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A survey of intestinal helminths of red foxes (*Vulpes vulpes*) in northern Belgium

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Abstract

Between 1994 and 1999, 219 red foxes (*Vulpes vulpes*) were collected in northern Belgium and examined for intestinal helminths. The effects of host-related (age, sex, body mass/size ratio) and temporal factors on the prevalence and on the number of parasite species per individual host were investigated. The following parasites were found: cestodes *Echinococcus multilocularis* (1.8%), *Dipylidium caninum* (0.9%) and *Taenia* spp. (2.7%), nematodes *Toxocara canis*, *Toxascaris leonina* (47.9%) and *Uncinaria stenocephala* (31.5%), and trematode species (0.9%). Of all foxes, 82 (37.4%) proved to be fully negative. We found no host-related (sex, age) nor temporal effect on parasite occurrence and on the number of parasite species per individual host. Unparasitised adult foxes had a higher body mass/size ratio than hosts with intestinal parasites and also the number of parasite species per individual was negatively related with this rough index of host body condition. The presence of these zoonotic parasites in a region with a very high human population density urges a close surveillance of these parasites as they may lead to expansions of helminthoses in (northern) Belgium. Finally, this study emphasizes the need to study the prevalence of intestinal helminths in regions that are recently colonised by red foxes since such studies may add to our understanding of the emergence, the temporal spread and the persistence of zoonoses in Europe.

Key words

Vulpes vulpes, intestinal helminths, epidemiology, zoonoses, Belgium

Introduction

As in many other European countries (e.g. Romig *et al*. 1999, Eckert *et al*. 2000), the distribution and density of the red fox (*Vulpes vulpes*) increased dramatically in northern Belgium over the last two decades (Vervaeke *et al*. 2003). In the early eighties foxes were only present in the middle and southern part of the provinces Vlaams-Brabant, Limburg and the northern and eastern part of Antwerpen. In 15 years time, the fox population density increased and the whole territory of Flanders (i.e. northern Belgium) was colonised (Vervaeke *et al*. 2003). The legal restriction on fox hunting, reduced mortality due to changes in rabies control programmes (i.e. the use of vaccine baits), nature conservation measures and the opportunistic behaviour of the fox are all plausible reasons for its explosive population growth (Vervaeke *et al*. 2003). The presence of foxes in Flanders, a region with a very high human population density, may have important epidemiological implications as foxes are a potential reservoir of some zoonotic intestinal helminth pathogens such as *Echinococcus multilocularis*, *Dipylidium caninum* and *Toxocara canis*, causing alveolar echinococcosis, dipylidiosis and toxocarosis, respectively (Glickman and Schantz 1981, Brandstetter and Auer 1994, Thompson and Lymbery 1995, Palmer *et al*. 1998, Rochette 1999, Van der Giessen and Borgsteede 2002). Despite the presence of these parasites in the regions that surround Flanders (Petavy and Deblock 1980; Petavy *et al*. 1985, 1991; Ballek *et al*. 1992a, b; Brochier *et al*. 1992; Wessbecher *et al*. 1994a, b; Losson *et al*. 1997; Pfeiffer *et al*. 1997a, b; Tackmann *et al*. 1998; Van der Giessen *et al*. 1999, 2001; Eckert *et al*. 2001; Van der Giessen and Borgsteede 2002) no surveys on fox intestinal helminths have been conducted in Flanders.

The aims of the present study were: (1) to describe the intestinal helminth population of red foxes in Flanders and to

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compare it with that of the surrounding countries/regions, and (2) to investigate the effects of host related (age, sex, body mass/size ratio) and temporal factors on helminth prevalence and the number of parasite species per individual fox.

Materials and methods

Sampling

Between 1994 and 1999, 219 foxes (181 adults, 38 juveniles; Table I) were collected as hunting and road casualties in Flanders. The animals were individually labeled and the locality, date and cause of death were noted. The carcasses were transported in sealed plastic bags and stored at –20°C. In order to exclude infection risk of *E. multilocularis* the animals were kept at –80°C for at least seven days before necropsy (Eckert *et al*. 2001). At necropsy the animals were sexed and separated in two age classes (juveniles, adults) by the extent of teeth abrasion: Foxes estimated younger than approximately eight months were considered as juveniles, all others as adults. As a measure of nutritional status, the ratio of body mass (± 0.001 kg) over body length (± 0.1 cm) was used (Rodriguez and Carbonell 1998).

The small intestine was isolated and ligatured at both ends, wrapped in plastic bags and stored at –20°C until examination. Before parasitological examination the small intestines were frozen for another seven days at –80°C.

Parasitological methods

The intestinal scraping technique was used to detect intestinal helminths (Deplazes and Eckert 1996, Eckert *et al*. 2001). The small intestine was placed on a plastic sheet, divided in five equal parts and each part was opened in full length with scissors. After removal of coarse material (stones, bones) and large parasites, deep mucosal scrapings were made using microscope slides. The mucosal material adhering to the slides was transferred to plastic Petri dishes and squashed to a thin layer by means of pressure on the slides. A mucosal scraping was taken at the proximal, middle and posterior third of each of the five parts of the small intestine resulting in a total of 15 scrapings per intestine. The mucosal squashes were then examined under a stereomicroscope. Helminths were identified by size and morphology following Soulsby (1982) and Thompson and Lymbery (1995). Because foxes and small intestines were frozen for several weeks, there was some autolysis of the foxes but this did not prevent us from recovering all intestinal parasites. However, the unambiguous identification of some trematodes and *Taenia* spp. was not always possible (see results).

Statistical analysis

Parasite prevalence could only be tested for *Uncinaria stenocephala*, *Toxocara canis* and *Toxascaris leonina*, as other parasites were too rare (see results). To estimate possible associations among the parasites we applied the method proposed by Howard *et al*. (2001) adapted for multiple years. We fitted a mixed log-linear model (log-link and Poisson errors) including a 2-way association among parasites. To test the significance of effects in mixed models, error terms must be constructed that contain all the same sources of random variation except for the variation of the respective effect of interest. Therefore, year was added to the models as a random variable to account for year-to-year variation in infestation. Temporal differences (day of the year) among sexes and age classes in parasite prevalence and parasite load (number of parasite species per individual host) were analysed using respectively, mixed model logistic regression (logit-link and binomial errors) and mixed model Poisson regression (log-link and Poisson errors). For parasite prevalence, different helminth species were tested separately and combined. The influence of parasitic infection on body condition was analysed with mixed model regression with normal distributed errors (Littell *et al*. 1996, Neter *et al*. 1996). Since body condition can also differ among sexes, ages and seasons, the latter were added to the model as co-variates. Again, year was added to the models as a random variable to account for year-to-year variation in infestation. The day of the year (day) is circular because early January will have low values, and late December will have high values, yet the climates at the two times will be somewhat similar. Because the available data were not uniformly distributed in time, it was impossible to convert the data to workable categories (e.g. season, month, week). Therefore we transformed the day to the new continuous variables "winterness" and "springness" (winterness = cos(day) with 1 = winter, –1 = summer, and springness = sin(day) with 1 = spring and –1 = autumn).

Mixed model regressions were calculated with the PROC MIXED module in SAS (SAS 8.02) in the case of normal errors, and with the GLIMMIX macro in the case of binomial or Poisson errors (Littell *et al*. 1996). The degrees of freedom of the fixed effects F-test were adjusted for statistical dependence using Satterthwaite formulas. Variance components were estimated by restricted maximum likelihood (REML).

Results

Of all foxes 137 (62.6%) proved to be positive, i.e. infected with one or more intestinal helminth species. Of all adult foxes, 62.1% of the males and 57.0% of the females were infected with one or more parasites. Of all juvenile foxes, 78.9% of males and 73.7% of females were infected with one or more parasites (Table I). The following parasite taxa were found: cestodes *E. multilocularis* (1.8% of foxes), *D. caninum* (0.9%) and *Taenia* spp. (2.7%); trematode species (0.9%) and nematodes *T. canis* and/or *T. leonina* (47.9%) and *U. stenocephala* (31.5%) (Table I). Hooks were missing from the seven recovered *Taenia* tapeworms making identification at the species level impossible (Soulsby 1982). The two trematodes were too autolysed to allow identification at the species

Table II. Prevalences (%) of intestinal helminths in red foxes (*Vulpes vulpes*) in Europe (1980–2004)

Locality	No. samples	*E. multilocularis*	*D. caninum*	*Taenia* spp.(1)	*T. canis*	*T. leonina*	*U. stenocephala*	Trematoda (1)	Reference
Intestinal scraping technique									
Mid-Germany	397	16.4	–	0.3–28.5	–	–	–	–	Ballek *et al.* 1992a
Karlsruhe, Germany	801	11.6	0.5	7.0–19.9	–	–	–	–	Wessbecher *et al.* 1994a
Germany	1300	0.3	0.2	0.2–17.7(a)	26.5	10.5	15.9	–	Pfeiffer *et al.* 1997a, b
Styria, Austria	500	3.6	–	0.2–14.6(d)	46.8	0.6	43.0	–	Lassnig *et al.* 1998
Northern Belgium	219	1.8	0.9	2.7(2)	*	*	31.5	0.9	present study
Intestinal sedimentation and counting technique									
Southwest Germany	3573	–	0.06	0.03–24.0	31.3	3.4	25.8	0.08	Loos-Frank and Zeyhle 1982
The Netherlands	137	–	–	53.3(2)	73.7	–	59.9	0.7–10.9	Borgsteede 1984
Massif Central, France	154	14.9	–	1.3–23.4	51.3	25.3	58.4	–	Deblock *et al.* 1987
Southern England	843	–	3.8	2.5–13.8	55.9	1.5	68.0	2.3–2.9	Richards *et al.* 1995
Copenhagen, Denmark	21	–	–	38.1(2)	81.0	–	85.7	–	Willingham *et al.* 1996
Greece	314	0	3.2	0.3–0.9(b)	28.6	2.5	43.9	1.6	Papadopoulos *et al.* 1997
Zürich, Switzerland	388	44.3	0.5	0.5–7.6(c)	47.4	–	66.8	–	Hofer *et al.* 2000
Poznan, Poland	92	–	–	–	16.3	–	–	–	Luty 2001
Dublin, Ireland	77	–	–	0.09(2)	37.7	–	92.2	2.6–27.3(3)	Wolfe *et al.* 2001
Great Britain	588	0	0.7	2.0–20.7(4)	61.6	0.3	41.3	–	Smith *et al.* 2003
Faecal flotation technique									
Southcentral Spain	20	–	0	10.0(2)	5.0	15.0	0	–	Rodriguez and Carbonell 1998
Poland	230	–	11.7	16.7(2)	17.0	6.5	–	–	Gund³ach *et al.* 1999
Central Tajo valley, Spain	67	–	–	1.5	4.4	52.2	58.2	1.5	Criado-Fornelio *et al.* 2000
Other technique(s)/technique(s) not mentioned									
Auvergne, France	69	7.2	–	1.4–27.5	27.5	33.3	68.1	–	Petavy and Deblock 1980
Mid-Wales	280	–	0.7	1.8–13.9	63.0	2.9	87.1	–	Hackett and Walters 1980
Central Germany	397	–	–	–	32.7	11.1	3.5	–	Ballek *et al.* 1992a
Saxony, Germany	400	–	–	–	56.5	4.5	13.0	–	Steinbach *et al.* 1994
Karlsruhe, Germany	801	–	–	–	30.2	2.0	24.3	–	Wessbecher *et al.* 1994b
Province Luxembourg, Belgium	145	51.0	–	–	34.5	–	–	–	Losson *et al.* 1997
Ebro valley, Spain	81	–	1.2	1.2–4.9	6.2	66.7	30.9	12.3	Gortazar *et al.* 1998
Hungary	68	–	–	2.9–33.8	26.5	11.8	11.8	48.5	Andras 2001
Stuttgart, Germany	492	16.8	–	13.4(2)	41.3	–	–	–	Deplazes *et al.* 2004

⁻No prevalence published, *pooled prevalence for *T. canis* and *T. leonina* = 47.9%, (1)ranges for different species, (2)total prevalence (i.e. no differentiation made between species), (3)the lower value is the total prevalence of other trematodes; the higher value is the prevalence for the most common species, *Alaria alata*, (4)the lower value is the prevalence of *Taenia pisiformis*; the higher value is the total prevalence of unidentified *Taenia* species, (a)prevalence of unidentified *Taenia* species of 9.2%, (b)prevalence of unidentified *Taenia* species of 1.6%, (c)prevalence of unidentified *Taenia* species of 8.4%, (d)prevalence of unidentified *Taenia* species of 17.4%.

Table I. Intestinal helminths recovered from 219 red foxes (*Vulpes vulpes*) in northern Belgium

	Adult males n = 95	Adult females n = 86	Juvenile males n = 19	Juvenile females n = 19	Total n = 219
Number of uninfected red foxes	36	37	4	5	82
Number of infected red foxes	59	49	15	14	137
Echinococcus multilocularis	2	2	0	0	4
Dipylidium caninum	1	1	0	0	2
Taenia spp.	3	2	0	1	6
Toxocara canis/Toxascaris leonina	46	37	12	10	105
Uncinaria stenocephala	27	24	8	10	69
Trematoda unidentified	1	0	0	1	2

level. *T. canis* and *T. leonina* could not be differentiated unambiguously, because many specimens were too autolysed or damaged. Identification by morphology of the oesophagus and the eggs of well-preserved specimens revealed that both *T. canis* and *T. leonina* were present. Yet, *T. leonina* is uncommon in northwest and central Europe whereas *T. canis* is very common (see Table II). Hence, we pooled the numbers of these two species in further analysis.

Single infections were more common than mixed ones and up to four different parasite species were found in the same individual host. Parasitism involving only one species was found in 42.0% (92/219) of the foxes, two species in 18.3% (40/219), three species in 1.8% (4/219) and four species in 0.5% (1/219).

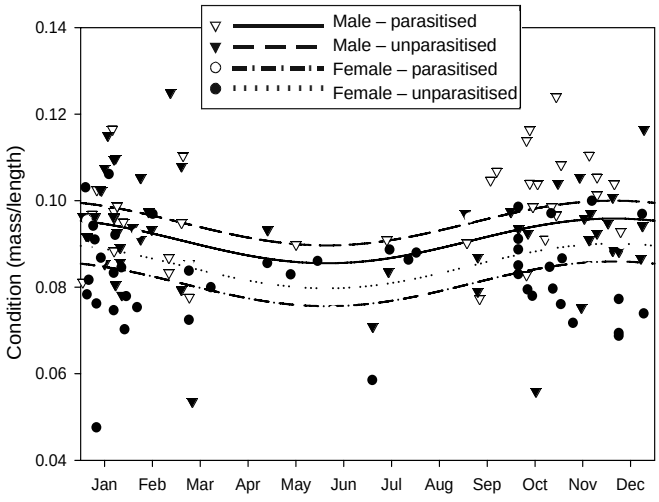

Fig. 1. Body mass/size ratio changes during the year for adult red foxes (*Vulpes vulpes*) in northern Belgium

The log-linear model showed no significant interaction among the two major parasite taxa (*U. stenocephala – T. canis*/*T. leonina*: $F_{1,9.7} = 1.17$; $p = 0.307$) indicating that the infestation by the parasites can be treated as independent. The logistic (single species prevalence) and Poisson (multiple parasite load) regression analyses showed no effect of sex, age or time of the year on parasite prevalence or load (all $p>0.1$). The analysis of the hosts mass/size ratio showed that adult males had a higher mass/size ratio than adult females ($F_{1,159} = 28.44$, $p<0.0001$), the ratio was higher during winter ($F_{1,145} = 7.33$, $p = 0.008$), and that unparasitised animals had a higher mass/size ratio than hosts with intestinal parasites ($F_{1,160} = 4.75$, $p = 0.031$) (Fig. 1). Moreover, for adults the number of parasite species per individual was negatively related with hosts' mass/size ratio ($F_{1,160} = 5.29$, $p = 0.023$; coefficient = –0.001720 ± 0.000747). For juveniles there was no significant effect of sex, helminth infection (all helminth species separately or combined) or the number of parasite species per individual host, on body condition (all $p>0.1$).

Discussion

The present study shows that the red fox in northern Belgium (Flanders) is a host to a range of intestinal helminth species, which include cestodes, trematodes and nematodes. *D. caninum* and *T. leonina* are reported for the first time in red foxes in Belgium. However, the two parasite species have been reported in stray dogs (*Canis lupus* f. *familiaris*) in Belgium (Gérin *et al*. 1980, Van Parijs *et al*. 1991). *E. multilocularis*, *T. canis* and *U. stenocephala* have been reported in red foxes in southern Belgium (Wallonia) (Bernard 1969, Brochier *et al*. 1992, Losson *et al*. 1997). *E. multilocularis* was found for the first time in northern Belgium and data concerning this parasite are discussed in detail elsewhere (Vervaeke *et al*. 2003).

Comparing the prevalence of intestinal helminths of red foxes in Flanders with those reported in other European surveys since 1980 (Table II) should be done with caution since techniques used to recover intestinal helminths may have a significant influence on prevalence estimates. The intestinal sedimentation and counting technique (SCT), and the intestinal scraping technique (IST) used in this survey, are considered to be the most reliable techniques (Thompson and Lymbery 1995, Eckert *et al*. 2001). In contrast, the faecal flotation technique (FFT) is known to severely underestimate the prevalence of gastrointestinal helminths in carnivores (Rodriguez and Carbonell 1998, Hofer *et al*. 2000, Eckert *et al*. 2001, Wolfe *et al*. 2001). Thus, comparisons with the prevalences given in Table II are only indicative.

Both *T. canis* and *T. leonina* are present in northern Belgium, but we are unable to speculate on their prevalence in this survey. Nevertheless, we suspect that the prevalence of *T. canis* is much higher than that of *T. leonina*. The prevalence of *T. canis* in northwest and central Europe varies widely, with high prevalences (ranging from 27 up to 81%) in southern Belgium, Germany, Austria, Switzerland, Ireland, the United Kingdom and Denmark, and lower prevalences in south Europe (i.e. Spain: ranging from 4 to 6%; but see Papadopoulos *et al*. 1997) and eastern Europe (i.e. Poland: 16–17%) (Table II). In contrast, the prevalence of *T. leonina* in northwest and central Europe is low (ranging from 0 to 11%), whereas this nematode species is highly prevalent (ranging from 25 up to 67%) in certain regions in Spain and southern France (Table II).

Prevalences of *U. stenocephala* in red foxes in Europe vary widely and range from 0% (south Central Spain) to 92.2% (Dublin, Ireland) (Table II). The prevalence of 31.5% found in this study is comparable with the infection rate in southwest Germany (25.8%) and in the Ebro valley in Spain (30.9%).

Dipylidium caninum was found in this survey in two fox intestines (0.9%) and is occasionally found in foxes in other European countries albeit at low prevalence (<4%).

Surprisingly, we did not detect the cestode *Mesocestoides* spp. although the prevalence of this genus is very high in red foxes in other European countries such as Germany (Ballek *et al*. 1992a: 4.3%, Wessbecher *et al*. 1994a: 16.6% and Pfeiffer *et al*. 1997a: 54.1%), Austria (Lassnig *et al*. 1998: 15.8%) and Poland (Ramisz *et al*. 2004: 63.7%).

We found no sex-specific differences in overall helminth prevalence and in the number of parasite species in adult foxes. The presence of intestinal nematodes and cestodes is primarily determined by the fox's prey selection and, thereafter, by the ability of the respective parasite species to become established (Grenfell and Dobson 1995, Richards *et al*. 1995, Papadopoulos *et al*. 1997). Several studies on the feeding habits of the red fox in Europe have shown that, in general, foxes are opportunistic and utilise food which is most abundant and easily obtainable at a particular time (Artois 1989, Papadopoulos *et al*. 1997). Data on the stomach contents of 119 red foxes from Flanders revealed that mammals were the major food source in the fox diet (48.7%), followed by birds (37%), vegetation material (14.3%), invertebrates (10.1%) and refuse (4.2%), and that the diet, and consequently the exposure to infection, was similar in both sexes (Vervaeke *et al*. unpubl. data). Other European surveys confirm the absence of differences in the food ecology of foxes between sexes (Rzebik-Kowalska 1972, Artois 1989 and references therein).

It is generally assumed that adult hosts are more resistant to helminth infections than young hosts due to immune mechanisms, which may be related to prior exposure to infection (Grenfell and Dobson 1995, Rodriguez and Carbonell 1998). Moreover, very young animals can be infected with helminths through direct transmission from their mother or through early acquisition in the environment. This could not be confirmed by our study since helminth prevalence and the number of parasite species per host did not differ significantly between adults and juveniles. Although an effect of small sample size of juveniles cannot be discarded, our findings correspond with those of Rodriguez and Carbonell (1998) who suggested that repeated exposure to free living infective stages, or to intermediate hosts along the life, can equal or even override the effects of increased resistance in adults.

Seasonal dependency in prevalences of cestode and nematode species in red foxes has been demonstrated in Germany and the United Kingdom (Loos-Frank and Zeyhle 1982; Richards *et al*. 1993; Pfeiffer *et al*. 1997a, b). The absence of significant temporal differences in our study may partly be explained by the non-uniform spread of sampled animals over the year because foxes were mainly collected during the hunting season, i.e. from September till the end of January.

The finding that unparasitised adult foxes have a higher mass/size ratio than parasitised hosts could be expected as infections with parasitic helminths often lead to increased metabolic rate and reduced body mass, which may have a long term impact on host fitness (Grenfell and Dobson 1995). Although these effects depend mostly upon the intensity of infection, Rodriguez and Carbonell (1998) also found that the number of parasite species in carnivores was negatively correlated with host physical condition. Our study supports this finding for adult red foxes in northern Belgium as the number of parasite species per individual was negatively correlated with fox body condition.

Several intestinal helminth species reported in this study are potential causative zoonotic agents. Infection with *E. multilocularis* results in alveolar echinococcosis which is usually fatal (Rausch 1995, Eckert *et al*. 2001). Both *T. canis* and *T. leonina* are a cause of visceral and ocular larva migrans (Rochette 1999). Most people infected with *U. stenocephala* are asymptomatic but this parasite may cause cutaneous larva migrans. Most infections with *D. caninum* in humans are asymptomatic but mild gastrointestinal disturbances may occur. Foxes may be a risk for human infections either directly by the contamination of soil with eggs or indirectly through infecting intermediate hosts and subsequently dogs and cats (Richards *et al*. 1993, Brandstetter and Auer 1994, Thompson and Lymbery 1995, Rochette 1999, Eckert *et al*. 2001). The presence of these zoonotic parasites in the fox population in northern Belgium urges a close surveillance of these helminths as (1) an increase of the fox population may be accompanied by a spread and increasing prevalence of helminths and (2) the human population density is very high in northern Belgium so that helminthoses may spread very rapidly. For example, the increase of the fox population in Belgium and the Netherlands in the last decade was followed by an increase of the prevalence of *E. multilocularis* (Vervaeke 2004). Unfortunately, substantial data for the spatio-temporal analysis of prevalence patterns are currently lacking for the other parasite species. Finally, this study emphasizes the need to study the prevalence of intestinal helminths in regions that are recently

colonised by red foxes especially since such studies may add to our understanding of the emergence, the spatio-temporal spread and the persistence of zoonoses in Europe.

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