ELSEVIER

Study on the prevalence of *Toxoplasma gondii* and *Neospora caninum* and molecular evidence of *Encephalitozoon cuniculi* and *Encephalitozoon* (*Septata*) *intestinalis* infections in red foxes (*Vulpes vulpes*) in rural Ireland

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Abstract

Thoracic fluid (pleural fluid and clotted blood) from 206 foxes were examined for antibodies to *Toxoplasma gondii* and 220 thoracic fluid samples were tested for *Neospora caninum* antibodies using indirect immunofluorescent antibody tests (IFAT). A total of 115 (56%) and six (3%) foxes had antibodies to *T. gondii* and *N. caninum*, respectively. The brains from 148 foxes were examined for histological lesions and pathological changes suggestive of parasitic encephalitis were observed in 33 (22%). Two thirds of these foxes had antibodies to *T. gondii* and one fox had antibodies to both *T. gondii* and *N. caninum*. PCR assays carried out on DNA extracted from the 33 brains with histological lesions were negative for *N. caninum* but one of the brains was positive for *T. gondii*. Microsporidian DNA was also amplified from the brains of two of these foxes. Sequencing these amplicons revealed 100% homology with *Encephalitozoon* (*Septata*) *intestinalis* in one fox and *Encephalitozoon cuniculi* in the second fox. This is the first report of *Encephalitozoon* infections in wildlife in Ireland.

1. Introduction

Red foxes (*Vulpes vulpes*) are members of the Canidae family and are ubiquitous in rural areas of Europe, Asia, Australia and North America. They are highly adaptable animals and easily colonise new habitats such as urban environments of towns and cities (Deplazes et al., 2004). Their wide-ranging diet and scavenging feeding habits results in them being reservoirs for a large number of helminth and protozoan parasites, many of which are pathogenic to man and animals.

The apicomplexan protozoa *Toxoplasma gondii* and *Neospora caninum* are important economic parasites in livestock production because they cause foetal mortality and abortion in sheep and cattle, respectively (Dubey

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and Lindsay, 1996; Trees et al., 1999). Both organisms have an indirect life cycle with a carnivore as definitive host. The cat is the definitive host for *T. gondii* and the sexual reproductive phase of *N. caninum* has been shown to occur in dogs and coyotes (McAllister et al., 1998; Basso et al., 2001; Hill and Dubey, 2002; Gondim et al., 2004). They utilise a wide range of carnivorous and herbivorous animals as intermediate hosts. Amongst the intermediate hosts, horizontal and vertical transmission can occur with *T. gondii* but in the case of *N. caninum* congenital infection appears to be the norm, especially amongst herbivores although in the dog, which can be both definitive and intermediate host, transplacental and lateral transmission can occur (Dubey, 1999). In humans *T. gondii* is a common latent infection, which can be reactivated into a life threatening disease in immunocompromised individuals (Hill and Dubey, 2002). *Neospora caninum* has also been shown to be an efficient opportunistic pathogen of HIV infected patients and patients with neurological disorders (Lobato et al., 2006).

Microsporidia are obligate intracellular parasites with a direct life cycle. They have a very extensive invertebrate and vertebrate host range. In recent years they have emerged as important opportunistic pathogens of immunocompromised humans and several genera and species including *Encephalitozoon cuniculi* and *Encephalitozoon intestinalis* have been described as causing disease in man (Weber et al., 1994; Mathis, 2000; Wasson and Peper, 2000; Mathis et al., 2005). The potential of *E. intestinalis* to produce a zoonosis remains to be clarified despite it producing severe enteritis in immunosuppressed humans and also been shown to infect farm and companion animals in Mexico and free-ranging gorillas in Uganda (Mathis et al., 2005). The zoonotic situation with *E. cuniculi* is more clear-cut and amongst animals, infection with this agent is usually asymptomatic. However, in dogs and especially farmed blue foxes, which are particularly susceptible to this parasite, severe neurological signs can sometimes occur caused by a granulomatous encephalitis (Plowright and Yeoman, 1952; Shadduck et al., 1978; Bjerkas and Nesland, 1987; McInnes and Stewart, 1991; Akerstedt et al., 2002). *Encephalitozoon cuniculi* may also on occasion be of clinical significance in laboratory and pet rabbits, as it can induce lethargy, tremors and generalised paresis in this species (Mathis et al., 1997; Wasson and Peper, 2000). It is also a common parasite of laboratory rodents. There are indications from Australia, Switzerland and Iceland that lagomorphs and rodents may be reservoirs of infection in the wild (Hersteinsson et al., 1993; Thomas et al., 1997; Muller-Doblies et al., 2002).

Foxes are at the top of the wildlife food chain and are considered sentinel animals for whatever disease agent or pollutant is present in the environment. They pose a risk for livestock and public health as their territorial behaviour and feeding activities brings them into close contact with animals and humans. The results of a limited study suggest that an *E. cuniculi* sylvatic cycle may exist in the UK (Wilson, 1979). With the exception of this report from Wilson (1979) and one from Muller (1998; quoted by Mathis et al., 2005) few data are available about *E. cuniculi* infection in red foxes. The prevalence of *T. gondii* and *N. caninum* amongst foxes varies depending on the country (Wanha et al., 2005; Jabubek et al., 2001; Dubey et al., 1999; Buxton et al., 1997). In Ireland there have been two published surveys of parasites in foxes with the most recent study limited to foxes in the suburbs of Dublin the capital city (Wolfe et al., 2001; Ross and Fairley, 1969). The present study was undertaken to determine the levels of protozoan infection amongst rural foxes.

2. Materials and methods

A total of 454 foxes were collected during the period of January to February 2003 for a national survey of Trichinosis amongst wildlife (Rafter et al., 2005). An average of 16 foxes were killed in each of the 26 counties in the Republic of Ireland and sent to the nearest Regional Veterinary Laboratory for examination. The foxes were shot as part of an annual vermin kill. Only foxes (345 in total) sent to the Regional Veterinary Laboratories at Cork, Dublin, Kilkenny and Limerick were available for this study. From this cohort, thoracic fluid (consisting of clotted blood and pleural fluid) and brain tissue were collected from 206 and 148 animals, respectively.

The samples of thoracic fluid were centrifuged at $1000 \times g$ and the supernatant which was assumed to contain serum proteins was stored at $-20\,^{\circ}\text{C}$ until screened for evidence of infection with *N. caninum* and *T. gondii*. They were tested for the presence of antibodies to *N. caninum* using a commercial indirect fluorescence antibody test (IFAT) (VMRD Inc. USA). Positive and negative control serum were supplied with the kit and used as per the manufacturer's instructions. Reagents for the *T. gondii* IFAT included rabbit anti-canine IgG (FC + Fab) FITC (Autogen Bioclear Ltd., UK) used at a 1:100 dilution, canine positive and negative serum (Biobest, UK) diluted 1:100 (as per manufacturer's instructions) and *Toxoplasma* antigen substrate slides (VMRD Inc., USA). The canine positive and negative control serum and fox thoracic fluid were

diluted with serum diluting buffer supplied in the VMRD *Toxoplasma* kit (VMRD Inc., USA). In both tests the samples were screened initially at a 1:50 dilution and any positive ones were end-tritrated with 2-fold dilutions. A thoracic fluid sample which exhibited no fluorescence at 1:50 dilution and whose corresponding formalin fixed brain sample was negative on histological examination was used as an additional negative control in both IFAT assays.

Only brains from 148 foxes were suitable for examination. One half of the brain was fixed in buffered formalin and processed by standard histological procedures to H & E slides. The remainder of the brain was stored at $-80\,^{\circ}C$ until required for DNA extraction.

DNA for the *T. gondii* and *N. caninum* PCR was obtained from those brains with histological evidence of parasitic encephalitis using commercial mini-prep extraction kits (Sigma, UK; Qiagen, UK). The *N. caninum* PCR was carried out using primers Np21 and Np6 (Yamage et al., 1996). The 50 µl reaction mixture contained 10 µl of extracted DNA solution, 10 µl Taq buffer (10X), 2 µl MgCl (50 mM), 4 µl dNTPs (10 nM) mix, 5 µl Np21 (20 pm/µl), 5 µl Np6 (20 pm/µl), 0.5 µl Taq Polymerase (5 units/µl, Bioline, UK) and 22.5 µl molecular biology grade water. The thermalcycling consisted of one cycle at 94 $^{\circ}C$ for 5 min followed by 40 cycles of denaturing (94 $^{\circ}C$: 1 min), annealing (58 $^{\circ}C$: 1 min) and extension (72 $^{\circ}C$: 3.5 min) with a final extension at 72 $^{\circ}C$ for 5 min. The *Neospora* PCR control consisted of DNA extracted from the brain of a sero-positive aborted bovine foetus with histological lesions suggestive of parasitic encephalitis and whose dam was also serologically positive for *N. caninum*.

The primers for the *T. gondii* B1 gene PCR have been previously described by Burg et al. (1989). The reaction mix for this PCR was as follows, 5 µl of extracted DNA, 5 µl Taq buffer (10X), 1.5 µl MgCl (50 mM), 2.5 µl dNTPS (10 mM mix), 1 µl forward primer (100 ng), 1 µl reverse primer (100 ng), 0.25 µl Taq Polymerase (5 units/µl, Bioline, UK), 33.75 µl molecular biology grade water. The thermocycling profile comprised of one cycle at 95 $^{\circ}C$ for 5 min followed by 35 cycles of denaturing (95 $^{\circ}C$, 1 min), annealing (55 $^{\circ}C$, 1 min) and extension (72 $^{\circ}C$, 1 min) with a final extension at 72 $^{\circ}C$ for 5 min. *Toxoplasma gondii* positive tissue from two sources was used to provide DNA controls. The tissues included ovine placenta from an aborted lamb, in which *T. gondii* tachyzoites were observed on histological examination and brain from a mouse experimentally infected with *T. gondii*. This latter tissue was a gift from Mr Stephen Wright (Moredun Institute, UK)

Amplification for both PCR reactions was performed in a PT-200 Programmable Thermal Controller (MU Research, USA). The amplicons were analysed on 2.0% agarose and visualised after staining with ethidium bromide.

DNA for the microsporidia PCR was extracted from the brains with histological lesions using QIAmp DNA mini kit (Qiagen GmbH, Germany). The PCR reaction using primers Mic3U and Mic421U was carried out with a few modifications according to Kock et al. (1997). These modifications included an amplication protocol of an initial denaturing cycle at 95 $^{\circ}C$ for 5 min and 35 cycles of denaturing (94 $^{\circ}C$, 29 s), annealing (67 $^{\circ}C$, 20 s), and extension (72 $^{\circ}C$, 20 s) with a final extension at 72 $^{\circ}C$ for 5 min. This reaction produces a 410–433 base pair (bp) fragment from the small subunit rRNA (ssrRNA) gene depending on the microsporidian genus and species. DNA extracted from *Enterocytozoon bieneusi* was used as a microsporidian positive control. In the negative control double distilled molecular grade water (Sigma, Austria) was substituted for the isolated test DNA.

The amplified products were visualised by silver staining after polyacrylamide electrophoresis (Amersham Pharmacia, Austria). The amplicons were purified with a purification kit (Amersham Pharmacia, Austria) and sequenced by direct sequencing using a sequencing kit (ABI PRISM BigDye sequencing kit, Applied Biosystems, Germany) and an automatic sequencer (310 ABI PRISM, PE Applied Biosystems, Germany). Sequences were obtained from both strands and sequence data were processed with the GeneDoc sequence editor (Nicholas et al., 1997). The sequences were compared to published sequences from various microsporidia by a BLAST search.

In order to further clarify these initial sequencing results two *Encephalitozoon* species specific PCR assays were run using SINTF and SINTR and ECUNF and ECUNR primer sets for *E. intestinalis* and *E. cuniculi*, respectively, according to the protocol described by Valencakova et al. (2005). Both sets of primers produce an amplicon of approximately 550 bp of the respective ssrRNA gene. In *E. intestinalis* this fragment is located between positions 366 and 869 and in *E. cuniculi* between positions 365 and 872. The amplicons were visualised by staining with ethidium bromide after electrophoresis in 2% agarose gel. The amplified products were excised from the gel, purified with a purification kit (Amersham Pharmacia, Austria) and sequenced as per the method previously described for the products from the initial microsporidia PCR.

Since the generic microsporidia primer pair MIC3U and MIC421U amplify a product which over laps with

the fragments produced by the SINT and ECUN primer sets their sequences were merged to produce a 845 bp fragment for *E. intestinalis* and 848 bp fragment for *E. cuniculi*. The resultant sequences were aligned with sequences from other micosporidia available in GenBank using multiple sequence alignment (Clustal X; Thompson et al., 1997). The alignments were assessed by eye and revised manually. Primer sites, unique gaps and ambiguously aligned sites were excluded from the analysis. Cluster analyses were performed with different evolutionary models including maximum likelihood, neighbour joining and maximum parsimony (Felsenstein, 1989). The cluster analysis was rooted using *Saccharomyces cervisiae* and *Ascocalyx abietina* as outgroups. The confidence of the branching order was assessed by the generation of 1000 bootstrap replicates.

Sequence data were deposited in GenBank and are available under the following accession numbers: DQ453122 (strain 1231), DQ453123 (strain 1268).

3. Results

Thoracic fluid was available for testing from foxes in 16 counties. The number of foxes tested in each county varied from two to 18. A titre of 1/100 or greater was taken as being indicative of infection. The overall prevalence of *N. caninum* and *T. gondii* was 2.7% (95% confidence interval (CI): 1.1–6.1%) and 55.8% (95% CI: 51.8–65.2%), respectively (Table 1). Antibodies to *N. caninum* were found in six foxes, two each from counties Tipperary and Wexford and one fox in each of the counties Westmeath and Wicklow. Four of these foxes were also seropositive to *T. gondii*. The prevalence of *T. gondii* infection varied from county to county and ranged from 18.7% to 88.9%. The counties with the highest prevalence included Kildare (88.9%, 95% CI: 54.2–99.4), Limerick (82.4%, 95% CI: 57.3–95.3), Louth (78.6%, 95% CI: 50.4–94.3%) and Clare (75.0%, 95% CI: 26.8–98.6%).

Pathological changes suggestive of mild parasitic encephalitis caused by protozoa were observed in 33 brains (22.3%, 95% CI: 16.0–30.0) of the 148 brains examined for central nervous system (CNS) lesions. These lesions ranged from focal to multifocal unilateral perivascular cuffing to foci of mononuclear cellular infiltration around an area of neurophil necrosis. In a few of the brains there was a diffuse infiltration of mononuclear cells in the cerebrum (Fig. 1).

Thoracic fluid was available from only 110 of the 148 foxes examined for brain lesions. A total of 30 (27.2%, 95%CI: 19.4–36.7) had histological changes and of these 19 (17.2%, 95% CI: 11.0–25.9) were seropositive to *T. gondii* and one fox (0.9%, 95% CI: 0.1–5.7) had antibodies to both *T. gondii* and *N. caninum* (Table 2). Eighty animals had no brain pathology and of these 55 (50.0%, 95% CI: 40.4–59.6) had antibodies to *T. gondii*. One fox (0.9%, 95% CI: 0.1–5.7) was seropositive to *T. gondii* and *N. caninum* and the remaining 24 (19.1%, 95% CI: 14.7–30.9) were serologically negative for both parasites.

The *T. gondii* PCR assay was positive in one fox and this animal was also seropositive (Table 2). The PCR assay for *N. caninum* was negative in all 33 foxes tested. However, microsporidian rRNA gene DNA was amplified from the brains of two foxes (Fig. 2a) giving a 421 bp amplicon in fox no. 1231 and a 419 bp product in fox no. 1268. Sequencing these amplicons revealed a 100% homology with *E. intestinalis* in fox no. 1231 and with *E. cuniculi* in fox no. 1268. The *Encephalitozoon* species specific PCRs using the SINT and ECUN primer

Table 1

Reciprocal immunofluorescence antibody test (IFAT) titres to *N. caninum* and *T. gondii* in thoracic fluid from 220 red foxes collected in rural Ireland

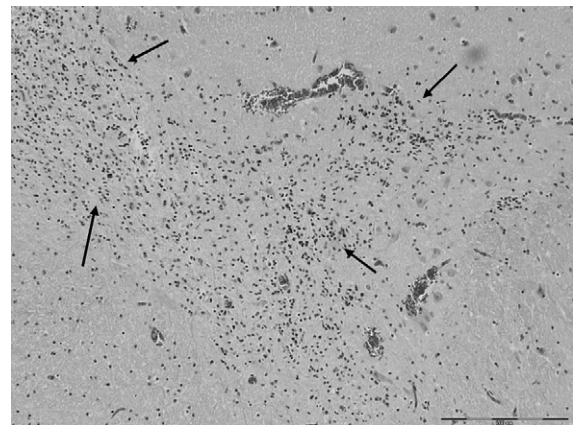| Reciprocal titre	N. caninum		T. gondii	
	Number of foxes	Percentage	Number of foxes	Percentage
<50	214	97.3	74	35.9
50	–	–	17	8.3
100	–	–	21	10.2
200	–	–	37	18.0
400	–	–	39	18.9
800	3	1.3	7	3.4
1600	–	–	5	2.4
3200	–	–	5	2.4
6400	3	1.3	–	–
12800	–	–	1	0.5

Fig. 1. Diffuse gliosis (arrows) in cerebrum of fox.

Table 2

Results of immunofluorescent antibody tests on thoracic fluid for antibodies to *N. caninum* and *T. gondii* and PCR amplification of *N. caninum*, *T. gondii* and *Encephalitozoon* DNA in brain tissue from foxes exhibiting histopathological lesions in their brains

Pathological lesions present in the brain	Serology		PCR		
Number of foxes	*N. caninum*	*T. gondii*	*N. caninum*	*T. gondii*	*Encephalitozoon* spp.
17	−	+	−	−	−
1	−	+	−	+	−
1	+	+	−	−	−
1	−	+	−	−	+
1	−	−	−	−	+
9	−	−	−	−	−
3[a]	ND	ND	−	−	−

[a] Thoracic fluid not available from these animals.

pairs were also positive. In the case of fox 1268 producing one clearly defined band of approximately 550 bp (Fig. 2b). In addition to the expected 550 bp fragment two further bands were also observed in fox 1231(Fig. 2b). These supplementary bands were probably due to the low annealing temperatures of
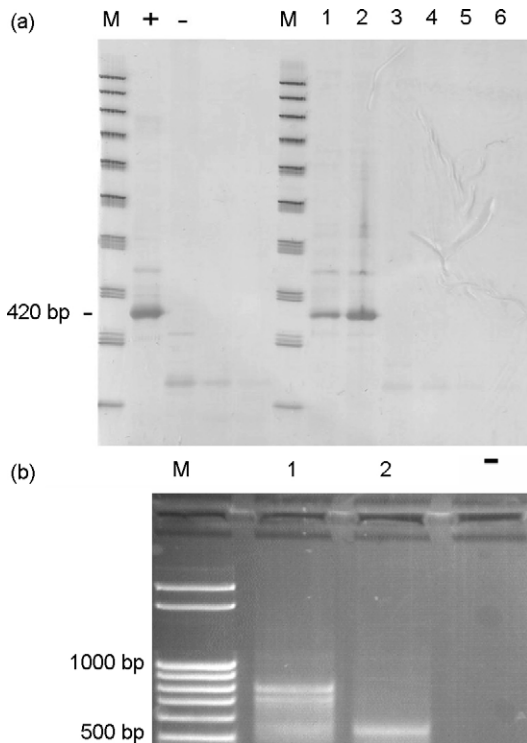

Fig. 2. (a) Silver stained polyacrylamide electrophoresis of the MicU3 and Mic421U amplified DNA fragment from the brain of fox no. 1231 and fox no. 1268, respectively. (M) marker; (+) positive control (amplified DNA fragment of *E. bieneusi*); (−) negative control; (1) fox no. 1231; (2) fox no. 1268; (3–6) negative foxes. (b) Agarose gel electrophoresis of the SINT and ECUN amplified DNA fragments from the brain of foxes no. 1231 and 1268, respectively. (M) marker; lane 1: fox 1231, SINT fragment; lane 2: fox no. 1268, ECUN fragment; (−) negative control.

the SINT primers. The sequence of the amplicon from fox 1268 showed a 100% base identity with five *E. cuniculi* sequences (GenBank accession nos: LI17072, AJ005581, X98467, AL590444 and AL391737). The sequence from fox 1231 had a 100% homology to the CDC:V297 *E. intestinalis* isolate (GenBank accession no: UO9929) and one base pair difference to another *E. intestinalis* isolate (GenBank accession no: L39113) and the unassigned *Encephalitozoon* sequences, L16866 and L16867. It also had a 10 bp difference with another *E. intestinalis* (kt1) isolate (GenBank accession no: L19567).

In the case of Fox 1231, the merged 850 bp sequence of the amplicon from MIC3U/MIC421U primer set with the product from the SINT primers had a 96–100% identity with *E. intestinalis* sequences listed in GenBank. Likewise, the merged sequence (sequence of the amplicon from MIC3U/MIC421U primer set merged with the sequence of the product from the ECUN primers) for fox 1268 was 99–100% homolous to *E. cuniculi* sequences in GenBank. The overall sequence identity between *E. intestinalis* and *E. cuniculi* is around 88% and between *E. intestinalis* and *Encephalitozoon hellem* around 89%. The *E. cuniculi* sequence is about 88% identical with the sequence of *E. hellem*. In cluster analysis the sequence from 1231 clustered with *E. intestinalis* and *E. hellem* and the sequence from 1268 clustered with *E. cuniculi*

In fox no 1231 a focal area of gliosis was observed in the cerebrum and it was seronegative for antibodies to *T. gondii* and *N. caninum* (Table 2). There were mild focal vascular cuffing lesions in the brain of fox no. 1268 and it was also seropositive for *T. gondii* (Table 2).

4. Discussion

In previous serological surveys of red foxes the IFAT titre selected as indicating specific antibody varied from

1/50 to 1/200 depending on the type of body fluid used (Trees et al., 1993; Bjorkman et al., 1994; Dubey and Lindsay, 1996; Jabubek et al., 2001; Hamilton et al., 2005; Wanha et al., 2005). In this study thoracic fluid containing clotted blood was used and notwithstanding the concentration of immunoglobulins in body fluids being less than in blood a titre of 1/100 was taken to indicate specific sero-conversion to either parasite. This cut off titre was selected to minimise any possibility of non-specific binding occurring due to microbial contamination or any extraneous proteins etc that may have been present in the thoracic fluid. On this basis 115 (56%) foxes were considered to have been exposed to *T. gondii*. Although, it is possible that a proportion of foxes with 1/50 titres may also have been infected. Similarly, only six (3%) were infected with *N. caninum*.

The presence of specific antibodies in wild carnivores is a sign that *T. gondii* and *N. caninum* are present in the environment and also emphasises the danger of infection to farm animals and humans. The results suggest that there is a high level of *T. gondii* circulating in rural Ireland and contaminating the diet of foxes. A study on perinatal lamb mortalities found that over 8% of the deaths were due to *T. gondii* (Dwyer, 1991). The prevalence of 56% reported here was comparable to a prevalence of 47% reported previously for a smaller cohort of 51 foxes caught in and around metropolitan Dublin (Wolfe et al., 2001). These results are slightly higher than Austrian, British and Swedish studies in which 20%, 35% and 38%, respectively of foxes had antibodies to *T. gondii* (Jabubek et al., 2001; Hamilton et al., 2005; Wanha et al., 2005). Higher exposure rates are present in Belgium and USA with seroprevalences of between 85% and 98% being recorded (Buxton et al., 1997; Dubey et al., 1999).

Three percent of foxes in this study had antibodies to *N. caninum*. Previous serological surveys in Ireland and the UK, two countries with similar rural environment and animal husbandry practices, have recorded a sero-prevalence of 1.4% and 0.9%, respectively (Wolfe et al., 2001; Hamilton et al., 2005). The infection rate amongst foxes in continental Europe varied between zero in Austria and Sweden to 17% in Belgium (Buxton et al., 1997; Jabubek et al., 2001; Wanha et al., 2005). This study continued the trend observed in previous studies that there is a lower prevalence of antibodies to *N. caninum* in wild canids than to *T. gondii* (Buxton et al., 1997; Wolfe et al., 2001; Jabubek et al., 2001; Hamilton et al., 2005; Wanha et al., 2005). This pattern may be an indication that *N. caninum* is less widespread in the environment, possibly, due to the definitive host, the

dog, excreting relatively few oocysts when infected (McAllister et al., 1998).

Although *N. caninum* shares several antigens with *T. gondii*, serological evidence suggests that the IFAT is specific (Dubey and Lindsay, 1996). Four of the foxes with antibodies to *N. caninum* had also seroconverted to *T. gondii* albeit at a lower titre. This finding is an indication that the habitat of these foxes was contaminated with both parasites. The foxes had been caught in Counties Kildare, Tipperary, Westmeath and Wicklow, areas where sheep and cattle rearing is the main agriculture activity.

It is often difficult to detect pathological changes induced by protozoa in larger animals because the number of parasitic cysts may be low and the size and weight of tissue that can be analysed by histology is small (Estobono-Reondo et al., 1999). Detection of circulating antibodies is more straightforward and appears to give a more accurate indication of infection and prevalence rates. In this study a limited number of brains was examined and over half of the foxes had no significant histological changes in their brains but had antibodies to *T. gondii*. Parasitic encephalitis lesions were observed in less than 20% of the sero-positive animals. However, the presence of CNS lesions may increase the likelihood of sero-conversion as two thirds of the foxes with pathological changes in this study had antibodies to either *T. gondii* or *N. caninum*.

PCR analysis of the brains with lesions failed to detect any *N. caninum* DNA and only one sample was positive for *T. gondii*. This result was not surprising as a previous study in the UK also reported negative PCR results with DNA extracted from the tongue of a cohort of 61 foxes sampled from a population with a *T. gondii* prevalence of 20% (Smith et al., 2003; Hamilton et al., 2005).

Encephalitozoon cuniculi is widely distributed in nature and infects a wide range of vertebrates (Canning and Lom, 1986). This is the first report of its presence amongst wildlife in Ireland. Its route of transmission is unknown but it is thought that carnivores get infected through predation. In the blue fox and dog the kidney is one of the predilection organs and urine from infected animals may be a source of environmental contamination with *E. cuniculi* (McCully et al., 1978; Akerstedt et al., 2002). The current trend of active recreation amongst wildlife habitats brings humans into closer contact with the sylvatic cycle of many infectious agents than heretofore. These recreational pursuits, such as hill and forest walking, increase the opportunities for parasites to cross the species boundary and cause disease in humans. In recent years *E. cuniculi* has

emerged as a pathogen of immunocompromised and immunocompetent patients (Weber et al., 1994; Didier, 2005).

Encephalitozoon intestinalis has been identified in the faeces of a number of animal species including goat, pig, cattle, dog and birds (Bornay-Llinares et al., 1998). It is the second most prevalent microsporidial species causing disease in immunosuppressed patients, although the role of animals in the epidemiology of human infections remains to be clarified (Mathis et al., 2005). This is the first account of *E. intestinalis* in wild mammals in Ireland and to our knowledge the first identification of this parasite in foxes in general. However, a previous study of waterborne zoonotic parasitic diseases found spores of *E. intestinalis* in the freshwater filter-feeder zebra mussel (*Dreissera polymorpha*) in the river Shannon (Graczyk et al., 2004). Taken together these two reports indicate that this parasite which, has zoonotic potential, is circulating in the environment in Ireland.

In conclusion this study has shown that the prevalence of *T. gondii* amongst red foxes in Ireland is greater than in the United Kingdom and in the majority of European countries from whom published reports are available. The number of foxes infected with *N. caninum* is also greater than the rest of Europe with the exception of Belgium. The results confirm the trend observed in most countries that *N. caninum* is less widespread in the environment than *T. gondii*. Evidence of the microsporidian species *E. cuniculi* and *E. intestinalis* infection amongst terrestrial wildlife in Ireland is presented for the first time. The importance of *Encephalitozoon* species in veterinary medicine appears to be increasing especially amongst animals reared in intensive production units, e.g. rabbits, artic foxes, fish, zoo animals and birds. The prevalence of microsporidia infection amongst AIDS patients is 15% and clinical microsporidiosis can also occur in immunocompetent individuals (Didier, 2005). In order to implement appropriate control and preventative strategies it is necessary to understand their epidemiology. At present the source and route of transmission of these organisms is uncertain. It is important that efforts continue to be made to identify domestic and wildlife animal reservoirs, sources of environmental contamination and also to clarify the zoonotic potential of microsporidia.

Finally, since all the parasites discussed in this study have been shown to be opportunistic pathogens of immunocompromised individuals, it behoves us to gain a better understanding of their distribution in nature so as to better prevent inadvertent infections of humans.

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