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Fox defecation behaviour in relation to spatial distribution of voles in an urbanised area: An increasing risk of transmission of *Echinococcus multilocularis*?

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ABSTRACT

Urbanisation of alveolar echinococcosis is a new phenomenon that has been highlighted during the last few decades. It has thus become necessary to understand the dynamics of transmission of *Echinococcus multilocularis* in urbanised areas. Spatial heterogeneity of infection by *E. multilocularis* has been explained as the result of a multifactorial dependence of the transmission in which the factors depend on the scale of the investigation. The aim of this study was to assess, in an urbanised area, the effect of such environmental factors as season, habitat type and the level of urbanisation, on the availability of two major intermediate hosts (*Microtus* spp. and *Arvicola terrestris*), the distribution of red fox faeces and the distribution of *E. multilocularis* as determined by detection of coproantigens in faeces. Results of the study revealed higher densities of *Microtus* spp. in rural than in peri-urban areas. Moreover this species was highly aggregated in urban wasteland. *Arvicola terrestris* densities did not appear to be linked to the level of urbanisation or to the type of habitat studied. Distribution of faeces was positively linked to distance walked and to *Microtus* spp. and *A. terrestris* distributions whatever the level of urbanisation. Such a distribution pattern could enhance the transmission cycle in urban areas. The Copro-ELISA test results on faeces collected in the field revealed that ODs were significantly negatively correlated with the abundance of *A. terrestris*. The larger population densities of *Microtus* spp. found in urban wastelands and the well known predominance of *Microtus* spp. in the red fox diet in the region suggest that *Microtus* spp. may play a key role in urban transmission of the parasite in the study area.

1. Introduction

The general increase in human populations and their increased migration from rural to urban areas during the last half of the 20th century has led to the development of an urban infrastructure (Shochat et al., 2006). Urbanisation implies modification of landscape (Miller and Hobbs, 2002) and leads to reduced biodiversity by promoting higher densities of species adapted to human environments (McKinney, 2006). Such ecological changes are likely to modify parasite transmission patterns. The emergence of pathogens, especially in areas with a high human density, could increase the risk of transmission to humans. The study of the adaptation of zoonoses in urban environments is therefore of great interest to public health administrators.

The red fox (*Vulpes vulpes*) with its highly flexible behaviour is observed in many European cities at high densities (Gloor et al., 2001; Deplazes et al., 2004). This expansion to urban areas seems to be related to the general increase in red fox populations in Europe during the last two decades (Romig et al., 1999) and some authors suggest that this phenomenon is a result of successful campaigns of oral vaccination against rabies (Breitenmoser et al., 2000; Chautan et al., 2000). In such a context, the urbanisation of the red fox highlights the increased potential risk of transmission of zoonoses to humans, particularly alveolar echinococcosis (AE). This lethal disease is caused by the tapeworm *Echinococcus multilocularis* in its cystic larval stage (Deplazes et al., 2004). The life cycle of the parasite involves carnivores as definitive hosts and several species of small mammals as intermediate hosts. Among the European species, the arvicoline sub-family (genera *Arvicola*, *Microtus* and *Myodes*) has the most susceptible hosts (Eckert and Deplazes, 2004). Because the transmission cycle of the parasite consists of three stages (adult/definitive host, larva/intermediate host and a free stage in the environment), the efficiency of transmission is modulated by many factors which themselves depend on each host species.

One of the factors affecting the efficiency of the transmission cycle of *E. multilocularis* is the intensity of the predator–prey relationship (Raoul et al., 2003; Guislain et al., 2008). Changes in the diet of foxes appear to be related to changes in the population

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density of prey in the field and studies have shown that dramatic increases in rodent populations were accompanied by an increased occurrence of these species in the diet of red fox (Weber and Aubry, 1993; Stien et al., 2010; Raoul et al., in press). Moreover, the availability of rodents is linked to the composition and structure of the landscape that influences the population dynamics of vole intermediate hosts (Delattre et al., 1992, 1996; Giraudoux et al., 1997, 2003). The latest study undertaken in Zürich revealed that the availability of *Arvicola terrestris* to the red fox was lower in meadows and pastures of urban areas than in those of the suburbs of the city (Hegglin et al., 2007). The density variation was related to a decrease in the consumption of this species by the red fox and to a decrease in the prevalence of the parasite in foxes. Thus, the lower predation of red fox on voles remains a possible explanation of the decreasing "rural to urban" infection gradient. The characterisation of urban areas with high densities of intermediate hosts is a basis for understanding the transmission dynamics of the parasite in an urbanised environment and for determining a strategy for controlling the intermediate host population.

Another aspect that significantly modulates the transmission dynamics of the parasite is the probability of infection of the intermediate hosts. The infection of intermediate and definitive hosts of *E. multilocularis* is spatially very heterogeneous. The prevalence among intermediate hosts on a regional scale is generally low, around 0.1% (Giraudoux et al., 2002). However on a local scale, micro-sites of infection have been reported with a prevalence of 10% in rural areas (Delattre et al., 1988, 1990a; Giraudoux et al., 2002) and up to 14% in Zürich (Hofer et al., 2000) and 28.6% in Geneva (Reperant et al., 2009) for arvicolids. Ecological studies have shown that a local micro-site of infection is produced by a high density of faeces, protection of eggs against drying and high densities of hosts (Giraudoux et al., 2002).

Due to the multifactorial context of parasite transmission efficiency, the purpose of this study was first to examine the relationship between the distribution of fox faeces and of rodents along an urbanisation gradient, in order to highlight the transmission of the parasite between the external environment and its intermediate hosts, *Microtus* spp. and *A. terrestris*. To our knowledge, no study has previously been conducted on the distribution of faeces and rodents along a gradient of urbanisation even though they are key parameters for parasite transmission efficiency.

The prevalence of *E. multilocularis* in the environment can be measured by detecting coproantigens with ELISA in fox faeces (Raoul et al., 2001). Such investigations in urban areas have shown an increase in the infection of urban to peri-urban environments (Stieger et al., 2002). However, the environmental characteristics of contaminated areas were not documented. In order to identify the risk of the parasite occupying an urban environment, it is of fundamental importance to clarify the influence of environmental variables, such as habitat, season and level of urbanisation on the prevalence of infection in faeces deposited in the field by red foxes. This study thus intends to evaluate the link between populations of *Microtus* spp. and *A. terrestris* and the prevalence of infection in fox faeces. The results could identify the risk of exposure to the parasite of the two vole populations which would then enable a more precise strategy for controlling the most important species in the urban life cycle of the parasite.

The aim of the study was to characterise the impact of environmental factors including urbanisation on three factors that are important for *E. multilocularis* transmission: (i) the densities of *Microtus* and *Arvicola* intermediate hosts, (ii) the densities of red fox faeces and (iii) the prevalence of infection in foxes. Finally, the study sought to determine whether there is a link between populations of intermediate hosts and fox faeces density in order to identify the intermediate host populations most likely to be infected in urbanised areas.

2. Materials and methods

2.1. Study site and habitat selection

The study was conducted from winter 2004–2005 to autumn 2006 in the urban agglomeration of Nancy (48° 41′ 36.96″ N, 6° 11′ 4.56″ E), in north-eastern France, an endemic area for *E. multilocularis* since 1987 (Aubert et al., 1987). The agglomeration is at an elevation ranging between 187 and 420 m above sea level. This large city of 263,000 inhabitants (INSEE, 2006, http://www.insee.fr/) is established between an agricultural landscape on its eastern side and a forest landscape on its western side. In order to assess the impact of urbanisation on densities of rodents and of faeces of red foxes, the 230 km^2 study area was divided into urban, peri-urban and rural areas (62, 75 and 93 km^2, respectively). The urban area was defined as a continuous built-up area including some recreational areas such as parks, gardens and small fields. The peri-urban area was a 1 km large buffer zone around the urban area. The landscape of the peri-urban area was composed of a mixture of recreational and agricultural areas with pastures, meadows and woodland while the rural land was principally farmland. Pasture was defined as a field used for grazing by livestock and meadows as fields used for growing hay. On the same study site, a previous study had revealed a prevalence of *E. multilocularis* in foxes of 54% in rural areas, 31% in peri-urban areas and of 4% in urban areas (Robardet et al., 2008). A total of 68 transects corresponding to different habitats were selected in different areas (Table 1 and Fig. 1). General transect size ranged from 180 to 1157 m with a mean of 446 m and a standard deviation of 170 m. All transects, defined by a start and end geo-referenced point, were sampled every season excluding summer (winter: from December to February; spring: from March to May; autumn: from September to November) in 2005 and in 2006, except for those in pastures and meadows which were sampled in 2006 only.

2.2. Estimation of the relative density of voles and red fox faeces

The relative density of voles was estimated with an index method calibrated against trapping methods in France for *Microtus arvalis* (Delattre et al., 1990b; Quere et al., 2000) and for *A. terrestris* (Giraudoux et al., 1995). This method enables determination of the relative density of the two principal intermediate hosts involved in the transmission of *E. multilocularis* in Europe. Since *M. arvalis* and *Microtus agrestis* were sympatric in our study area, we were not able to differentiate their individual indices; they were thus referred to collectively as *Microtus* spp. The indices revealing the presence of *Microtus* spp. and of *A. terrestris* were identified while walking along the longest diagonal of each transect (Table 1 and Fig. 1). Observation was restricted to a 2 m wide band and the diagonal was divided into 10 m intervals delimited with the aid of a manual Global Positioning System (GPS); the presence or absence of voles was then checked every 10 m. The indices of presence were defined as runways in vegetation and burrow entries associated with fresh faeces or recent vegetation consumption for *Microtus* spp. (Delattre et al., 1990b) and fresh earth tumuli for *A. terrestris* (Giraudoux et al., 2005). In this study area, a trapping session revealed that *M. agrestis* was much less abundant than *M. arvalis* with, respectively, two and 45 trapped animals.

On the same line transects, fox faeces were identified by shape and odour. They were safely and carefully transferred into a plastic tube using a single-use wood spatula, with mask and gloves being worn to prevent the risk of infection. Each sample location was recorded using a GPS. The transects were covered once each season from the winter of 2005 to the autumn of 2006. Rodent and fox faeces density was not estimated in summer as the vegetation was too high to allow appropriate visibility of the rodent indices.