Rodents as shared indicators for zoonotic parasites of carnivores in urban environments

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(*Received 24 July 2008; revised 11 November 2008; accepted 13 November 2008; first published online 21 January 2009*)

SUMMARY

Rodents are shared intermediate or paratenic hosts for *Echinococcus multilocularis*, *Toxocara* spp. and *Toxoplasma gondii*, and may serve as valuable indicators for assessing the occurrence and the level of environmental contamination and infection pressure with free-living stages of these zoonotic parasites. We investigated 658 non-commensal rodents for parasite infections in the canton of Geneva, Switzerland. The prevalence of infection with *E. multilocularis* was highest in *Arvicola terrestris* captured in the north-western area (16·5%, CI: 10·1%–24·8%), possibly reflecting a higher red fox density due to the low incidence of sarcoptic mange in this part of the canton. The exposure rate to *Toxocara* spp. was highest in the urban area (13·2%, CI: 7·9%–20·3%), and may account for higher densities of domestic carnivore and red fox definitive hosts within the city. Exposure to *T. gondii* was widespread (5·0%, CI: 3·2–7·4%), indicating a ubiquitous distribution of infected cat definitive hosts. Interestingly, a widespread distribution of *Taenia taeniaeformis*, a parasite mainly transmitted by cats, was similarly evidenced in *A. terrestris*. Distinct spatial patterns for the different zoonotic parasites likely reflected differences in distribution, abundance, and habitat use of the respective definitive hosts. These results highlight the potential value of rodents as shared indicators for these pathogens.

Key words: *Arvicola terrestris*, *Echinococcus multilocularis*, rodent, *Taenia taeniaeformis*, *Toxocara*, *Toxoplasma gondii*, urban.

INTRODUCTION

Anthropogenic environments favour animal species, such as medium-sized generalist carnivores and rodents, which are able to adapt to or exploit anthropogenic resources. Their populations can reach higher densities than in natural environments due to the great abundance of food resources and shelters (McKinney, 2002). For instance, urban red fox populations (*Vulpes vulpes*) have increased drastically during the last decades and have been reported in major cities of continental Europe (Deplazes *et al*. 2004), with higher densities than in rural environments (Harris, 1981; Contesse *et al*. 2003; Hegglin *et al*. 2007). Similarly, populations of domestic cats and dogs reach higher density in urban than in rural environments (Afonso *et al*. 2006, Calhoon and Haspel, 1989; Matter and Daniels, 2001). The presence of non-commensal rodents in anthropogenic environments is strongly influenced by the density and degree of fragmentation of the vegetation cover (Dickman, 1987; Dickman and Doncaster, 1987). Small mammal species richness may be preserved in urban areas provided a system of small (>0·65 ha) patches of habitats with a well-developed layer of ground vegetation is maintained (Dickman, 1987). Such patches might be present in urban and peri-urban (or residential) areas, e.g., along streams, in natural city parks, gardens and churchyards. For example, non-commensal rodents, including *Apodemus sylvaticus*, *Myodes* (formerly *Clethrionomys*) *glareolus*, and *Microtus agrestis*, were widespread and abundant throughout the city of Oxford, UK, (Dickman and Doncaster, 1987). Likewise, *Arvicola terrestris* were abundant in a city park and in peri-urban areas of the city of Zurich, Switzerland (Stieger *et al*. 2002).

Red foxes, domestic carnivores (dogs and cats), and non-commensal rodents are definitive, intermediate or paratenic hosts for zoonotic parasites of public health significance, i.e., the cestode *Echinococcus multilocularis*, the nematodes *Toxocara canis* and *T. cati*, and the coccidian *Toxoplasma gondii*. Arvicolid rodents (notably *A. terrestris* and *Microtus arvalis*) are the main intermediate hosts of *E. multilocularis* in Europe (Eckert and Deplazes, 2004), while both arvicolid and murid rodents and other animal species are suitable paratenic or intermediate hosts for *Toxocara* spp. and *T. gondii*, respectively (Hill and Dubey, 2002; Despommier, 2003). In contrast, sexual reproduction of these parasites occurs in more restrictive definitive host ranges. Red

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Parasitology (2009), **136**, 329–337.
doi:10.1017/S0031182008005428 Printed in the United Kingdom

foxes are the main definitive hosts of *E. multilocularis* in Europe (Eckert and Deplazes, 2004). Domestic dogs and cats can act as definitive hosts but are not considered important for transmission of the parasite to rodent intermediate hosts because of low prevalence in dogs (Deplazes *et al.* 2004) or low egg excretion in cats (Kapel *et al.* 2006). Canids (mainly domestic dogs and red foxes in Europe) and felids (mainly domestic cats in Europe) are the definitive hosts of *T. canis* and *T. cati*, respectively (Despommier, 2003). Felids (mainly domestic cats in Europe) are the only definitive hosts of *T. gondii* (Hill and Dubey, 2002).

Carnivore definitive hosts are responsible for the contamination of the environment by the parasites' free-living stages, which are infectious for humans, after shedding eggs or oocysts with their faeces. *E. multilocularis*, *T. canis*, *T. cati*, and *T. gondii* infect different definitive hosts. Therefore, the environmental contamination by infectious free-living stages, and thereafter the infection pressure by these parasites, is likely to differ and depend on the distribution, abundance and habitat use of the respective definitive hosts, notably in urbanized areas. High prevalences of infection with *T. canis* in domestic dogs and red foxes in urban areas (O'Lorcain, 1994; Habluetzel *et al.* 2003; Reperant *et al.* 2007), and high prevalences of infection with *E. multilocularis* in red foxes in peri-urban areas (Hofer *et al.* 2000; Fischer *et al.* 2005; Robardet *et al.* 2008) have notably been reported, suggesting high levels of environmental contamination in these environments.

Non-commensal rodent intermediate or paratenic hosts become infected after ingestion of infectious free-living stages of *E. multilocularis*, *Toxocara* spp. or *T. gondii* present in the environment. Because they are shared hosts for zoonotic parasites of carnivores and become infected by free-living stages that are also infectious for humans, we investigated the potential use of non-commensal murid and arvicolid rodents as shared indicators for the occurrence and the level of environmental contamination by these parasites. Contrary to measures of environmental contamination based on the detection of eggs or infectious free-living stages in soil or fecal samples, seroprevalence or prevalence of infection in rodents may represent more direct indicators of the level of infection pressure for humans.

MATERIALS AND METHODS

Study area

The study was carried out in the canton of Geneva, Switzerland. This conurbation is densely populated with 400 000 inhabitants living in an area of 240 km^2. The study area was subdivided into three zones of increasing level of urbanization according to human density, as described by Fischer *et al.* (2005). Rapidly, the categorization of zones was based on a grid of 100 m^2 and an adaptive Kernel method was used to smooth the shapes of the three areas. They are subsequently referred to as rural (51% of total area; human population of 0–40 inhabitants per km^2), peri-urban (26%; 40–220 inhabitants per km^2) and urban areas (23%; up to 3790 inhabitants per km^2). Additionally, two geographical areas were distinguished. The north-western area, located north of the Rhone river, presented a low incidence of sarcoptic mange in the red fox population during the entire study period (C. Fischer, personal communication). The south-eastern area, located south and east of the Rhone river, experienced in contrast a severe epidemic of sarcoptic mange, which affected the red fox population of the westernmost part of the study area since 1997, and over the entire area, since the beginning of 2003. The red fox population of the westernmost part of the study area decreased from 4·6 to 5·6 individuals per km^2 in 1997 to 0·5 to 1·3 individuals in 2001 (C. Fischer, personal communication).

Rodent samples

Rodents were trapped in 15 trapping sites located in the three urban areas (five sites per area), of which six were located in the north-western and nine in the south-eastern geographical area (Fig. 1). Trapping sites presented both open and covered habitats. Open habitats were characterized by areas of >300 m^2 of short grass (up to 10 cm high); covered habitats were characterized by the presence of light to dense undergrowth over an area of >300 m^2. Trapping sites were selected on the presence of signs and tracks of targeted rodent species, i.e., earth monticules characteristic of *Arvicola terrestris*; holes and grass tracks characteristic of *Microtus* spp.; holes and other signs characteristic of the presence of *Myodes glareolus* (formely *Clethrionomys glareolus*), and/or *Apodemus* spp. The presence of *A. terrestris* and rodent species of covered habitats was detected over the entire study area. In contrast, few sites presented signs of the presence of *Microtus* spp., suggesting low population densities over the study area.

We trapped 466 *Arvicola terrestris* and 35 *Microtus arvalis* in autumn 2003 and spring 2004, using Topcat traps (TOPCAT Gmbh, Wintersingen, CH), which are highly selective for *A. terrestris* and *Microtus* spp. We trapped 58 *Myodes glareolus*, and 99 *Apodemus flavicollis* in autumn 2003, using Longworth traps (Penlon Ltd, Oxon, GB), which are highly selective for rodent species of covered habitats (Table 1). Identification of rodent species was done following Brohmer (1988). Rodents were frozen at -20 °C until dissection. Prior to dissection, rodents were measured, weighed and sexed.

Because the *A. terrestris* sample was the most suited for statistical analyses, (sero)prevalences of

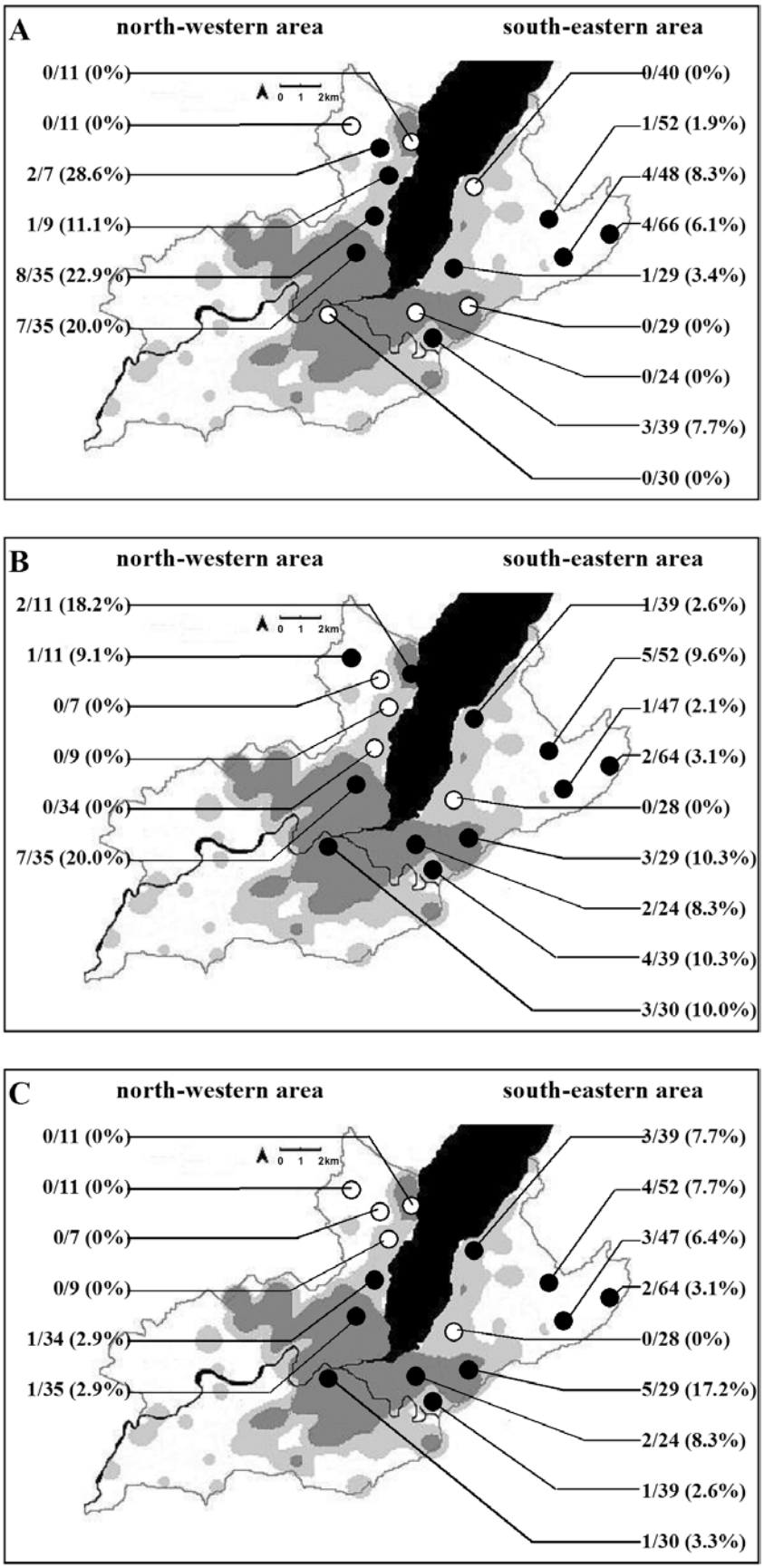

Fig. 1. Prevalence of infection with *Echinococcus multilocularis*, and seroprevalence of *Toxocara* spp. and *Toxoplasma gondii* in *Arvicola terrestris* from 15 trapping sites in the canton of Geneva, Switzerland. Given are the number of positives/total number of investigated animals and prevalence in parenthesis. Black circles: positive sites; white circles: negative sites for given parasite. Black: lake and rivers; dark grey: urban area; light grey: peri-urban area; white: rural area. (A) Prevalence of infection with *E. multilocularis*. (B) Seroprevalence of *Toxocara* spp. (C) Seroprevalence of *T. gondii*.

Table 1. Species of rodents captured in the canton of Geneva, Switzerland in autumn 2003 and spring 2004

		Total	Rural area	Peri-urban area	Urban area	North-western area	South-eastern area
Autumn 2003	*Arvicola terrestris*	236	99	53	84	73	163
	Microtus arvalis	10	10	0	0	7	3
	Myodes (*Clethrionomys*) *glareolus*	58	51	5	2	21	37
	Apodemus flavicollis	99	44	26	29	47	52
Spring 2004	*Arvicola terrestris*	230	85	100	45	36	194
	Microtus arvalis	25	15	4	6	12	13

parasites and their variations were analysed in this species only and for mid- to highly abundant parasites. Two age classes (subadult/adult) were determined based on weight, body length and development of sexual organs. Adult *A. terrestris* were defined as individuals of >60 g and >120 mm as described in detail (Stieger *et al.* 2002).

Parasitological and serological examinations

Careful examination was performed at the opening of thoracic and peritoneal cavities, and organs, in particular the liver, were attentively examined for lesions. Metacestodes were collected and identified after morphological characteristics and number, shape and length of rostellar hooks. If morphological identification was not possible, liver lesions were squashed and digested using proteinase K, and *E. multilocularis* infections were diagnosed using PCR as described (Stieger *et al.* 2002). *E. multilocularis* cysts containing protoscoleces were cut into small pieces, squashed and washed with PBS through a sieve of 1 mm mesh size. Flow-through material was investigated under light microscopy and protoscoleces were counted over the entire sample if <100 were present. Their numbers were otherwise estimated from three subsamples of 100 μl.

Transudate was collected from the thoracic cavity and pooled with coagulated blood from the heart, centrifuged for 5 min at 1000 g and the supernatant (further referred to as serum) was frozen until use. Enzyme-linked immunosorbent assays (ELISAs) were performed as described (Deplazes *et al.* 2005) with the following specifications. *T. canis* excretory/secretory antigen (TcE/S-Ag) was prepared following the method described by Speiser and Gottstein (1984), and a commercially available *Toxoplasma gondii* tachyzoite antigen preparation (TgT-Ag, Institute Virion Ltd, Rüschlikon, Switzerland) was used. Optimal antigen concentrations were determined by titration experiments in 0·1 M carbonate/bicarbonate coating buffer (pH 9·6) containing 0·02% NaN_3 (TcE/S-Ag diluted 1 : 1000, TgT-Ag 4 mg/ml). Serum samples were tested in a 1 : 200 dilution in phosphate-buffered saline (pH 7·2) containing 0·02% NaN_3, 0·05% bovine haemoglobin and 0·2% (v/v) Tween-20 (PBS-T). As conjugate, goat anti-mouse polyvalent antibodies linked to alkaline phosphatase (Sigma) were used in a dilution of 1 : 500 in PBS-T, and the substrate was a 1 mg/ml solution of p-nitrophenyl phosphate (Sigma) in 0·05 M carbonate/bicarbonate buffer (pH 9·8) containing 1 mM $MgCl_2$. Reactions were stopped after 10 min at $+37$ °C by addition of 50 μl per well of 3 M NaOH. Absorbance values were read at 405 nm (OD_{405}). Positive control sera of mice experimentally infected with *T. gondii* or *T. canis* and negative control sera of helminth-free mice were included in all test runs.

Specificity of the TcE/S-Ag in ELISA has been reported to be high in mice and no cross-reactions were observed with other experimental larval ascarid infections such as *Toxascaris leonina* or *Ascaris suum* (Cuéllar *et al.* 1995). A previous validation of this antigen in our laboratory using sera of 2 groups of 6 *Microtus arvalis* experimentally infected with 100 embryonated eggs of *T. canis* and *T. cati*, respectively, resulted in strong antibody reactions (63 days post-inoculation) in both groups, and proved a good sensitivity for both nematode species (data not presented). Validation of the TgT-Ag-ELISA was performed with 22 seropositive and 31 seronegative *A. terrestris* from another study by performing a *T. gondii* specific PCR (Müller *et al.* 1996) after DNA preparation (Qiaamp DNA mini kit, Qiagen, Hilden, Germany) of half of the brain. One of the seronegative samples (3%) and 14 of the 22 seropositive samples (64%) yielded *T. gondii* PCR positive results. The specificity of the PCR used was determined to be 100% (Müller *et al.* 1996), however, its sensitivity for the detection of *T. gondii*-DNA in tissue samples of infected animals was rather low in previous investigations (Wyss *et al.* 2000). Therefore, PCR confirmation of 14 out of a total of 22 seropositive animals and only 1 PCR positive reaction in 31 seronegative animals confirm the good concordance between the two tests. Cut-off values for both tests were calculated as described (Greiner *et al.* 1994).

Table 2. Number of trapping sites and rodents found positive for metacestodes, *Toxocara* spp. and *Toxoplasma gondii* in the canton of Geneva, Switzerland

	Parasite identification						Serology		
	N	*Echinococcus multilocularis**	*Taenia taeniaeformis*	*Taenia crassiceps*	*Taenia martis*	*Mesocestoides spp.*	*N***	*Toxocara* spp.	*Toxoplasma gondii*
Trapping sites	15	9	15	7	2	1	15	12	11
Arvicola terrestris	466	31	153	12	0	0	460	31	23
Microtus arvalis	35	3	6	1	0	1	32	3	1
Myodes (*Clethrionomys*) *glareolus*	58	6	3	0	0	0	43	2	0
Apodemus flavicollis	99	0	8	1	2	0	80	4	2

* Identified morphologically or by PCR (Stieger *et al*. 2002).
** Missing samples are due to insufficient quantity of collected serum for 6 *A. terrestris*, 3 *M. arvalis*, 15 *M. glareolus*, and 19 *A. flavicollis*.

Statistical analysis

Statistical analyses were performed with SPSS–PC version 11.5. Factors affecting prevalences were investigated using a backward-stepwise logistic regression procedure based on the likelihood ratio test. Variables included in the initial models were the season (autumn: October–November and spring: April–May), the urbanization area, the geographical area, weight and sex. Results were considered significant when $P<0{\cdot}05$. Exact binomial 95% confidence intervals (CI) were calculated following (Clopper and Pearson, 1934).

RESULTS

Larval cestode infections

Metacestodes of *E. multilocularis* were morphologically identified in 2 adult *A. terrestris* containing 6850 and 30 710 protoscoleces. These two animals were trapped in spring in a peri-urban site located in the north-western geographical area. Additionally, 29 out of 79 *A. terrestris*, 3 out of 4 *M. arvalis* and 6 out of 9 *M. glareolus*, which presented small liver lesions that could not be identified morphologically, were PCR-positive for *E. multilocularis*. None of 99 *A. flavicollis* were infected with *E. multilocularis* (Table 2).

Strobilocerci of *T. taeniaeformis* were morphologically identified in the four rodent species (Table 2). Up to 11 strobilocerci were found in *A. terrestris* and up to 6 in *A. flavicollis*, yet most infected rodents harboured one or few strobilocerci.

Cysticerci of *T. crassiceps*, located in the pleural cavity or subcutaneously, were found occasionally (Table 2). Infected *A. terrestris* were captured in one rural, one peri-urban and three urban trapping sites. Two *A. flavicollis* captured in rural and peri-urban sites were infected in the pleural cavity with two and one specimens of *T. martis*, respectively. Four specimens of *Mesocestoides* spp. were found in the pleural and peritoneal cavities of 1 *M. arvalis* trapped in the urban area (Table 2).

Variations of prevalence of infection with E. multilocularis *in* A. terrestris

A highly significant model was found to explain the variations of prevalence of infection with *E. multilocularis* in *A. terrestris* ($\chi^2=31{\cdot}2$, D.F.$=2$, $P<0{\cdot}0001$). It included the body weight (LR$=22{\cdot}9$, $P<0{\cdot}0001$) and geographical area (LR$=14{\cdot}0$, $P<0{\cdot}0001$) as significant variables (Table 3). Only two subadult *A. terrestris* were found to be infected with *E. multilocularis* (prevalence of 1·3%), whereas prevalence in adults was 9·2%. The level of urbanization of the habitat had no influence on the prevalence of infection with *E. multilocularis* but *A. terrestris* were more frequently infected in the north-western area (prevalence of 16·5%) than in the south-eastern area (3·6%). Local prevalences in *A. terrestris* ranged from 11·1% to 28·6% in the north-western area (Fig. 1A).

Variations of prevalence of infection with T. taeniaeformis *in* A. terrestris

Body weight (LR$=83{\cdot}5$, $P<0{\cdot}0001$) and season (LR$=11{\cdot}5$, $P=0{\cdot}001$) were included in the highly significant model explaining the variations of prevalence of infection with *T. taeniaeformis* ($\chi^2=85{\cdot}8$, D.F.$=2$, $P<0{\cdot}0001$; Table 3). Adult *A. terrestris* were more frequently infected than subadults (prevalence of 44·3% and 9·2%, respectively). Higher *T. taeniaeformis* prevalence was found in autumn (36·0%) than in spring (29·6%), but no spatial variation of prevalence was evidenced. *T. taeniaeformis* infections were found at all trapping sites throughout the study area with local prevalences in *A. terrestris* ranging from 9·1% to 58·3%.

Table 3. Selected models of the backward-stepwise logistic regression procedure explaining the variations of prevalence or seroprevalence of the 4 main parasite infections diagnosed in *Arvicola terrestris* from the canton of Geneva, Switzerland

(OR: odd-ratio; CI 95: 95% confidence interval.)

Variables	Category/ Ref. Category	*Echinococcus multilocularis*		*Taenia taeniaeformis*		*Toxocara* spp.		*Toxoplasma gondii*	
		OR	CI 95	OR	CI 95	OR	CI 95	OR	CI 95
Body weight (g.)		1·040	1·017–1·063	1·052	1·038–1·065	1·040	1·018–1·062	1·032	1·010–1·055
Season	Autumn/Spring	—		2·164	1·369–3·421	—		—	
Sex	Female/Male	—		—		—		—	
Urbanization area	Rural/Urban	—		—		0·321	0·132–0·783	—	
	Peri-urban/Urban	—		—		0·214	0·074–0·623	—	
Geographical area	North-western/ South-eastern	7·353	3·217–16·803	—		—		—	

—, Not significant.

Variations of seroprevalence of Toxocara spp. *in* A. terrestris

Forty rodents tested positive for antibodies against *Toxocara* spp., i.e., 31 of 460 *A. terrestris*, 3 of 32 *M. arvalis*, 2 of 43 *M. glareolus* and 4 of 80 *A. flavicollis* (Table 2). A highly significant model of logistic regression explained the variations of seroprevalence in *A. terrestris* ($\chi^2 = 24{\cdot}1$, D.F. = 3, $P < 0{\cdot}0001$; Table 3). It included the body weight (LR = 15·4, $P < 0{\cdot}0001$) and the urbanization area (LR = 10·7, $P = 0{\cdot}005$) as significant variables. All sero-positive *A. terrestris* were adults. Seroprevalence was higher in the urban area (13·2%) than in the rural and peri-urban areas (4·9% and 3·3%, respectively). Sero-positive *A. terrestris* were found in all five urban sites, with local prevalences ranging from 8·3% to 20·0% (Fig. 1B).

Variations of seroprevalence of T. gondii *in* A. terrestris

In total, 26 rodents were found sero-positive for antibodies against *T. gondii*, i.e., 23 of 460 *A. terrestris*, 1 of 32 *M. arvalis* and 2 of 80 *A. flavicollis* (Table 2). The significant model explaining the variations of seroprevalence of *T. gondii* in *A. terrestris* ($\chi^2 = 8{\cdot}8$, D.F. = 1, $P = 0{\cdot}003$) included the body weight as the only significant variable (LR = 8·8, D.F. = 1, $P = 0{\cdot}003$; Table 3). Seroprevalence was 6·4% in adults and 2·1% in subadults. Infected *A. terrestris* were trapped in rural, peri-urban and urban sites, with local prevalences ranging from 2·9% to 17·2% (Fig. 1C).

DISCUSSION

Rodent species may serve as valuable shared indicators for assessing the occurrence and the level of environmental contamination and infection pressure with free-living stages of zoonotic parasites of carnivores. However, the variability in the abundance and distribution of the various species of rodents, and in their infection rates makes the sampling design of critical importance. Due to time and logistical constraint, 15 sites were selected over the study area. It allowed for the detection of spatial trends in the occurrence and sero-prevalence of the investigated parasites, with regards to geographical areas and urbanization level of the environment. The selection of the trapping sites in rural, peri-urban and urban areas was based on the detection of the presence of rodent species of both open and covered habitats in order to maximize the trapping success of several rodent species occurring over a wide habitat range. Thus, four species of non-commensal rodents were trapped in the canton of Geneva, and *Arvicola terrestris*, an arvicolid rodent susceptible to infection with *Echinococcus multilocularis*, *Toxocara* spp., and *Toxoplasma gondii*, was trapped in high numbers throughout the canton, in both rural and urbanized areas. This species therefore appears suitable for surveillance studies of these zoonotic parasites in a wide range of habitats. Few *Microtus arvalis* were trapped over the entire study area. The rare presence of signs characteristic of *Microtus* spp. suggested low population densities over the study area. *A. terrestris* has been shown to be red foxes' main prey in western Switzerland (Weber and Aubry, 1993). It therefore may be the most important intermediate host of *E. multilocularis* in this region.

A higher prevalence of infection with *E. multilocularis* was found in *A. terrestris* trapped in the north-western area. This may be explained by a significant decrease in red fox density in the south-eastern area at the time of the study, caused by an epizootic of sarcoptic mange affecting, since early 2003, the entire area located south of the Rhone river (C. Fischer, personal communication). Sarcoptic mange is fatal in wild carnivores and short-term

mortality rates may reach up to 90% in affected red fox populations (Pence and Ueckermann, 2002). Thus, red fox densities in the westernmost part of the study area, which was affected by the epidemic of sarcoptic mange since 1997, decreased by up to 10-fold. Variations in the level of environmental contamination with eggs of *E. multilocularis* are associated with variations in the density of fox faeces, which is influenced by fox density and social structure (Pleydell *et al*. 2004). A reduction in the number of red foxes has notably been shown to reduce the level of environmental contamination by eggs of *E. multilocularis* (Raoul *et al*. 2003). Interestingly, a cluster of infection with the nematode *Capillaria hepatica* in *A. flavicollis*, *M. glareolus* and *A. terrestris* has also been reported in the north-western area (Reperant and Deplazes, 2005), and may also be associated with a higher red fox density. *C. hepatica* develops in the hepatic parenchyma of rodents mainly, and carnivore species are believed to amplify its cycle, as infectious eggs are liberated in their faeces upon digestion of infected rodents (Childs *et al*. 1988).

High prevalences of infection with *E. multilocularis* were found in urbanized areas north of the Rhone River, demonstrating a significant infection pressure in these environments. Prevalences of infection with *E. multilocularis* were similarly found high in red foxes from peri-urban areas of the canton of Geneva (Fischer *et al*. 2005; Reperant *et al*. 2007) and in red foxes and arvicolid rodents from peri-urban areas in Zurich (Hofer *et al*. 2000; Stieger *et al*. 2002). The risk of infection for humans is thus perceived to be maximal in environments at the interface of rural and urban areas (Deplazes *et al*. 2004). The permanent presence of *E. multilocularis* in urbanized areas may lead to major changes in the epidemiology of the disease in humans (Vuitton *et al*. 2003). Importantly, the density of *A. terrestris* has been found to be a risk factor for infection with *E. multilocularis* in humans (Viel *et al*. 1999), and the occurrence of this rodent up to urbanized areas may contribute to the establishment of the parasite in cities and residential areas. The presence of suitable habitats for rodent intermediate hosts appears indeed to be primordial in the transmission dynamics of *E. multilocularis* in Europe and China (Danson *et al*. 2006). Over the present study area, *A. terrestris* infected with the parasite were trapped in only 1 out of 5 urban sites, which is in accordance with lower prevalence of infection with *E. multilocularis* in urban red foxes in Geneva (Fischer *et al*. 2005; Reperant *et al*. 2007).

High prevalence of infection with *E. multilocularis* was also found in *M. glareolus*, which is an arvicolid rodent occurring preferentially in covered habitats, such as bushes, hedges and woods. Prevalences of infection with *E. multilocularis* in this species were typically lower in other studies (Loos-Frank and Zeyhle, 1982; Bonnin *et al*. 1986; Stieger *et al*. 2002). Our findings suggest that red foxes may intensively use wooded areas of the canton of Geneva, resulting in a significant environmental contamination with eggs of *E. multilocularis*. The risk of infection for humans in these areas intensively used for leisure activities may thus be significant.

The seroprevalence of *Toxocara* spp. in *A. terrestris* increased significantly with the level of urbanization, suggesting a high infection pressure by these nematodes in urban areas. Soil contamination with *Toxocara* eggs has been found higher in urban than in rural areas (Mizgajska, 1997, 2001; Giacometti *et al*. 2000; Dubna *et al*. 2007). High densities of wild and domestic carnivores within cities, in particular in green areas where establishment of rodent prey populations is possible, are likely to result in high focal levels of environmental contamination in urban areas (Mizgajska, 1997, 2001). High prevalences of infection with *T. canis* in red foxes have notably been reported in the city of Geneva (Reperant *et al*. 2007). However, no differences in fox prevalence rates were detected between rural, peri-urban, and urban areas, further suggesting that domestic carnivores may contribute to environmental contamination in urban areas. Few studies have investigated the natural exposure of populations of wild rodents to *Toxocara* spp. by a serological method comparable to that used here, and reported likewise higher seroprevalences in urban or peri-urban rodent populations (Dubinsky *et al*. 1995; Antolova *et al*. 2004). Serological surveys of rodents, e.g., *A. terrestris*, may thus represent another tool for monitoring the environmental contamination and infection pressure by *Toxocara* spp. up to most urbanized areas.

A ubiquitous occurrence of *T. gondii* and of the cestode *Taenia taeniaeformis* was found as determined by seroprevalence and prevalence of infection in *A. terrestris*, respectively. No spatial trend could be detected for these two parasites which appeared widely distributed throughout rural, peri-urban and urban parts of the canton of Geneva. The cestode *T. taeniaeformis* develops to its patent stage nearly exclusively in the cat whereas foxes or dogs are rarely infected with non-reproductive stages (P. Deplazes, unpublished observations). Patent infections with excretion of *T. gondii* oocysts occur strictly in felids (Hill and Dubey, 2002), and therefore cats only are responsible for environmental contamination with oocysts in the area investigated. These results suggest a ubiquitous spatial distribution of infected domestic cats, up to most urbanized environments. It has been proposed that infection rates of *T. gondii* in cats reflects infection rates in prey, notably rodents (Hill and Dubey, 2002). Because no practical methods for the sensitive detection and isolation of oocysts from environmental samples have yet been implemented (Dumetre and Darde, 2003), seroprevalence of *T. gondii* in rodent intermediate

hosts may represent a most valuable tool for the monitoring of the level of environmental contamination and infection pressure by this coccidian (Aramini *et al*. 1999).

We are grateful to the Natural History Museum of Chur, Graubünden for providing the Longworth traps, and to V. Bersot and F. Rohr for their help with trapping. We thank L. Kohler for her help with the laboratory work, and A. Mathis and J.-M. Weber for fruitful discussions. We acknowledge the support of our research activities by the Swiss Federal Office for Education and Science and the European Commission (Project Echinorisk, Contract QLK2-CT-2001-01995/BBW Nr. 00.0586-2) and by the Service des Forêts, de la Protection de la Nature et du Paysage (SFPNP) and the Service Vétérinaire of the canton of Geneva.

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