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ORIGINAL PAPER

A coprological survey of parasites of wild carnivores in Ireland

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Abstract The increasing movement of people to wilderness areas, shrinking of wildlife habitats and the resulting urbanisation of wildlife has led to growing concerns about the transfer of parasitic diseases, particularly from contaminated faeces. Faecal samples from wild carnivores in Ireland were examined for the presence of protozoan and nematode parasites. Red fox (*Vulpes vulpes*) samples ($n=91$) were positive for *Uncinaria stenocephala* (38 %), *Eucoleus aerophilus* (26 %), *Toxocara canis* (20 %), *Trichuris vulpis* (4 %) and *Isospora*-like oocysts (9 %). Badger (*Meles meles*) samples ($n=50$) were positive for *Uncinaria criniformis* (40 %), *E. aerophilus* (6 %) and *Isospora*-like oocysts (16 %). No parasites were observed in pine marten ($n=48$; *Martes martes*) faeces. Approximately 5 % of American mink (*Mustela vison*) samples were positive for *Cryptosporidium* by polymerase chain reaction (identified as *Cryptosporidium andersoni* ($n=3$) and 'mink' genotype ($n=1$)). The results suggest that wild carnivores in Ireland have a range of parasites, although it is unclear from the present study to what extent these infections are associated with morbidity. While it can be expected that, via their faeces, wild carnivores contribute to the spread of these parasites, they are unlikely the primary source of environmental contamination. Therefore, they should not always be the principal target of control measures.

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Introduction

Opportunities for the transfer of parasitic diseases from wildlife to humans and domestic animals, and vice versa, have increased due to shrinking wildlife habitat, human population expansion and wildlife urbanisation (Gortázar et al. 2007; Crawford 2007). Contamination of food sources and drinking water with animal faeces may go undetected, resulting in the spread of disease (Daniels et al. 2003; Coffey et al. 2007).

Toxocara canis is an ascarid nematode of zoonotic importance that is commonly found in canids. Canids (including dogs) are the definitive host, shedding large numbers of eggs in their faeces. Although not as common as *T. canis*, *Trichuris vulpis* also infects dogs as definitive hosts, shedding eggs into the environment that can survive for long periods. As in toxocariasis, *T. vulpis* can cause visceral larva migrans in both final and intermediate hosts, including humans (Masuda et al. 1987; Mizgajska 1997).

Another trichuroid nematode frequently associated with wildlife is *Eucoleus aerophilus* (syn. *Cappilaria aerophilus*). It is primarily associated with foxes, but is also found in dogs and other carnivores, rarely in humans (Cross 1992; Kahn and Line 2010). *E. aerophilus* is contracted through the ingestion of eggs shed in the faeces of its hosts. Clinical signs include coughing and sneezing and in severe cases cause chronic respiratory disease (Nevarez et al. 2005).

One of the most common parasitic species found in the faeces of foxes globally is *Uncinaria stenocephala*. *Uncinaria criniformis* is closely related to *U. stenocephala*, but is host specific to mustelids, where the adult forms can be distinguished from *U. stenocephala* (Ransom 1924). It is primarily found in badgers (Torres et al. 2006; Millán et al. 2004; Rosalino et al. 2006). *U. stenocephala* is a hookworm of veterinary importance as it can also infect dogs and cats, which may lead to diarrhoea and cutaneous lesions (Menelaos

and Smaragda 2006). It may also cause larva migrans in humans (Beaver 1956).

Cryptosporidium is an obligate intracellular protozoan parasite of medical and veterinary importance. It infects a wide spectrum of vertebrate hosts including humans and livestock, but disease is principally observed in the young and immunocompromised (Angus 1983; O'Donoghue 1995; Feng 2010). The two species which have the most impact on human health are *Cryptosporidium parvum* and *Cryptosporidium hominis*. Ireland has one of the highest reported infection rates of cryptosporidiosis in Europe (Health Protection Surveillance Centre. Annual report: Infectious intestinal diseases, 2008. 3.2 Cryptosporidiosis, pp. 43–44; www.hpsc.ie/hpsc/AboutHPSC/AnnualReports/) and, due to the predominant use of surface waters for drinking water abstraction, is considered particularly vulnerable to waterborne outbreaks.

The detection of *Cryptosporidium* spp. in wildlife has led to speculation that wildlife could be an important source of environmental contamination, particularly of drinking water supplies (Méndez-Hermida et al. 2007). However, although some of the 19 or so *Cryptosporidium* species identified to date (Fayer 2010), have been shown to be infectious to multiple host species, wild mammal species identified with human pathogenic species are rare and few in number (Zhou et al. 2004; Appelbee et al. 2005; Feng et al. 2007; Feng 2010). Therefore, when evaluating the potential health risk of zoonotic cryptosporidiosis arising from wild mammals, it is important to identify the parasite to species or gentotype (Feng 2010).

When conducting research into parasites in wildlife, carnivores not only present the opportunity to identify species for which they serve as hosts but also to characterise parasites of prey species, which may have been acquired through ingestion (Feng et al. 2007; Hamnes et al. 2007). Therefore, studies of pathogenic parasites in wild carnivores are a good indicator of potential wildlife reservoirs. However, when investigated, the prevalence of some of these parasites, have been found to be lower in wildlife species, relative to domestic hosts (Smith 1943). Therefore, it is not only important to investigate which wild animals serve as hosts to these parasites, but also their prevalence in order to help determine their role in the epidemiology of a disease. This is of particular concern where control measures such as culling are being implemented based on the assumption that a reduction in the wildlife reservoir will decrease exposure to humans and/or domestic species.

The aim of this study is to investigate the presence of protozoan and nematode parasites in the faeces of selected wild mammal carnivores in Ireland. While this is a continuation of two previous studies on parasites of foxes on the island (Ross and Fairley 1969; Wolfe et al. 2001), its scope goes much further as it includes a much broader host range. An additional focus of our study was the screening of semi-aquatic mammalian carnivores for the presence of *Cryptosporidium* species. As well as assessing the role of wildlife as a reservoir of human and livestock disease, we aimed to evaluate the likely effects of the various parasites on the wild hosts' fitness and health, as some parasitic diseases have been identified as potential causes of species endangerment (Daszak et al. 2000; Smith et al. 2006).

Methods

Collection of material

Red foxes (*Vulpes vulpes*), American mink (*Mustela vison*), European badgers (*Meles meles*), pine martens (*Martes martes*), European otters (*Lutra lutra*) and Irish stoats (*Mustela erminea hibernica*) were sampled throughout Ireland, while all stoats came from Booro bog, Co. Offaly (N 17146 17882). The majority of the animals were donated by hunters or found as road kill. No animals were killed specifically for the purposes of this study. Faecal samples were extruded from the rectum of cadavers. In the case of pine martens, samples were collected as scats as part of the Irish National Pine Marten Survey (O'Mahony et al. 2006). Faecal samples were stored without preservative at 4 °C until examination. Whether the faecal sample was tested for all parasites or just *Cryptosporidium* spp. depended on the size of the sample obtained.

Ageing was carried out by cementum analysis by Matsons Laboratory (www.matsonslab.com). Foxes were assumed to be born on 1 April and grouped into three age classes; cubs (1–6 months), sub-adults (6–12 months) and adults (over 12 months), after Richards et al. (1995). Pine martens were grouped according to their actual age in years. All other animals' age was unknown.

Detection and identification of enteric parasites

A qualitative flotation technique using a zinc sulphate solution (sp. gr. 1.18 at room temperature) was used to concentrate parasites in 3 g faecal matter from 91 foxes, 50 badgers and 48 pine martens each. Helminth eggs and protozoan oocysts were identified using keys and descriptions of their morphology (Quinn et al. 1997; McGarry and Morgan 2009; Soulsby 1982) and confirmed from photographs and measurements by experienced diagnostic parasitologists. Exposure time to the zinc solution was reduced to a minimum to prevent damage to the eggs and oocysts by hyperosmotic pressure.

Screening for the presence of *Cryptosporidium* spp. using PCR

DNA was extracted from faecal material of 81 American mink, 25 European otters, 30 Irish stoats, 13 red foxes, 7 European badgers and 7 pine martens (foxes, badgers and martens were the same samples as those listed in the previous section). Faeces from foxes, badgers and pine martens were concentrated prior to DNA extraction using Sheather's sugar solution (sp. gr. 1.18 at 4 °C; Current 1990). Since insufficient mink and stoat faecal material was available for sugar concentration and the jelly-like consistency of otter faeces precluded reliable oocyst flotation, DNA was extracted directly from faeces in those species. DNA extraction was carried out according to the methods described by Boom et al. (1990) as modified by McLauchlin et al. (1999).

A nested polymerase chain reaction (PCR) was performed according to Xiao et al. (1999, 2001). PCR products were fractionated on 2 % agarose gels and visualised by staining with SYBR Safe DNA gel stain (Invitrogen, Paisley, UK). Amplicons were purified using the QIAquick PCR purification kit (Qiagen) and sequenced (GATC Germany).

Results

Presence of helminths according to flotation

Of the fox faecal samples examined, 32 % had single, 18 % had dual and 10 % had triple infections. The most prevalent parasites found in foxes were *U. stenocephala* (38 %), *E. aerophilus* (26 %) and *T. canis* (19 %; Fig. 1). *T. vulpis* (4 %) and *Isospora*-like oocysts (9 %) oocysts were also observed in the fox faecal samples.

In badgers, the parasite distribution was similarly overdispersed with 30 % with single, 10 % with dual and 2 % with triple infections. In this host, the most prevalent parasite was *U. criniformis* (40 %). *E. aerophilus* (6 %) and *Isospora*-like oocysts (17 %) were also observed. No parasites were identified in the pine marten faecal samples examined.

No significant association was detected between the foxes' gender and infection frequencies for *T. canis* (males, 19 %; females, 21 %; $\chi^2=0.02$, $1df$; $p>0.05$), *U. stenocephala* (males, 35 %; females, 41.02 %; $\chi^2=0.17$, $1df$; $p>0.05$) and *E. aerophilus* (males, 19 %; females, 35.89 %; $\chi^2=2.39$, $1df$; $p>0.05$). Furthermore, there was no significant association between the foxes' age class and infection frequencies for *T. canis* (cubs, 38 %; sub-adults, 19 %; adults, 12 %; $\chi^2=4.77$, $2df$; $p>0.05$; Fig 1). However, cubs were found to have significantly higher frequencies of infection with *T. canis* than adults ($p=0.04$). A significant association was observed between the foxes' age class and infection frequencies for *U. stenocephala* (cubs, 46 %; sub-adults, 65 %; adults, 22 %; $\chi^2=10.73$, $2df$; $p<0.05$). While there was no significant difference between cubs and sub-adults ($p=0.31$) or between cubs and adults ($p=0.16$), the difference between sub-adults and adults was significant ($p=0.0004$). No significant association was detected between the foxes' age class and infection frequencies for *E. aerophilus* (cubs, 31 %; sub-adults, 23 %; adults, 27 %; $\chi^2=0.17$, $2df$; $p>0.05$). When comparing parasite prevalences between host species, foxes were observed to have a significantly higher prevalence of *E. aerophilus* infection than badgers ($p=0.01$).

Cryptosporidium in wildlife

PCR resulted in amplicons of the expected size in five of the mink samples and one of the otter samples. Four of the mink samples were sequenced successfully. By comparison with published sequences using NCBI BLAST, one of the mink

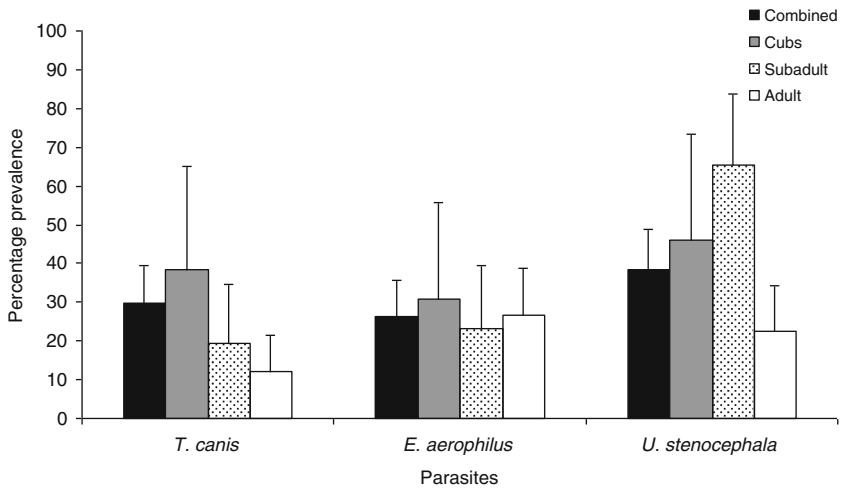

Fig. 1 Prevalences of *Toxocara canis*, *Eucoleus aerophilus* and *Uncinaria stenocephala* found in fox faecal samples

samples was identified as *Cryptosporidium* 'mink genotype', 100 % identical to published sequence EF641015. Three of the samples were identified as *Cryptosporidium andersoni* (100 % identical to published sequence EU245042). The remaining two amplicons could not be identified due to poor sequencing data. The prevalence of *Cryptosporidium* spp., *T. vulpis* and the *Isospora*-like oocysts were too low for appropriate statistical analyses.

Discussion

T. canis was detected in 20 % of faecal samples taken from foxes in this study (Fig. 1). The prevalence observed in this study is slightly lower (although not significantly (χ^2=0.428, 1*df*; p>0.05)) than the prevalence of 27 % found by Wolfe et al. (2001) in faecal samples from Dublin and neighbouring counties in Ireland. However, it is higher than the prevalence reported from fox faecal examinations (Criado-Fornelio et al. 2000; Martínez-Carrasco et al. 2007) and intestinal examinations (Smith et al. 2003; Brochier et al. 2007; Di Cerbo et al. 2008a; Magi et al. 2009; Miterpáková et al. 2009) reported from other European countries. Similarly, Holland (1997) found the seroprevalence of toxocariasis in humans to be high in Ireland relative to other European countries. They suggested that the mild climate and the high numbers of dog owners in Ireland may be the cause of the high levels of *T. canis* infections (Holland 1997). A study of *T. canis* in stray dogs in Ireland also found the prevalence of *T. canis* to be high (O'Lorcain 1994). Another factor that could explain the high prevalences of *T. canis* in Ireland is the common occurrence of foxes in all habitats in Ireland (Ross and Fairley 1969; Harris and Yalden 2008).

The high prevalence of *T. canis* eggs in fox faeces in conjunction with the previously high abundance of *T. canis* eggs recorded in foxes in Ireland by Roddie et al. (2008) suggests they are a potential public health concern. Measures should be put in place to stop the transmission of the parasite from foxes to humans, especially to children who are more vulnerable to infection (Holland 1997). The *T. vulpis* (4 %) prevalence is similar to that recorded by Wolfe et al. (2001) showing foxes are also a persistent reservoir host of this zoonotic parasite in Ireland.

U. stenocephala was the parasite most frequently found in fox faecal samples in the present study (Fig. 1). In comparison to other studies (58 % (Criado-Fornelio et al. 2000) and 67 % (Wolfe et al. 2001)) the prevalence recorded in the present study is low (38 %). Richards et al. (1995) found the prevalence of *U. stenocephala* in foxes to decrease from July to November. Although foxes in the present study were sampled throughout the year, the majority (86 %) were obtained during the latter half of the year possibly explaining the low prevalence encountered.

E. aerophilus was the second most common parasite identified in the fox samples (26 %; Fig. 1). While this is similar to the prevalence of 37 % reported for Ireland previously (Wolfe et al. 2001), the infection rate was considerably higher than in other parts of Europe (Richards et al. 1995; Criado-Fornelio et al. 2000; Martínez-Carrasco et al. 2007; Di Cerbo et al. 2008b, 7 % Magi et al. 2009; Miterpáková et al. 2009), with the exception of Scandinavia (Willingham et al. 1996; Saeed et al. 2006). As in the case of *T. canis*, Ireland's mild and wet climate may facilitate transmission of *E. aerophilus* which can use animals that favour damp conditions, such as snails and earthworms, as paratenic hosts.

The prevalence of *T. canis* was observed to be highest in cubs (38 %), decreasing in sub-adults (19 %) and lowest in adults (12 %; Fig. 1), with a significant difference found between the frequency of infection in cubs and adults. This pattern has previously been recorded in foxes in other countries (Richards *et al.* 1993; Saeed and Kapel 2006; Brochier *et al.* 2007; Reperant *et al.* 2007) and, similarly, O'Lorcain (1994) observed the prevalence of the infection to decrease markedly in dogs over 48 weeks old. The high level of infection in cubs is thought to be due to the ability of *T. canis* to infect cubs in utero (Richards *et al.* 1993) and transmammarily. Although the prevalence may be lower in adult foxes, egg output is high in both adult foxes and cubs (Richards and Lewis 2001); therefore, given their larger ranges, adults still have a significant role in the epidemiology of toxocariasis. In contrast to *T.canis*, sub-adult foxes were found to have a significantly higher prevalence of *U. stenocephala* infection than adults (Fig. 1). *U. stenocephala* infections cannot be acquired in utero, unlike *T. canis*. Foxes probably ingest the third-stage larvae in faecal-contaminated soil, when they leave the breeding earth and go above ground (Richards *et al.* 1995) resulting in the high levels seen in sub-adults. By the time they reach adulthood, they are likely to have acquired a degree of immunity. No significant association was detected between the fox's age class and infection frequencies of *E. aerophilus* (Fig. 1) indicating that in spite of a high rate of exposure, the foxes did not appear to develop a noticeable degree of resistance or possibly that the parasite is long lived in foxes and infections acquired when the animal is young persist to adulthood.

No significant association was found between frequency of *T. canis*, *U. stenocephala* or *E. aerophilus* infection and a fox's sex in the present study or by Borgsteede (1984), Richards et al. (1995), Luty (2001), Wolfe et al. (2001), Davidson et al. (2006) and Di Cerbo et al. (2008a). In contrast, an association between a fox's sex and prevalence of infection of *T. canis* has previously been identified by some authors (Richards et al. 1993; Saeed et al. 2006; Saeed and Kapel 2006; Di Cerbo et al. 2008b). This discrepancy

may be caused by the size of home ranges which in turn influence parasite exposure (Torres et al. 2006) and the fact that they are subject to seasonal changes (Kolb 1984; White et al. 1996). As a result, variations in the time of year the animals are sampled may result in the disagreement of the published literature. Richards et al. (1993) found that difference in male in female prevalences of *T. canis* was only significant during the autumn and winter months.

This is the first time *U. criniformis* (which is restricted to mustelid hosts) has been reported in a badger from Ireland. *U. stenocephala* (a species that does not infect mustelid hosts), which is believed to have a similar lifecycle and environmental requirements (Jancev 1986), has previously been found in Ireland in this and previous studies. In contrast, badgers are not an important host for *E. aerophilus* (Di Cerbo et al. 2008b) as was verified by the significantly lower prevalence observed in badgers (6 %) than in foxes (27 %) in the present study.

It is surprising that no parasites were identified from pine marten faecal samples, as it is known that *U. criniformis* and *E. aerophilus* both occur in pine martens, with *E. aerophilus* in particular being common (Segovia et al. 2007). However, species with low density and a restricted geographic area have lower parasite pressure (Torres et al. 2006). Pine martens in Ireland currently occupy a reduced range following extensive persecution (O'Mahony et al. 2006). It may be that as the population expands, the prevalence of parasites will also increase.

This is the first time *Cryptosporidium* has been identified in faeces from mink or otters in Ireland. The most common species of *Cryptosporidium* identified in the mink faeces was *C. andersoni* ($n=3$). This species of *Cryptosporidium* has not previously been identified from American mink (Feng et al. 2007; Gómez-Couso et al. 2007; Ziegler et al. 2007; Wang et al. 2008). The species is primarily associated with adult cattle where low levels of infection may persist without any apparent ill effects although some reports suggest that the parasite may reduce milk production (Lindsay et al. 2000; Kvác et al. 2008). Cattle have been identified as a major source of environmental contamination with several species of *Cryptosporidium* (including the important zoonotic agent, *C. parvum*; Sturdee et al. 2003), and animal manure is a common source of water pollution in Ireland (Sherwood 1986). Therefore, contaminated manure runoff into fresh water is a likely source of infection for the positive mink samples identified in the present study. Similarly, Ziegler et al. (2007) found that wildlife from agricultural areas were more likely to be infected with *Cryptosporidium* than those from non-agricultural areas. The mink may acquire *C. andersoni* directly through the ingestion of contaminated surface water or through infected prey as *C. andersoni* also occurs in rodents (Kvác et al. 2008). Moreover, it can be accumulated by filter-feeding invertebrates (Chalmers et al. 1997). At the same time, it is possible that the oocysts pass through the minks' digestive tract as part of their prey without causing any infection. Given the primary association of *C. andersoni* with cattle, it is more likely that positive mink faeces indicate contaminated manure runoff rather than the mink being an important source of contamination for that water course. This highlights the need to identify *Cryptosporidium* to species level so that correct control measures can be put in place.

The other positive sample of *Cryptosporidium* that could be identified was the mink genotype. This has previously been identified in 17 % of mink in a New York (USA) watershed (Feng et al. 2007). This genotype has not been found to infect humans or cattle and to date is believed to be host specific to mink.

Unfortunately, repeated attempts to sequence the PCR amplicons from one mink and otter sample, respectively, failed. This may have been due to non-specific amplification and/or the presence of a mixture of specific (and non-specific) products. However, further studies of *Cryptosporidium* spp. in otters from Ireland is warranted to establish if otters could be a source of *Cryptosporidium* spp. and therefore are of public health concern, but also if the parasite could be a contributing factor to the current decline of otters in Ireland (Marnell et al. 2009). Méndez-Hermida et al. (2007) reported a prevalence of 3.9 % for *Cryptosporidium* spp. when testing the faeces of European otters.

Conclusions

Wild carnivores occur at much lower densities than the domestic animals that they may share parasites with. However, free-ranging animals may have greater opportunities to transfer parasites throughout their environment. The ecology of a wildlife host has an important role in their ability to transfer parasites. For example, due to its semi-aquatic lifestyle, the mink can potentially contaminate water sources with *Cryptosporidium* and due to its urbanisation the red fox may frequent areas such as parks and lawns where transfer of *T. canis* can occur between humans and their animals. However, there are caveats to this theory. The *Cryptosporidium* species identified in mink to date are not pathogenic to humans. It also seems mink are contracting *C. andersoni* from infected cattle as opposed to transferring the parasite to them. Moreover the higher level of *T. canis* in stray dogs in Ireland (83 %; O'Lorcain 1994) relative to foxes suggests stray dogs are the primary control concern. In such cases, it may be the transmission of diseases from domestic animals to wildlife hosts which is the prime concern as opposed to transmission in the other direction.

It can be seen that Irish carnivores carry many parasites that can be spread via their faeces. The presence of some of

these parasites are of concern as they are pathogenic to humans and domestic animals and therefore these carnivores should be included in plans to limit the spread of these parasites. However, it is important to note that they are usually not the primary source of infection and identifying and combating the primary source should be the priority control measure.

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