ECHINOCOCCUS MULTILOCULARIS INFECTIONS OF RURAL, RESIDENTIAL AND URBAN FOXES (*VULPES VULPES*) IN THE CANTON OF GENEVA, SWITZERLAND

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Summary: s

We examined 267 red foxes (Vulpes vulpes) from the canton of Geneva, Switzerland, for intestinal infections with *Echinococcus multilocularis*. This region is situated in the core area of the endemic range of this zoonotic cestode in Central Europe. Several factors were taken into account and urbanisation level appeared to be the most explicative to describe observed differences. The prevalence decreased significantly from rural and residential areas (prevalence of 52 %, CI 43-62 %, and 49 %, CI 38-59 %, respectively) to the urban area (prevalence of 31 %, CI 19-42 %). A few juvenile foxes harboured very high burdens up to more than 120,000 worms and were significantly more heavily infected than adults. The intensity of infection decreased from rural and residential areas to the city, suggesting a lower contamination of the urban environment.

KEY WORDS : *Echinococcus multilocularis, Vulpes,* red fox, urbanisation, zoonosis, Switzerland.

Résumé : ECHINOCOCCOSE ALVÉOLAIRE CHEZ LE RENARD ROUX, EN MILIEUX RURAL, RÉSIDENTIEL ET URBANISÉ, DANS LE CANTON DE GENÈVE, SUISSE

*Nous avons examiné 267 renards (*Vulpes vulpes*) provenant du canton de Genève, Suisse, en vue d'évaluer le taux d'infection par le cestode* Echinococcus multilocularis. *Cette région est située au cœur de l'aire de distribution européenne de cet helminthe zoonotique. Nous avons considéré plusieurs facteurs potentiels et le taux d'urbanisation est apparu être le plus explicatif. La prévalence diminue significativement des zones rurale et résidentielle en allant vers la zone urbaine. Quelques individus juvéniles abritaient de très hautes charges parasitaires allant jusqu'à plus de 120 000 vers et ils étaient significativement plus infectés que les adultes. La charge parasitaire allait en diminuant du secteur rural vers les secteurs résidentiels et urbains, ce qui suggère une contamination de l'environnement plus faible en milieu urbain.*

MOTS CLÉS : Echinococcus multilocularis, Vulpes, *renard roux, urbanisation, zoonose, Suisse.*

INTRODUCTION

The small fox tapeworm *Echinococcus multilocularis* is one of the most serious zoonotic helminths leading to alveolar echinococcosis in humans (Eckert & Deplazes, 2004; Giraudoux *et al.*, 2001; Vuitton *et al.*, 2003). This liver disease caused by the metacestode stage of *E. multilocularis* is rare (0.02 to 1.4 cases per 100,000 inhabitants in Europe, Vuitton *et al.*, 2003, 11 to 40 cases per 100,000 inhabitants in endemic foci), but severe with a fatal outcome if left untreated (Amman & Eckert, 1995). Gottstein (1992) concluded that 56 % to 94 % of declared and untreated cases of alveolar echinococcosis result in death, and letality was still 10 % to 14 % when early diagnosed and treated.

The cestode *E. multilocularis* is a dixenous helminth with carnivores as definitive hosts. *E. multilocularis* is maintained in a wild animal cycle including red foxes as definitive hosts and a range of rodents as intermediate hosts (Eckert & Deplazes, 2004; Vuitton *et al.*, 2003). Domestic dogs and cats are also potential definitive hosts (synanthropic transmission) though at a much lower extent (Eckert *et al.*, 2001a; Gottstein *et al.*, 2001). However, in Europe, red foxes are considered as the main source of contamination of the environment (Eckert & Deplazes, 2004).

After the successful vaccination campaigns against rabies, red fox populations in Western and Central Europe recovered to densities similar to what was observed before the epidemics, or even increased (Breitenmoser *et al.*, 1995) according to a trend towards an increase already observed before the rabies epidemic (Chautan *et al.*, 1998). A new phenomenon appeared at the same time: as already observed in Great Britain several decades before (Macdonald & Newdick, 1982), red foxes began to colonize cities and by now "urban foxes", with populations living and reproducing in the cities, are also a reality in continental Europe (Gloor *et al.*, 2001a; Deplazes *et al.*, 2004).

Red foxes in urban areas are often more abundant than in rural or natural habitats (Harris, 1981; Gloor *et al.*, 2001b), and have closer contact with humans. This proximity and the observed densities are a concern for

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public health. *E. multilocularis* could thus represent a public health risk especially for urban inhabitants. However, because of a subsequent incubation of five to 15 years in humans, the correlation between the increased risk related to fox population new dynamics and a potential increase in the incidence of alveolar echinococcosis is difficult to assess at that time (Deplazes *et al.*, 2004).

Only few studies have so far been conducted in urban areas of Central Europe to investigate transmission of *E. multilocularis*. What is interesting in Geneva, is that the situations in neighbouring France (Pétavy *et al.*, 1990) and Switzerland (Siegenthaler & Brossard, 1996) are already documented. In addition, the structure of the city (*cf.* study area) and the apparently less appropriate climate for the survival of *Echinococcus* eggs (warmer and drier conditions as compared to surrounding rural areas) known as the Urban Heat Island effect (Shashua-Bar & Hoffman, 2000; Unger *et al.*, 2001) also make Geneva a unique study opportunity. Furthermore, a preliminary study, conducted on a small sample size (N = 160 foxes) already identified the presence of the small-fox tapeworm in the canton of Geneva, and showed a difference along a gradient of variable human density (Fischer *et al.*, 2003). However, the method used to differentiate urban, residential and rural areas was not precise enough. The method used in the city of Zurich is not satisfactory either, with a "border zone" being considered arbitrarily to have a width of 250 m between urban and rural zones (Stieger *et al.*, 2002). This method determines a buffer area rather than a real identifiable residential habitat.

In this paper, *E. multilocularis* prevalence and abundance were investigated following a gradient of urbanisation.

STUDY AREA

The canton of Geneva is located at the western tip of Switzerland, at the end of the lake Geneva (Fig. 1). Altitudes range between 350 and 500 m and the climate is temperate with a Mediterranean influence. Average annual rainfall is less than 900 mm and mean temperatures is around 12° C. Mean relative humidity is less than 75 % (Annuaire statistique du canton de Genève, 2003). Snow periods are rare and if snow is present, there are only a few centimetres and it rarely lasts for more than a couple of days.

The canton is highly urbanised, with 400,000 inhabitants living in an area of 240 km^2. Over 84 % of the inhabitants live in the agglomeration of Geneva itself, which is located in the centre of the canton, around the end of the lake. The rest of the population is scattered within 16 small villages. The urbanized areas account for half the canton's area, the rest is mostly devoted to intensive agriculture (cereal crops and vineyards) and has a limited portion of permanent grassland (18.6 %). Forests represent less than 10 % of the area. An extensive road network with heavy traffic criss-crosses the rural areas and forests.

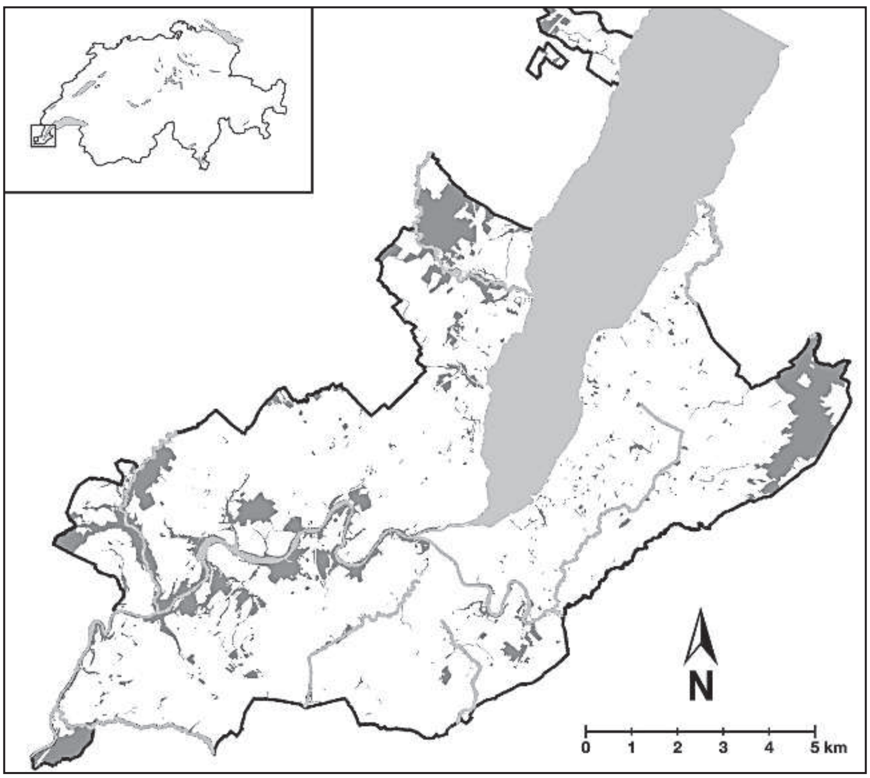

Fig. 1. – Situation of the canton of Geneva.

The agglomeration of Geneva is characterized by a particularly extensive residential area organised as a belt around the core of the city. Individual housing with private gardens accounts for 20 % of the canton's area (*cf.* methods). Its width ranges from 200 m to 2 km. This zone appears to be particularly favourable for the red fox, as it provides abundant food and shelter possibilities. Furthermore, the shores of the lake and riverbanks constitute sheltered corridors for the movements of the fauna and give it the possibility to penetrate deep into the core of the city.

METHODS

SAMPLING OF FOXES

In the canton of Geneva hunting was abolished in 1974. Only game keepers are allowed to shoot animals. Regarding red fox management, only individuals which are injured, sick or which pose recurrent problems are shot. Game keepers also collect all wild animals found dead. Data collection is not bound to hunting pressure and the collaboration of hunters. Data collection is thus similar from year to year.

Dead and shot foxes were collected all over the canton from 1998 onward. They were located exactly on a map or by GPS, weighed and sexed. Age and reproductive status were defined. Age was determined counting annuli in the cementum of the canine of the lower jaw. Age in months was calculated assuming birth date being the first of April (Wandeler, 1976). Foxes are considered juveniles when less than one year old. Carcasses were dissected and organs collected and deep frozen for four days at – 80° C following safety precautions described in Eckert *et al*. (2001c), and then conserved at – 20° C. before further analysis. The digestive tract was isolated from the pyloric sphincter to the anus.

E. MULTILOCULARIS DETECTION

E. multilocularis was recovered with the intestinal sedimentation and counting technique (SCT). Species identification was based on typical morphological characteristics. The sediment fraction was examined in small portions of about 5 ± 10 ml in square Petri dishes. If more than 100 worms were found the total worm burden was calculated from the count of one subsample (for details: Hofer *et al*., 2000).

Aggregation was measured using the corrected moment estimate of k as:

$$k = (x^2 - S^2/N)/(S^2 - x)$$

where x is mean of intensity, S^2 is variance of intensity and N is sample size, as described in Elliot (1977).

Confidence interval (95 %) of prevalence was calculated as:

$$95\ \%\ CI = 1.96\ [(p\ (1-p))/N]^{1/2}$$

where p is prevalence and N is sample size. Intensity of infection refers to parasite distributions in infested animals only.

URBANISATION

Regarding urbanisation, we defined three different sectors: rural, residential, and urban. We considered the total number of inhabitants of private housing and of employees working in industries, offices and administrations, i.e. the number of persons registered at a given adress (following data of the cantonal administration based on a population census made in summer 2002), and selected a grid of squares of 100 × 100 m. Rural sectors were then defined by a human density of ≤ 40 people per km^2 during the day or night, residential areas by a density ranging between 40 and 220 humans per km^2, and urban areas by a density of more than 220 human per km^2 (maximum density being 3,790). The three resulting concentric areas, with the most urbanised, the city, in the core and around the end of the lake, then a belt of residential area, and finally, the rural areas towards the borders of the canton (Fig. 2) were smoothed with the adaptive Kernel method (applied with the least-squares crossvalidation as smoothing parameter; Worton, 1989). Several small villages scattered within the rural area represent residential islands. In the south, the residential area continues towards France up to the small town of Annemasse, interrupting the surrounding rural sectors.

STATISTICAL ANALYSIS

Statistical analyses were performed with SPSS–PC version 10.0. To evaluate the effect of urbanisation on *E. multilocularis* prevalence in foxes, irrespective of potentially confounding variables, a multiple logistic regression model was calculated with the factors year, season (winter: December to February; spring: March to May; summer: June to August; fall: September to November), age (juvenile or adult) and sex. Furthermore, as 27 % of the investigated foxes were infected with sarcoptic mange, and as it could bias dead fox sampling procedure, the factor mange (infected or not infected) was added to the model. In a second step the factor urbanisation sector was added to the full model and the difference of the two models was tested by a Log-Likelihood test. Finally, a stepwise backward procedure (using likelihood ratio) was performed to find the most relevant factors affecting the *E. multilocularis* prevalence. The Mann-Whitney U-test was applied for the comparison of parasite distribution (intensity of infection), or age distribution in months. Tests were considered significant when $p < 0.05$.

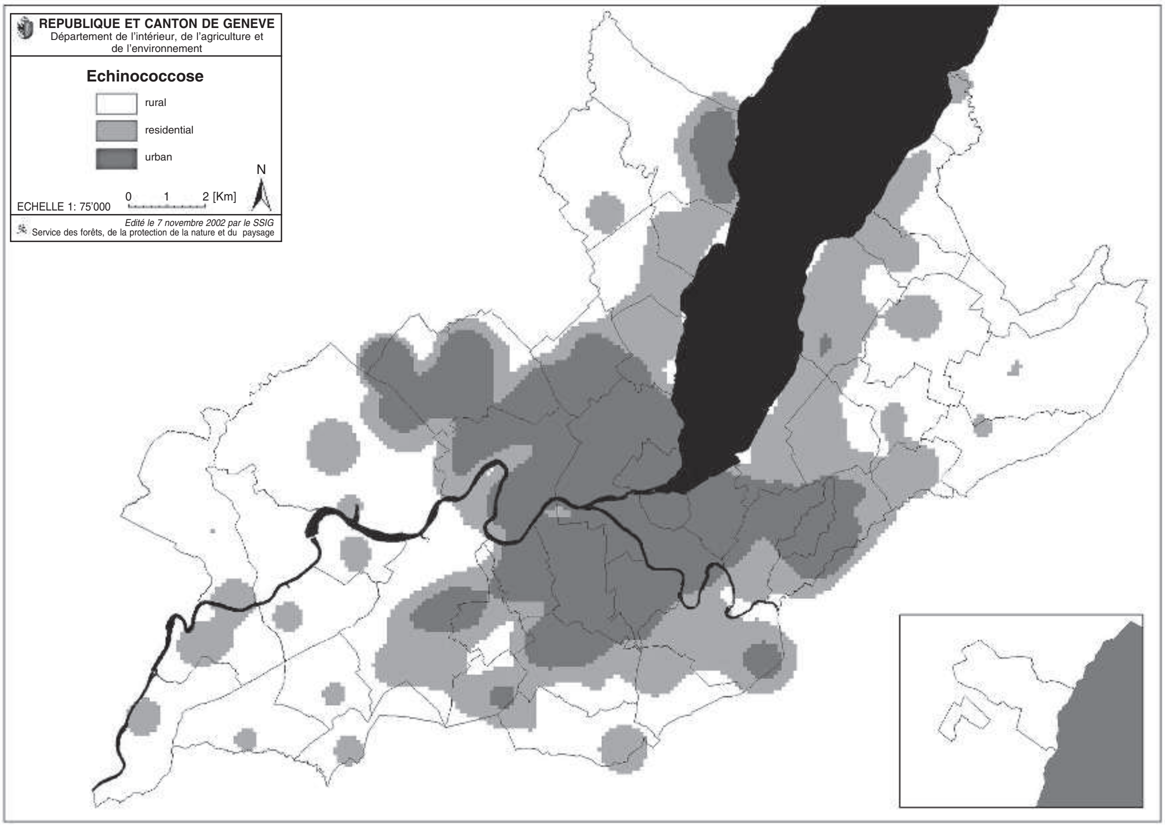

Fig. 2. – Representation of the three considered areas: white = rural; light grey = residential; dark grey = urban.

RESULTS

RED FOXES

From January 1998 to December 2002, 454 red foxes, found dead or shot were collected from all parts of the canton of Geneva, but only 267 could be analysed for parasitical survey. Mortality was mainly due to collisions with vehicles (over 75 % of instances) and often it was not possible to collect intact inner organs, or carcasses were too old to be evaluated. Sex and age were determined for 253 animals, distributed in 137 males and 116 females. Juveniles counted for 134 animals and adults for 119.
A total of 103 carcasses came from the rural area, 88 from the residential area and 62 from the urban area; location of 14 remaining foxes was not known. No statistical difference in age (ratio juveniles:adults) or sex distribution between the three urbanization areas was detected.

PREVALENCE OF *E. MULTILOCULARIS*

E. multilocularis was recovered at a prevalence of 45.7 % in red foxes from the canton of Geneva. A full logistic regression model revealed significant differences of *E. multilocularis* prevalence between the different years and between the different urbanisation sectors (Table I). A Log-Likelihood test revealed that the addition of the factor urbanisation sector improved the model only at

Explanatory variables (test category/ reference categorie)	OR	95 % CI for OR	P-value
Sex			
male/female	1.22	0.69-2.13	0.498
Age			
adult/juvenile	0.84	0.47-1.49	0.556
Mange			
yes/no	0.92	0.49-1.69	0.786
Season			0.229
Dec.-Feb./Sep.-Nov.	1.44	0.59-3.44	0.418
Mar.-May./Sep.-Nov.	0.86	0.38-1.95	0.727
Nov.-Aug./Sep.-Nov.	1.89	0.79-4.47	0.151
Year			0.062
1998/2002	0.58	0.25-1.34	0.205
1999-2002	**0.36**	**0.15-0.83**	**0.018**
2000/2002	0.39	0.15-1.02	0.056
2001-2002	**0.31**	**0.12-0.76**	**0.011**
Urbanisation sector			0.039
rural/urban	**2.73**	**1.24-5.97**	**0.012**
border/urban	**2.32**	**1.03-5.18**	**0.04**

Table I. – Odds-Ratios, 95 % Confidence intervals and P-values of a full logistic regression model for the prevalence of *E. multilocularis* in foxes of Geneva ($P \leq 0.05$ given in bold).

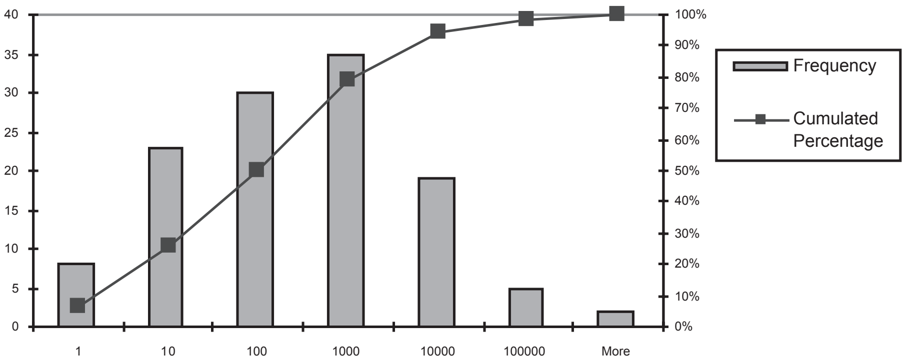

Fig. 3. – Prevalence of *E. multilocularis* along the increasing gradient of urbanization (represented with 95 % Confidence interval).

a significance level of $\alpha \leq 0.1$ ($p = 0.057$). However, urbanisation sector remained the only significant factor (Wald Statistics 6.6, df = 2, $p = 0.037$) in the model when the stepwise backward procedure was applied to the full model. Prevalence was highest in the rural (52 %, CI 43-62 %) and lowest in the urban area (31 %, CI 19-42 %; Fig. 3).

INTENSITY OF INFECTION WITH *E. MULTILOCULARIS*

Worm burden of *E. multilocularis* in foxes was highly aggregated, with a corrected moment estimate of k = 0.025. Worm burden ranged from one to 120,020 specimens. Fifty percent of infected foxes harboured less than 100 worms. 26 foxes (9.7 %) had high worm burdens (more than 1,000 worms) and carried 97.3 % of total worm biomass. Five foxes (1.9 %) carried more than 55,000 worms, representing 75.8 % of total worm burden (Fig. 4).

A significant decrease in the intensity of infection was found between rural and urban areas (Mann-Whitney U-test, $p = 0.043$) and between residential and urban areas (Mann-Whitney test, $p = 0.035$; Table II).

Whereas prevalence of *E. multilocularis* in juveniles (prevalence of 47.7 %) and adults (prevalence of 42.9 %) presented no statistical difference, the intensity of infection was higher in juveniles than in adults (Mann-Whitney test, $p = 0.013$), with 64 juveniles harbouring 93.8 % and 51 adults harbouring 6.2 % of total worm burden. All foxes infested with more than 55,000 worms (five individuals) were juveniles from two to five and a half months-old.

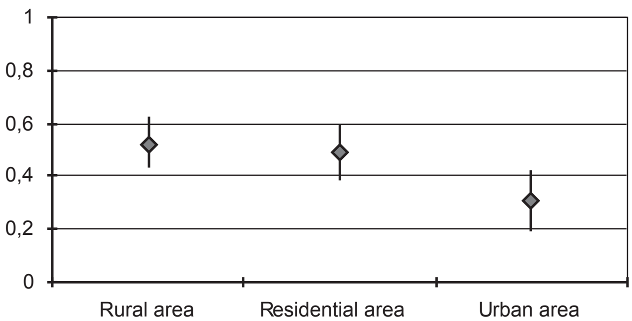

Fig. 4. – Worm burden distributions of *Echinococcus multilocularis* in infested red foxes (intensity of infection) from the canton of Geneva, Switzerland.

DISCUSSION

As we already pointed out in a preliminary study (Fischer *et al.*, 2003), the small-fox tapeworm is present in the canton of Geneva up to the urbanised areas of the city. Considering research conducted in neighbouring France and canton of Vaud (Switzerland) we could expect the presence of *E. multilocu-*

	Adults			**Juveniles**		
	Rural area	**Residential area**	**Urban area**	**Rural area**	**Residential area**	**Urban area**
Sample size*	27 (52)	16 (36)	6 (25)	23 (44)	26 (50)	(10 (30)
Mean	1,062.6	417.9	55.5	12,002.1	10,120.7	699.1
Standard deviation	4,631.5	538.0	73.2	31,056.0	26,885.9	1,384.9
Geometric mean	45.1	99.2	21.6	566.0	182.1	46.7
Minimum	1	3	1	2	1	1
Maximum	24,200	1,798	199	120,020	104,000	4,218
Total worm burden	28,690	6,686	333	276,048	263,139	6,992
Corrected moment estimate of k**	0.008	0.177	0.064	0.051	0.050	0.043

Table II. – Worm burden distribution (intensity of infection) of *E. multilocularis* along the increasing gradient of urbanization, in Geneva, Switzerland.

laris, at least in the rural areas, but the high prevalences we observed and the presence of the parasite within the city are quite astonishing. In Haute-Savoie, Contat (1984) and Pétavy *et al.* (1990) mention prevalences of 43 % and 22 % respectively over the whole "Département". In neighbouring areas of the canton of Vaud, Siegenthaler & Brossard (1996) determined a prevalence of 11 %, whereas they observed a mean prevalence of 30 % for the whole of western Switzerland.

The canton of Geneva is situated in the core area of the endemic range of *E. multilocularis* in Central Europe (Eckert & Deplazes, 2004; Vuitton *et al.*, 2003). In this region the prevalence was high in the nearby Jura Mountains where climatic conditions are more favourable for the survival of *Echinococcus* eggs, as mean temperature is rather low and humidity high (Veit *et al.*, 1995). Siegenthaler & Brossard *op cit.* observed that conditions were most favourable between 500 and 900 m. Furthermore, landscape composition was described as playing an important role, prevalence being positively correlated to the ratio of permanent grassland, as population outbreaks of the two main intermediate hosts of *E. multilocularis*, *Microtus arvalis* and *Arvicola terrestris*, are favoured (Giraudoux *et al.*, 1997; Raoul *et al.*, 2003).

However, the prevalence of *E. multilocularis* in Geneva appeared to be much higher than what was observed in the adjacent areas, despite low altitude with a relatively dry and warm climate and a limited proportion of permanent grassland. This could be a sign of annual variations, or of a general increase within the last decades, as the studies mentioned (Contat *op. cit.* and Pétavy *op. cit.*) were achieved in the mid 80s and in the 90s. Furthermore, they had a supra-regional character and did not concentrate on local specificities. Thus, there might be temporal differences, dependent of the cyclic evolution of rodent populations for example, which could explain the marked difference between the rural areas of Geneva and the adjacent territories of Haute-Savoie (F) and the canton of Vaud (CH).

E. multilocularis distribution is known to be heterogeneous. In Switzerland, prevalence ranges between 3 % and 53 % in 21 of the 23 cantons (Eckert *et al.*, 2001a). At smaller scales, prevalence of the parasite in foxes reaches high levels in high endemic foci. A prevalence of 49 % was found in the canton of Fribourg (Siegenthaler & Brossard, 1996) and of 67 % in the rural area surrounding the city of Zurich (Hofer *et al.*, 2000). The prevalence of 45.7 % in our study, reaching 68 % in the rural area in 2002, indicates that Geneva can be considered as a high endemic area of the parasite.

Differences in urbanisation appeared to be the most explicative factor for differences in prevalence between foxes, when using multiple logistic regression on various potential intrinsic and extrinsic factors. The small-fox tapeworm was found up to the centre of the city of Geneva, though at a much lesser extent than in rural and residential areas. In Zurich, Hofer *et al.* (2000) showed a similar decrease in prevalence from the surroundings of the city to the urban zone, but they did not record any difference in worm burden distributions between rural and urban foxes, suggesting an equivalent contamination of the two habitats when comparing infested sites. In Geneva, the decrease in the intensity of infection suggests that a lower parasite biomass occurs in the urban area and thus a lower contamination characterizes the infested sites of the urban environment. These findings imply either unsuitable conditions in the city for egg survival (Urban Heat Island effect); unsuitable conditions to maintain a sustainable intermediate host population; or a different intermediate host community.

Age-related difference was revealed for the infestation rate of *E. multilocularis*, with juveniles infected with heavier worm burdens than adults. Higher susceptibility of juveniles suggests that an acquired but partial immunity appears in adults (Hofer *et al.*, 2000; Raoul *et al.*, 2003).

Compared to rural and residential areas, the urban area of the canton of Geneva may represent a less suitable habitat for rodents, especially voles. Fragmentation of the urbanized environment may result in disparate sustainable vole populations. Density of rodent population and predation rate of urban foxes on rodents might be responsible for the decrease in prevalence and at least partially for the decrease in the intensity of *E. multilocularis* infection. Diet of urban foxes has been investigated in several European countries (*e.g.* Doncaster *et al.*, 1990; Contesse *et al.*, 2004). Whereas rural foxes have seasonal preferences for rodents especially voles, birds, fruit and crops (Artois, 1989; Weber, 1996), urban foxes prey less frequently on small mammals and ingest more scavenged food (Contesse *et al.*, 2004).

Finally, risk factors of the infestation of humans with *E. multilocularis* are reported as working in agriculture, dog ownership (Kern *et al.*, 2004), contact with foxes (Eckert *et al.*, 2001c) and densities of voles (Viel *et al.*, 1999). Main route of contamination is believed to be the ingestion of eggs from contaminated soil or plants, or from direct contacts with infected definitive hosts. Prevalence in domestic carnivores is generally low but can reach relatively high levels in hyperendemic foci (Eckert & Deplazes, 2004). However, red foxes are the main source of contamination of the environment, especially young animals (Eckert & Deplazes, 2004). In our study, five foxes representing less than 2 % of the sample carried more than 75 % of the parasite biomass and were less than six month-old. One adult of twelve months-old carried more than 22,000 worms and was the only adult out of fifteen foxes harbouring more than 2,000 worms. As foxes enter the city, potential risk arises for humans with the contamination of their highly used environment. An urban cycle of *E. multilocularis* is possible and has been recognized in the city of Zurich (Deplazes *et al.*, 2004). Secondly, domestic animals preying on infectious rodents and living directly in the vicinity of humans represent another non-negligible, risk for humans as closer contacts occur between them and their owners (Petavy *et al.*, 2000; Gottstein *et al.*, 2001; Deplazes *et al.*, 2004). The concern about a potential increase of the infection risk for human population with *E. multilocularis* eggs has led to trials to control this zoonosis. In Germany, mass treatment of red foxes with baits containing praziquantel was applied using light aircrafts (Romig *et al.*, 1999). In the city of Zurich, where a high contamination with *E. multilocularis* was identified (Stieger *et al.*, 2002), a more intensive but locally restricted strategy for urban areas was tested (Hegglin *et al.*, 2003). As observed in many studies (Hofer *et al.*, 2000; Eckert *et al.*, 2001a; Hansen *et al.*, 2003), and also in Geneva, juvenile foxes have a significantly higher worm burden than adults. Furthermore, they defecate within a restricted area around the reproductive den during their first months of life. Environmental contamination with eggs in this area is thus likely to be very elevated. In Geneva, we observed several reproductive dens located in or close to gardens of family houses with children. These gardens are thus primary risk areas, and we suggest that they should be considered as a priority when planing treatment strategies.

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