of the presence of *E. multilocularis* in such samples by parasite-specific coproantigen detection (Craig, Rogan & Allan, 1996; Morishima *et al.* 1999; Eckert *et al.* 2001b) or copro-DNA (Mathis & Deplazes, 2002) opened up a new strategy for the direct assessment of the environmental contamination in a defined area.

Nevertheless, the strategy for collecting specifically fox faecal samples had to be scrutinized, as fox faeces may be confused, particularly with dog faeces, which are very frequent in urban areas. Our results clearly confirm that differentiation of dog and fox faeces can reliably be achieved.

The EM-ELISA used in this study was originally validated with intestinal contents of foxes (Deplazes *et al.* 1999). A cluster analysis (Greiner *et al.* 1994) of our fox environmental samples yielded an intrinsic cut-off value (A_{405nm}) slightly higher than in the former study. This might be due to different environmental influences on these faeces. The evaluation of this proceeding by isolating taeniid eggs followed by PCR identification (Mathis *et al.* 1996) in 40 of these samples resulted in a sensitivity of 88.2% for patent infections, which is comparable to the sensitivity of 83.6% determined with intestinal contents of 55 infected foxes (Deplazes *et al.* 1999). In 4 of 40 samples free of taeniid eggs DNA amplification by PCR was negative, but the EM-ELISA was positive due to either cross-reactions or the presence of prepatent infections that cannot be detected with our PCR strategy (Mathis *et al.* 1996). False negative results of the EM-ELISA can be explained with the high proportion of foxes infected with low worm burdens in the study area (Hofer *et al.* 2000). Neither the relative age of samples nor the intake of different food affected the detection of *E. multilocularis* coproantigens. This is consistent with observations made with the same coproantigen test with environmental faecal samples from rural endemic areas in France (Raoul *et al.* 2001). Stability of helminth coproantigens has been documented in 2 studies: *T. hydatigena* antigens were stable for at least 5 days at room temperature (Deplazes *et al.* 1990), and the detection of *E. granulosus* coproantigens was not influenced by exposing the faeces during 6 days and nights to sun-exposed places in the Australian Capital Territory (Jenkins *et al.* 2000).

Little is known about the correlation of the infection pressure of *E. multilocularis* on the incidence of human AE. Gottstein *et al.* (2001) found an increased seroprevalence in blood donors living in an *E. multilocularis* 'hot spot' area over a period of 10 years without increase in clinical cases. There are no indications of a higher incidence of alveolar echinococcosis in urban dwellers in Switzerland (Renner-Schneiter *et al.* 2000), but the recent invasion of foxes in urban habitats (Gloor *et al.* 2001), and the

considerable contamination of this area with *E. multilocularis*-infected fox faeces, could change the situation. Urban inhabitants use the border zone of the built-up area intensively for recreational activities, and this zone may therefore represent an area of increased risk for acquiring AE. Furthermore, the many free-roaming domestic cats and dogs in this area might prey on infected voles and hence could represent a potential source of infection for humans (Eckert & Deplazes, 1999). Based on the incubation time of 5–10 years in humans, changes in the epidemiology of AE would only be noticed in a long-term surveillance which could be achieved in the framework of a reporting system.

The cycle of *E. multilocularis* in urban settings seems to be determined by the small homeranges of foxes and the distribution of suitable intermediate hosts. Therefore, local interaction in the cycle reducing the infection pressure in defined areas (e.g. public parks, swimming pool areas, private gardens) should be feasible. Field studies in Zurich on the control of the *E. multilocularis* infection in urban areas, by distributing praziquantel-containing baits manually, are currently being carried out.

The authors thank all collaborators involved in the Integrated Fox Project. We especially thank Christian Stauffer and the game wardens of the city of Zurich for their assistance and for their generous collaboration. We thank Professor J. Eckert for valuable comments on the manuscript. The study was supported by the Swiss Federal Veterinary Office (FVO Nr. 1.99.03) and the Swiss National Science Foundation (grant no. 31-47031.96). This work represents the dissertation of Claudia Stieger, veterinarian.

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