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Short communication

Echinococcus multilocularis and *Toxocara canis* in urban red foxes (*Vulpes vulpes*) in Brussels, Belgium

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Abstract

During the last decades, European red foxes (*Vulpes vulpes*) have been implicated in the transmission of several viral or parasitic pathogenic agents to domestic animals and humans. In urban areas, risks of zoonoses transmission are likely to increase as a result of a higher rate of intra- and inter-species contacts. Foxes occur on 35% of the Brussels-Capital Region area and local densities reach up to 4 family groups per square kilometre. According to the directive 2003/99/ECC, a first survey for the presence in foxes of *Echinococcus multilocularis* and *Toxocara canis* was conducted in Brussels from 2001 to 2004. None of 160 foxes were found to be infected with *E. multilocularis* and 24 of 134 foxes were found to be infected with *T. canis*. Considering numbers of examined foxes, the sensitivity and the specificity of tests used for diagnosis, the 95% credibility intervals for the true prevalence of *E. multilocularis* and *T. canis* were estimated in a Bayesian

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framework to be 0 to 1.87% (median value of 0%) and 12.7 to 26% (median value of 18.7%), respectively. For *T. canis*, a significantly higher risk to be a carrier occurs in cubs and a significantly lower risk in adults.

Keywords: Red fox; Urban; Zoonosis; *Echinococcus multilocularis*; *Toxocara canis*; Bayesian approach

1. Introduction

An increasing number of studies showed that the red fox *Vulpes vulpes* (Linnaeus, 1758) has entered and colonised several large cities in continental Europe, North America and Australia (Artois, 1989; Gloor et al., 2001). The fox colonisation of Brussels goes back to the 1980s (Brochier, 1990). A recent study has shown that foxes occurred in 35% of the Brussels-Capital Region (BCR) area and that densities ranged in function of the habitat from 0.6 up to 4 fox family groups per square kilometre (De Blander et al., 2006).

During last decades, red foxes have been implicated in the transmission of viral or parasitic pathogenic agents of public health concern and veterinary significance. In some countries of continental Europe, the red fox acts as reservoir of rabies virus, *Echinococcus multilocularis* and *Toxocara canis* in humans, respectively, responsible for rabies, human alveolar echinococcosis (AE) and toxocariasis *larva migrans*. In urban and suburban areas, risks of zoonoses transmission are likely to increase as a result of a higher rate of intra- and inter-species contacts (close contact between dense populations of foxes, pets and humans).

A successful program of fox vaccination resulted in the complete elimination of rabies from Belgium (Brochier et al., 2001). With regards to the current epidemiological situation in Western Europe and the fact that Brussels is situated north of Meuse-Sambre valley, where rabies has never been detected since active surveillance began in 1966, the risk of rabies spread in Brussels is presently not considered.

In Europe, the life-cycle of *E. multilocularis* (*Cestoda*, *Taeniidae*) is predominantly sylvatic, i.e. involving wild carnivores (mainly foxes of the genera *Vulpes* and *Alopex*) as final hosts and several species of rodents, mainly water vole (*Arvicola terrestris*), common vole (*Microtus arvalis*) and muskrat (*Ondatra zibethicus*) as prey/intermediate hosts (Eckert et al., 2001a).

Larval stages of *E. multilocularis* are responsible for AE, a rare but very severe liver condition in humans (Eckert and Deplazes, 1999). Recent studies have shown that *E. multilocularis* has a wider geographical range than was previously thought. At present, the known distribution of the parasite in Europe includes regions in 14 countries (Eckert and Deplazes, 2004; Deplazes et al., 2004; Hanosset et al., 2004). The prevalence of infection in foxes shows a wide variability between countries and in endemic areas, ranging from below 1 to more than 60% (Eckert and Deplazes, 1999).

In Belgium, the carriage of *E. multilocularis* by foxes was reported for the first time in 1991 (Brochier et al., 1992). Since then, further surveys conducted in foxes from rural areas of the whole country reported a high prevalence (33%) on the south-eastern high plateau of Ardennes and revealed a decreasing north–west gradient in prevalence (Losson et al., 1997,

2003; Vervaeke et al., 2003). In the north-western low land, where the altitude rarely exceeds 100 m, the prevalence of *E. multilocularis* was the lowest: 1.7% in Flanders and 1.6% on the low plateau of Hesbaye. This could be related to the milder climatic conditions and the different nature and utilization of the soil that might be less suitable for the maintenance of the cestode life-cycle.

Humans are at risk of infection in all areas where the cestode occurs in foxes, since these are responsible for contaminating the environment with infective eggs. Cats and dogs are also considered to be a possible source of infection for human beings, although the prevalence in these hosts is lower than in foxes (Eckert and Deplazes, 1999). Since 1999, eight human cases of AE were diagnosed in Belgium, the infection being most probably acquired locally (Delbecque et al., 2002).

In Europe, the occurrence of *E. multilocularis* in urban foxes was recently reported in several cities such as Zürich (Hofer et al., 2000), Geneva (Fischer, 2003), Copenhagen (Kapel and Saeed, 2000) and Stuttgart (Romig et al., 1999).

The life-cycle of the ascarid nematode *T. canis* is complex, involving direct, transplacental and transcolostral transmission. The complete development of this nematode can only occur in canid hosts such as dogs and foxes. Development is restricted to the second-stage larvae in paratenic hosts such as rodents and humans. Although the biology of *T. canis* has been more extensively studied in domestic dogs, numerous surveys were conducted in foxes from rural areas of several European countries. Less documented prevalence data of *T. canis* in urban foxes were reported from large European cities such as Berlin (Schöffel et al., 1991), Dublin (Wolfe et al., 2001), Bristol (Richards et al., 1993, 1995) and Zürich (Hofer et al., 2000).

These surveys provide evidence that urban foxes can act as a wild reservoir of *T. canis*, and may represent a source of infection for domestic pets. In addition, the environmental contamination with eggs (soil of gardens and public parks) poses a potential health threat for humans, particularly children (Glickman and Schantz, 1981).

So far, the occurrence of *E. multilocularis* and *T. canis* in urban fox populations has not been investigated in Belgium. According to the directive 2003/99/ECC (European Parliament and Council, 2003), the aim of the present study was to survey these zoonotic parasites in foxes from Brussels.

2. Materials and methods

The Brussels-Capital Region is an agglomeration of 161 km^2, including 960,000 inhabitants and is one of the three Regions of the federal Belgian State. The study area reflected the range of fox distribution in Brussels and had a surface area of approximately 56 km^2 (De Blander et al., 2006).

Road and rail killed foxes were sampled from the study area from January 2000 onwards for *E. multilocularis* ($N = 160$) and from October 2000 onwards for *T. canis* ($N = 134$). Both samplings were finished in April 2004. The animals were individually labelled with an identification number and information about the exact locality, date and cause of death.

Regarding the variables ‘‘space’’ (place of origin), ‘‘age’’ (adult versus juvenile) and ‘‘season’’ (spring-summer versus fall-winter), the sample, as a whole, was homogeneously

distributed. Specimens were collected from all parts of the study area. Taking into account the sampling period and the size of the study area, the mean fox sample density was 0.87/km^2/year.

In line with previous studies, cubs were assumed to be born on April first.

Adults, sub-adults and cubs were distinguished either by visual examination of teeth (cubs aged 1–6 months) or by measuring the relative width of the pulp cavity of a lower canine tooth by X-rays (sub-adults aged 6–12 months, adults older than 12 months of age).

The age of 146/160 collected foxes could be determined. Fifty-five percent (81/146) were adults and amongst juveniles 23 (34/146) and 22% (31/146) were cubs and sub-adults, respectively (Table 1).

Forty-six percent (73/160) of foxes were collected during the spring and summer months and 54% (87/160) during the fall and winter months.

According to the framework of a national programme of rabies surveillance, collected foxes were also submitted to the reference laboratory diagnosis and attested negative.

At necropsy, the small intestine was isolated and ligatured at both ends, wrapped in plastic bags and frozen at −80 °C until examination at least 3 days in order to inactivate the infective material (Eckert et al., 2001b). The intestinal scraping technique (IST) (Eckert et al., 2001b) was used to detect both intestinal helminths. The identification of *E. multilocularis* was performed according to the size and the appearance of the uterus of the last mature segment. During each analysis session, small intestines from positive foxes collected in the highly endemic area of Belgium were used as positive controls. The absence of the *E. multilocularis* was confirmed in 20 foxes by using the more sensitive technique of sedimentation and counting (SCT) (Eckert et al., 2001b). *T. canis* was identified by size and morphology following Soulsby (1982).

Echinoccocus multilocularis allows an unequivocal diagnosis in most cases (Eckert et al., 2001b). For the detection of *E. multilocularis*, we selected a Beta (alpha = 1000, beta = 10) distribution (2.5th percentile = 0.983; median = 0.99; 97.5th percentile = 0.995) as prior information for the specificity and a Beta (alpha = 69, beta = 103) distribution (2.5th percentile = 0.329; median = 0.40; 97.5th percentile = 0.475) as prior information for the sensitivity of the IST (Hofer et al., 2000). For the detection of *T. canis*, the

Table 1
Prevalence of *Echinococcus multilocularis* and *Toxocara canis* carriage in urban foxes in Brussels

Age	*Echinococcus multilocularis*			*Toxocara canis*		
	Number of animals examined	Number of positive animals	Apparent prevalence % (95% CI[a])	Number of animals examined	Number of positive animals	Apparent prevalence % (95% CI)
Adults	81	0	0 (0–3.6)	69	4	5.8 (1.6–14.2)
Juveniles	65	0	0 (0–4.5)	54	18	33 (21.1–47.5)
Cubs	*34*	*0*	*0 (0–8.4)*	*25*	*10*	*40 (21.1–61.3)*
Sub-adults	*31*	*0*	*0 (0–9.2)*	*29*	*8*	*28 (12.7–47.2)*
Age undetermined	14	0	0 (0–19.3)	11	2	18 (2.3–51.8)
Total	160	0	0 (0–1.85)	134	24	18 (11.8–25.5)

[a] The 95% confidence interval (binomial exact).

specificity of the applied test is fixed to be equal to 1 as the microscopic differentiation of *T. canis* from *Toxascaris leonina* is easy to perform and very reliable (Gasser et al., 2006). The sensitivity of the applied test was estimated to be at least 0.95 as the worms measure several centimetres and are obvious. However, small developing immature worms could probably be missed. We selected a truncated Beta (alpha = 1, beta = 1) distribution as prior information for this sensitivity.

The sensitivity and the specificity of the applied tests and the true prevalence of *E. multilocularis* and *T. canis* were estimated in a Bayesian framework (Berkvens et al., 2006), using WinBUGS 1.4. (Spiegelhalter et al., 2003).

The statistical analysis of the apparent prevalence (Agresti, 1990) and the distribution of the number of the *T. canis* carrier foxes in function of their age were carried out in Stata/SE 8 (StataCorp, 2003).

3. Results and discussion

E. multilocularis could not be detected in any of the 160 collected foxes (Table 1). Based on a Bayesian approach and using the above prior information (Appendix A), the true prevalence of *E. multilocularis* was estimated to have a median value of 0% with a 95% credibility interval of 0 to 1.87%. The sensitivity of the IST was estimated to have a median value of 40% with a 95% credibility interval of 32.9 to 47.4%. The specificity of the IST was estimated to have a median value of 99.2% with a 95% credibility interval of 98.5 to 99.6%. In the urban area of Brussels, the true prevalence of *E. multilocularis* was very low. The very low prevalence of *E. multilocularis* within the Brussels fox population might be explained by both ecological and behavioural factors. As the life-cycle of *E. multilocularis* is based on the predator–prey relationship between final and intermediate hosts, key-factors are the habitat-related abundance of suitable intermediate prey-hosts and the importance of the latter in the fox diet. The occurrence of *Microtus arvalis*, *Arvicola terrestris* and *Ondatra zibethica* is related to the presence of permanent grasslands (pastures and meadows) and/or water habitats (rivers, ponds). A census of mammalian species within the Brussels Region showed that the latter rodent species were not abundant and poorly distributed as a consequence of the scarcity of favourable habitats (Devillers and Devillers-Terschuren, 1997). The part of micro-mammals in the fox diet is a second key-factor. De Blander et al. (2006) showed that the proportion of rodents in the BCR urban fox diet is lower than observed in rural foxes. This latter observation is also proposed by Hofer et al. (2000) as explication of the difference between the city of Zürich and the surrounding rural zones. In Zürich, the prevalence of *E. multilocularis* infection increased from 47 in the urban to 67% in the adjacent recreational area.

T. canis was detected in 24 of the 134 collected foxes (Table 1). The true prevalence of *T. canis* (Appendix B) was estimated to have a mean value of 18.9% with a 95% credibility interval of 12.7–26% (median value of 18.7%). The sensitivity of the ICT was estimated at 97.5% with a 95% credibility interval of 95.1–99.9% (median value of 97.5%). In the model, the specificity of the ICT was fixed at 100%. Prevalence of *T. canis* varied from 5.8 in adult foxes to 40% in cubs. *T. canis* infections are age-related with higher risk in cubs

(odds ratio cubs/adults = 24.375, 95% CI = 6.7–88.4; see also ref. Saeed et al., 2006). Most of worms were adult female worms (numbers lying between 1 and 30), the sole contributors to the contamination of the environment and source of infection for dogs. It is thus very important to treat dogs regularly. Moreover, playgrounds should be closed from animals (e.g. foxes and dogs). Equally, children should not be allowed to play in sand excavations made by foxes, particularly in breeding holes in gardens or park areas. However, when this fox and child eco-ethological high risk behaviour can be excluded, the contribution of foxes to contamination of the environment with *T. canis* can be considered as minor compared to the contribution of dogs (estimated as 327 dogs per square kilometre) because of their very much lower density (Belgian Association for Identification and Registration of dogs, 2005) and the estimated mean prevalence of 17.4% *T. canis* in dogs (Vanparijs et al., 1991).

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Appendix A. Bayesian model for the estimation of the true prevalence of *Echinoccocus multilocularis*.

```
model
{
res[1:2] ~ dmulti(p[1:2], n)
p[1] <- prev*(1-se)+(1-prev)*sp
p[2] <- prev*se+(1-prev)*(1-sp)
prev  <- numinf/n
se ~ dbeta(69,103)
sp ~ dbeta(1000,10)
numinf2 ~ dpois(mu)
mu ~ dexp(1)
numinf <- trunc(numinf2)
for (i in 1:2)
{
      d[i] <- res[i]*log(max(res[i],1)/(p[i]*n))
}
G0 <- sum(d[])
res2[1:2] ~ dmulti(p[1:2], n)
for (i in 1:2)
{
      d2[i] <- res2[i]*log(max(res2[i],1)/(p[i]*n))

}
Gt <- sum(d2[])
bayesp <- step(G0 - Gt)
}
list(res=c(160,0), n = 160)
```

Appendix B. Bayesian model for the estimation of the true prevalence of *Toxocaras canis*.

```
model
{
res[1:2] ~ dmulti(p[1:2], n)

p[1] <- prev*(1-se)+(1-prev)
p[2] <- prev*se

prev  ~ dbeta(1,1)
se ~ dbeta(1,1)I(.95,1)

for (i in 1:2)
{
      d[i] <- res[i]*log(max(res[i],1)/(p[i]*n))
}
G0 <- sum(d[])
res2[1:2] ~ dmulti(p[1:2], n)
for (i in 1:2)
{
      d2[i] <- res2[i]*log(max(res2[i],1)/(p[i]*n))
}
Gt <- sum(d2[])
bayesp <- step(G0 - Gt)

}

list(res=c(110,24), n = 134)
```

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