



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Seroprevalence of *Encephalitozoon cuniculi* in Wild Rodents, Foxes and Domestic Cats in Three Sites in the United Kingdom

Citation for published version:

Meredith, AL, Cleaveland, SC, Brown, J, Mahajan, A & Shaw, DJ 2015, 'Seroprevalence of *Encephalitozoon cuniculi* in Wild Rodents, Foxes and Domestic Cats in Three Sites in the United Kingdom' *Transboundary and Emerging Diseases*, vol. 62, no. 2, pp. 148-156. DOI: 10.1111/tbed.12091

Digital Object Identifier (DOI):

[10.1111/tbed.12091](https://doi.org/10.1111/tbed.12091)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Peer reviewed version

Published In:

Transboundary and Emerging Diseases

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



Seroprevalence of *Encephalitozoon cuniculi* in wild rodents, foxes and domestic cats in three sites in the United Kingdom

Meredith A. L¹, Cleaveland S.C², Brown J³, Mahajan, A¹, Shaw D.J.¹.

1 Royal (Dick) School of Veterinary Studies & The Roslin Institute, University of Edinburgh, Easter Bush Campus, Roslin, Midlothian, EH25 9RG.

2 Institute of Biodiversity, Animal Health and Comparative Medicine, The University of Glasgow, Graham Kerr Building, Glasgow, G12 8QQ,

3 School of Clinical Sciences, University of Edinburgh, The Chancellor's Building, 49 Little France Crescent, Edinburgh, EH16 4SB

Corresponding author: Anna Meredith, Royal (Dick) School of Veterinary Studies, Easter Bush Campus, Roslin, Midlothian EH25 9RG. Email: Anna.Meredith@ed.ac.uk

Short title: *E. cuniculi* in UK wildlife

Summary

Encephalitozoon cuniculi is an obligate intracellular microsporidian that is the causal agent of encephalitozoonosis, an important and emerging disease in both man and animals. Little is known about its occurrence in wildlife. In this study, serum samples from 793 wild rodents (178 bank voles, 312 field voles, 303 wood mice) 96 foxes and 27 domestic cats from three study areas in the UK were tested for the presence of antibodies to *E. cuniculi* using a direct agglutination test (DAT). Seroprevalence in the wild rodents ranged from 1.00% to 10.67% depending on species (overall 5.31%), and was significantly higher in foxes (49.50% (50/96)). None of the 27 cats sampled were found to be seropositive. This is the first report of seroprevalence to *E. cuniculi* in bank voles, field voles, wood mice, foxes and cats in the UK, and provides some evidence that foxes could act as sentinels for the presence of *E. cuniculi* in rodents. The study demonstrates that wildlife species could be significant reservoirs of infection for both domestic animals and humans.

Key words

Encephalitozoon cuniculi, wildlife reservoir, sentinel

Introduction

Encephalitozoon cuniculi is an obligate intracellular microsporidian that is the causal agent of encephalitozoonosis, an important and emerging disease in both man and animals (Halanova et al., 2003; Mathis et al., 2005; Wasson and Peper, 2000). Microsporidia are now considered to be highly derived fungi that have one of the smallest eukaryotic genomes described (Katinka et al., 2001). The spores contain a unique long, coiled tubular extrusion apparatus, the polar tube, which distinguishes microsporidia from all other organisms (Müller 1997).

E. cuniculi is the most widely studied mammalian microsporidian and infections in domestic and laboratory rodents and rabbits are well documented (Wasson and Peper, 2000; Thomas et al., 1997). It is also known to infect shrews, cats, dogs, sheep, goats, pigs, horses, and captive nonhuman primates (Mathis et al., 2005; Canning and Lom, 1986). Human infection occurs in immunosuppressed individuals and *E. cuniculi* has emerged as a zoonosis with the advent of HIV/AIDS (Didier et al., 1996a; Fournier et al., 2000; Kodjikian et al., 2005; Rossi et al., 1998; Schwartz et al., 1994; Snowden et al., 1999). Despite this little is known about the occurrence of *E. cuniculi* in wildlife.

The life cycle of *E. cuniculi* is simple and direct, and, like other microsporidia, involves a proliferative merogonic stage, followed by a sporogonic stage resulting in rupture of the host cell and release of small (1.5 x 2.5µm), environmentally resistant, infective spores. Infection is usually horizontal by ingestion or inhalation of spores, which are shed mainly in the urine. Spores are extremely resistant in the environment and can survive for many months in humid environments (Li et al., 2003). Vertical transmission also occurs and is well documented in rabbits, dogs and foxes (Wasson and Peper, 2000).

In animals the main target organs are the central nervous system and the kidney, which can result in a granulomatous encephalitis and nephritis. Infection in rabbits and rodents is

frequently subclinical, but infected animals can also exhibit neurological signs such as head tilt, paralysis and seizures, and renal failure. In carnivores, encephalitozoonosis is a severe neurological disease of neonatal animals, due to transplacental infection from chronically infected dams (Wasson and Peper, 2000), and is a major endemic disease problem and cause of economic losses in farmed blue foxes in Scandinavia (Akerstedt, 2002). In man, symptoms include fever, abdominal pain, nausea, vomiting, renal failure, ocular problems such as keratoconjunctivitis, pneumonitis, and neurological signs, and the organism can be recovered from urine, kidney, CSF and lung (Mathis et al., 2005). Despite the zoonotic potential and opportunistic nature of *E. cuniculi* being well recognised (Mathis et al., 2005) (Deplazes et al., 1996), the exact source of human infections is unclear. Some human patients infected with strain I (rabbit strain) recall exposure to rabbits (Mathis et al., 1997) (Weber et al., 1997), and some of those infected with strain III (dog strain) owned dogs (Didier et al., 1996b; Teachey et al., 2004), but infection in the respective animals was not proven. As the spores of *E. cuniculi* are highly resistant in the environment (Li et al., 2003), direct contact with infected animals is not necessary for transmission to occur.

The main host for *E. cuniculi* is believed to be the rabbit (*Oryctolagus cuniculus*), with seroprevalence ranging from 15% to 75% in healthy farmed and pet rabbits (Ashmawy et al., 2010; Dipineto et al., 2008; Harcourt-Brown and Holloway, 2003; Igarashi et al., 2008; Keeble and Shaw, 2006; Okewole, 2008; Santaniello et al., 2009), . However, seroprevalence in wild rabbits has been poorly studied. *E. cuniculi* has also been described in laboratory rodents, including in the UK (Gannon, 1980), but again prevalence in wild rodents is poorly described and has never been reported in the UK. In farmed blue foxes infection is attributed to ingestion of food contaminated with infected rodent urine or faeces as well as transplacental transmission ((Akerstedt, 2002; Canning and Lom, 1986). However, there is limited information on the disease in wild carnivores, with the only two reports being of *E. cuniculi* detected in the brains

of foxes; one in the UK (Wilson, 1979), and one in Ireland (Murphy et al., 2007).

Antibody detection by serology is the most important tool in the diagnosis of *E. cuniculi* in living animals (Kunzel and Joachim, 2010) and man (Mathis et al., 2005), as histological examination of neural or renal tissue is generally not possible and shedding of spores in urine or faeces, for detection by direct or molecular techniques, can be intermittent. A direct agglutination test (DAT) methodology has recently been developed (Jordan et al., 2006), which does not require species-specific reagents and avoids the use of any specialised equipment, and is therefore recognised as a useful approach for examining multiple exotic or wildlife species, as in this study. Furthermore, this test has been validated in rodents (mice) and canids (dogs), and has also been used in wild raccoons, beavers and lemurs (Jordan et al 2006; Yabsley et al 2007). While ELISA and IFA testing are the two serologic tests commonly used in research and hospital laboratories, with IFA considered the "gold standard", these methods have distinct disadvantages, in that each method requires the use of species-specific antibodies and specialized equipment, and much cross reactivity occurs between *Encephalitozoon* species using IFA, with genetic analysis often required to confirm the infective species. The DAT methodology has been published as being highly sensitive and specific for antibodies to *E. cuniculi*, and cross-reactivity is reduced compared to IFA (Jordan et al 2006).

The current status of *E. cuniculi* infection in UK wildlife is unknown, There have been no published reports detecting *E. cuniculi* in UK wildlife since 1979, and then only in three rabbits and one fox (Wilson, 1979). The aim of this study was to investigate the seroprevalence of *E. cuniculi* in three species of wild rodents, foxes and also domestic cats known to hunt and consume wild rodents in three geographically distinct areas in the UK using a DAT.

Materials and methods

As part of a study looking at the seroprevalence of several diseases of wildlife in the UK, serum samples were collected from wild rodents, foxes and domestic cats in three rural study sites of varying habitat in northern England (Cumbria) and Scotland (Pentlands and Borders) (Figure 1) over a twenty-nine month period (April 2007- October 2009). The three areas are representative of a variety of habitats where these wildlife species are to be found, ranging from semi-urban to rural.,

Wild rodents were trapped in Ugglan No.2 traps with a storm roof (Grahnb, Sweden) set out in grids of 25 traps (5 x 5; approx. 10m spacing). Traps were placed, prebaited with carrot and grain and left open for 3 days, then set for 3 days and checked daily. Rodents were humanely euthanased using an overdose of volatile anaesthetic (isoflurane) and cervical dislocation, and blood was immediately collected by cardiac puncture. Rodent trapping was carried out over four seasons (April-August 2007; November 2007 - March 2008; April - August 2008; November 2008-December 2008).

Foxes were shot as part of routine pest control operations in the three areas and blood collected from the thoracic or abdominal cavity within 24 hours of death. Cat blood samples were obtained via veterinary surgeons with practices in the three areas, from animals presented for veterinary examination where a blood sample was routinely taken, and surplus serum was submitted for the study. An owner questionnaire was used to confirm that the cats submitted for the study were known to hunt and consume wild rodents.

For all samples, serum was separated by centrifugation at 4,000rpm within 24 hours of collection and stored at -70°C. Foxes and cat samples were collected opportunistically throughout the study period. Location (grid reference), age class (adult/ non-adult), and sex were recorded for all animals.

Direct agglutination test

A direct agglutination test was developed in accordance with the protocol described by Jordan *et al.* (2006). Briefly, Madin Darby canine kidney (MDCK) cells were cultured and maintained in 150cm² flasks at 37°C in a 5% CO₂ incubator, and passaged every 2-3 days using MDCK growth medium containing penicillin and streptomycin under biosafety level 2 conditions.

Spores of canine subtype species *E. cuniculi* were imported from the USA (American Type Culture Collection, Manassa, VA, USA; ATCC no 50522™) and inoculated aseptically onto confluent MDCK cells with fresh medium without penicillin/streptomycin. The *E. cuniculi* culture was maintained over 6 weeks by removing the medium containing the spores every 2-3 days, centrifuging at 1300g for 10 minutes, resuspending the pellet in fresh medium and transferring this to a fresh flask of confluent MDCK cells. The microsporidia could be visualised with light microscopy within the MDCK cells as refractile intracytoplasmic structures. In parallel to regular passage into fresh flasks, infected flasks were maintained for several weeks, in order to build up large amounts of microsporidial spores.

E. cuniculi spores were harvested and killed by collecting supernatant, centrifuging at 1300g, resuspending the pellets and fixing in 2ml 4% formaldehyde solution for 10-15 seconds, then diluted with PBS and storing at 4°C overnight. The fixed spores were then centrifuged at 1300g and the pellets washed in PBS twice to remove the formaldehyde, and the final pellets resuspended in 1ml PBS and pooled to make a total volume of 10ml in PBS. 0.1ml 10% sodium azide was added to this final antigen solution to prevent bacterial growth. Spores were stained with 0.1% SYBR® green 1 (Applied Biosystems, Life Technologies Corporation, California, USA) and counted using fluorescent microscopy.

The DAT was conducted in 96-well round-bottom plates. An optimum concentration of spores for the test was determined by making up a solution containing 1×10^7 spores/ml using an

alkaline-eosin buffer plus 0.2 mM mercaptoethanol. A test plate was set up with doubling dilutions of this antigen solution tested in duplicate against a 1:10 dilution in PBS of both a positive control rabbit serum (1:640 titre as obtained by a commercial ELISA, Pinmoore Animal Laboratory Services Ltd) and a negative control normal rabbit serum (R9133, Sigma). 25 µl of control serum was combined with 75 µl antigen solution and mixed thoroughly in each well by pipetting up and down several times. The plate was sealed and incubated at 37°C overnight.

On visualisation over a lightbox, positive sera formed a diffuse opacity across the central portion of the well, whereas negative sera formed a discrete point in the central portion of the well as described (Jordan et al., 2006). The optimum concentration for antigen solution was determined empirically as a 1:6500 dilution of the stock solution (10^4 spores/ml). This was then used to determine an optimum dilution for test sera of 1:25, by titration of the positive and negative control serum. At this dilution on repetition of the test the positive and negative controls were clearly interpretable.

Test sera were diluted 1:25 and 25µl added to 75 µl alkaline-eosin buffer solution and incubated overnight at 37°C. Positive and negative control rabbit sera were tested on each plate. Photographic images of plates were recorded using a Kodak Image station 440 with UV illumination.

Data analysis

For the statistical analysis of variation in seroprevalence between host species and within host species R (v 2.15.0 The R Foundation for Statistical Computing) was used. Generalised linear mixed-effect models with binomial errors (*GLMEb*) were used for the analysis. For the main set of analyses which study area the samples came from was entered as a random effect to accommodate area related infection pressures. In addition, the individual study sites within areas were entered as a random effect nested within study area for rodent species. The sex and age of

the host species and the season the animals were sampled were incorporated as fixed effects in different univariate models. For age, animals were classed as either adult or non-adult (juvenile and subadult). In addition, differences between the seroprevalences of the 3 rodent species within each study site were also considered in separate *GLMEb* with just study site as the random effect. The spatial clustering of cases in each study area in both rodents and predator species was investigated using Bernoulli statistical spatial models of the number of positive animals at a particular location (Kulldorff 1997) in SaTScan (v9.1.1 www.satscan.org). Statistical significance level was placed at $P < 0.05$.

Results

A total of 916 serum samples were tested for antibodies to *E. cuniculi*, from 793 rodent species (178 bank voles (BV), 312 field voles (FV), 303 wood mice (WM)), 27 cats and 96 foxes (**Table 1, Figure 1**). The majority of samples for both rodents and foxes were collected in seasons 1 to 3, and in season 4 only the Cumbria study area was sampled for rodent species (**Figure 2**). Unfortunately, most of the cat samples were obtained after season 3, with cat samples from the Borders and Pentlands study areas collected up to over a year after the last rodent sampling in those areas. Fox samples were collected throughout the study. All 3 rodent species were found in the 3 study areas with 17/28 (61%) of the individual rodent trapping locations within study areas having all 3 rodent species and only 3 rodent trapping sites had one rodent species (**Figure 1**).

Overall seroprevalence

Overall, all 3 rodent species were found to be seroprevalence for *E. cuniculi*. Seroprevalence varied from 10.67% in bank voles, 5.77% in field voles to 1.0% in wood mice (5.04% for all rodent species, **Table 1**). Foxes were also found to be seroprevalence, with much higher levels – 50/96 foxes (52.1%). In contrast, none of the 27 cats were found to be seropositive.

Rodent species

Within individual rodent species, seroprevalence was significantly higher overall in bank voles and field voles compared to wood mice ($P < 0.014$), but there was no difference in seroprevalence between field and bank voles ($P = 0.126$) or within study areas ($P > 0.075$). In addition, there was no significant difference in seroprevalence in bank voles (10.0-15.8%) or field voles (3.9-6.9%) between study sites ($P > 0.616$), though only 19 bank voles were obtained from the Pentlands site. No wood mice were found to be seropositive in the Pentlands or Borders study sites compared to 3.1% in the Cumbria site. However, the seroprevalence of the wood mice in Cumbria was not significantly different from that of the field and bank voles from Cumbria ($P > 0.173$, **Table 1**).

There was no difference in seroprevalence between either the 4 trapping seasons (overall $P > 0.987$; 4.8-6.5%) or if only the 3 full trapping seasons were considered ($P > 0.937$; 4.8-5.5%). This lack of difference was maintained if seasonal variation for each rodent species ($P > 0.346$) were considered. Eighteen of the individual rodent trapping locations had rodents with a seroprevalence $> 0\%$, with less than 5 rodents obtained from 5 of the trapping locations with 0% seroprevalence. There was no evidence of spatial clustering of infection within rodents in any of the study areas ($P > 0.076$, **Figure 3**). Finally, seroprevalence did not differ significantly between males (16/405; 4.0%) and females (22/380; 5.8%, $P = 0.228$), nor between sexes in individual rodent species (BV M=7.8%, F=12.2%; FV M=5.6%, F=6.0%; WM M=0.6%, F=1.6%, $P > 0.342$). Only adult rodents were seropositive (38/703; 5.4%),

Foxes

There was also no significant difference in seroprevalence in foxes between the study areas (44.7-60%, $P > 0.417$), though only 15 foxes were obtained from the Pentlands study site. There was also no statistical evidence of spatial clustering of infection within foxes within each study area ($P > 0.165$, **Figure 3**). In addition, seroprevalence was not significantly different between

sexes (M=17/31=64.3%, F=27/41=54.8%, P=0.416) and seroprevalence did not differ significantly between adult (62.3%) and non-adult foxes (52.4%, P=0.436), though there were only 21 non-adult foxes.

Foxes and rodent comparison

Seroprevalence was statistically significantly higher in foxes than in rodents for the study overall (P<0.001, **Table 1, Figure 3**). Furthermore the seroprevalence in foxes was statistically significantly higher than all individual rodent species (P<0.001).

Discussion

The DAT employed in this study was, as described in the report by Jordan *et al* (2007), relatively quick and simple to perform and thought to be ideal for the current study due to its previous validation in mice and dogs. However, the poor quality of the serum from foxes, due to post mortem sampling, did cause some initial difficulties, as the opacity of the serum and particulate material made detection of the agglutination pellet less clear. Operator experience in looking at test results quickly overcame these initial difficulties. Although antigen solution was specifically prepared for this study, microwell plates pre-coated with *E. cuniculi* antigen are now commercially available (Medicago, Uppsala, Sweden) and could be utilised as the basis for a DAT.

This study is the first report of seroprevalence of *E. cuniculi* in wild rodents and foxes in the UK, indicating that wildlife are a potential reservoir for this infection. *E. cuniculi* has only been detected the brains of one hand-reared fox and three rabbits in the UK, in one study performed over 30 years ago (Wilson, 1979), and it has also been detected more recently in the brain of one fox in rural Ireland (Murphy et al., 2007).

Seropositive wild rodents have been detected previously in Switzerland where 13% (3/23) of

wild rats were seropositive, and the organism was isolated from the brain of one of these (Müller-Doblies et al., 2002). Specific antibodies against *E. cuniculi* were found in Iceland in 4% and 9% of *Apodemus sylvaticus* and *Mus musculus*, respectively, and these rodents were proposed to be a potential reservoir of infection and possible factor in the decline of the arctic (blue) fox population in this country (Hersteinsson et al., 1993). In an area at the Czech Republic-German Border 14.5% (42/289) faecal samples from wild house mice (*Mus musculus musculus* and *M.m. domesticus*) were positive by PCR for *E. cuniculi* (Sak et al., 2011).

Cats were not found to have any evidence of infection with *E. cuniculi* in this study. Cats have been shown to raise a detectable IgG response to *E. cuniculi* infection (Csokai et al., 2010; Hsu et al., 2011) so this finding either indicates a lack of sample size, a lack of exposure to this pathogen, or that the DAT is not appropriate for use in this species. A seroprevalence of 24% (17/72) of cats has been reported in Eastern Slovakia (Halanova et al., 2003) and a recent study in Virginia, USA found a seroprevalence of 6.5% (15/232) (Hsu et al., 2011); therefore a larger sample of cats would be required to have more confidence in the lack of infection result. Clinical disease in cats is reportedly rare and there are only three case reports of *E. cuniculi* associated with clinical disease in cats in the literature, in South Africa (van Rensburg and du Plessis, 1971), the USA (Buyukmihci et al., 1977) and Austria (Csokai et al., 2010). , Although the DAT has not been validated in domestic cats or other feline species, its validation and use in a wide variety of other domestic and wild species suggests that it would be likely to be appropriate. Further studies to validate its use in cats would be desirable.

E. cuniculi is primarily recognised as a disease of domestic rabbits, but it was not possible to test wild rabbits in this study. It has been demonstrated that the seroprevalence in domestic rabbits is high, with reported levels of 37%-68% (Kunzel and Joachim, 2010), with UK studies demonstrating a seroprevalence of 23% (Harcourt-Brown and Holloway, 2003) and 52% (Keeble and Shaw, 2006) in healthy pet rabbits. One Australian study found none of 823 wild

rabbits to be seropositive to *E. cuniculi* (Cox et al., 1980; Cox and Ross, 1980), but a later study found 20/81 (24.6%) of wild rabbits seropositive (Thomas et al., 1997). In the UK, only one study in 1979 reports the finding of *E. cuniculi* in wild rabbits, in three out of three rabbits tested from the Pentland hills in Scotland (Wilson, 1979), although a subsequent study in 1980 found no serological evidence of *E. cuniculi* in 175 wild rabbits from other areas of Scotland and England (Cox and Ross, 1980). Current seroprevalence in their wild counterparts in the UK remains unknown.

This study also demonstrated that seroprevalence to *E. cuniculi* is significantly higher in predators (foxes) than in their rodent prey. This could be interpreted as providing some evidence for possible bioconcentration by ingestion or close contact for this pathogen. Specific experimental studies demonstrating ingestion of infected prey by a predator as a means of establishing *E. cuniculi* infection have not been performed to the authors' knowledge, but ingestion of feed contaminated by infected urine, is well recognised as the main route of transmission for *E. cuniculi* spores (Canning and Lom, 1986), and rodent or fox carcasses are also traditionally implicated as a source of infection (Akerstedt and Kapel, 2003), so this is a plausible route of infection for the predator species in the present study. Experimental oral infection of adult arctic foxes has been shown to result in an antibody response that is detectable for at least one year after infection (Akerstedt, 2003). Indeed, the absence of rodents in the diet is cited as a possible explanation for the absence of *E. cuniculi* in wild Arctic foxes in Greenland (Akerstedt and Kapel, 2003), whereas in Iceland, where rodents have been shown to be seropositive, seroprevalence in Arctic foxes varied from 2-27% (Hersteinsson et al., 1993). In the UK, a seroprevalence of 13.3% has been found in stray dogs (Hollister et al 1989), and ingestion of infected rodents is a possible route of infection. *E. cuniculi* spores are also highly resistant, and predators are also likely to become infected by ingestion of infected urine in the environment. In farmed blue (Arctic) foxes, and other species such as rabbits, vertical

transmission *in utero* is believed to be an important route of infection (Wasson and Peper, 2000). Arctic fox pups infected in utero also produce a strong humoral immune response (Mohn, 1982), and so serological detection in the foxes in this study could also indicate vertical transmission and may be one means of perpetuating infection in a predator species.

There were marked species differences in seroprevalence between all species tested – among the rodents seroprevalence was 1% in wood mice, 5.77% in field voles and 10.67% in bank voles. Field voles and bank voles were seropositive in all three study areas but wood mice were only seropositive in the Cumbria study area. Of the two predator species tested the 96 foxes had a high seroprevalence of 52.1% compared to 0% in the 27 cats. This may reflect the different habitats from which fox and cat samples were obtained and level of exposure to wild rodents, rabbits or other sources of infection.

Overall, the study indicates that exposure to *E. cuniculi* in wildlife species is occurring in the UK, and that wildlife species have the potential to be significant reservoirs of infection for both domestic animals and humans. The finding that seroprevalence in foxes is significantly higher than in rodent prey species provides some evidence that foxes have the potential to act as a sentinel species for *E. cuniculi* infection.

References

- Akerstedt,J., 2002. An indirect ELISA for detection of *Encephalitozoon cuniculi* infection in farmed blue foxes (*Alopex lagopus*). Acta Vet. Scand. 43, 211-220.
- Akerstedt,J., 2003. Humoral immune response in adult blue foxes (*Alopex lagopus*) after oral infection with *Encephalitozoon cuniculi* spores. Vet. Parasitol 113, 203-210.
- Akerstedt,J. and C.M.Kapel, 2003. Survey for *Encephalitozoon cuniculi* in arctic foxes (*Alopex lagopus*) in Greenland. J Wildl. Dis 39, 228-232.
- Ashmawy,K.I., S.S.Abuakkada and A.M.Awad, 2010. Seroprevalence of Antibodies to *Encephalitozoon cuniculi* and *Toxoplasma gondii* in Farmed Domestic Rabbits in Egypt. Zoonoses. Public Health 58, 357-364.
- Buyukmihci,N., R.W.Bellhorn, J.Hunziker and J.Clinton, 1977. *Encephalitozoon (Nosema)* infection of the cornea in a cat. J Am Vet Med Assoc. 171, 355-357.
- Canning,E.U. and J.Lom, 1986: The microsporidia of vertebrates. Academic Press. London, UK.
- Cox,J.C., D.Pye, J.W.Edmonds and R.Shepherd, 1980. An investigation of *Encephalitozoon cuniculi* in the wild rabbit *Oryctolagus cuniculus* in Victoria, Australia. J Hyg. (Lond) 84, 295-300.
- Cox,J.C. and J.Ross, 1980. A serological survey of *Encephalitozoon cuniculi* infection in the wild rabbit in England and Scotland. Res. Vet. Sci. 28, 396.
- Csokai,J., A.Fuchs-Baumgartinger and G.Maass, 2010. Detection of *Encephalitozoon cuniculi*-infection (strain II) by PCR in a cat with anterior uveitis. Wiener Tierarztliche Monatsschrift, 210-215.
- Deplazes,P., A.Mathis, R.Baumgartner, I.Tanner and R.Weber, 1996. Immunologic and molecular characteristics of *Encephalitozoon*-like microsporidia isolated from humans and rabbits indicate that *Encephalitozoon cuniculi* is a zoonotic parasite. Clin. Infect. Dis 22, 557-559.
- Didier,E.S., G.S.Visvesvara, M.D.Baker, L.B.Rogers, D.C.Bertucci, M.A.De Groote and C.R.Vossbrinck, 1996. A microsporidian isolated from an AIDS patient corresponds to

Encephalitozoon cuniculi III, originally isolated from domestic dogs. J Clin. Microbiol 34, 2835-2837.

Didier,E.S., G.S.Visvesvara, M.D.Baker, L.B.Rogers, D.C.Bertucci, M.A.De Groote and C.R.Vossbrinck, 1996. A microsporidian isolated from an AIDS patient corresponds to *Encephalitozoon cuniculi* III, originally isolated from domestic dogs. J Clin. Microbiol 34, 2835-2837.

Dipineto,L., L.Rinaldi, A.Santaniello, M.Sensale, A.Cuomo, M.Calabria, L.F.Menna and A.Fioretta, 2008. Serological survey for antibodies to *Encephalitozoon cuniculi* in pet rabbits in Italy. Zoonoses. Public Health 55, 173-175.

Fournier,S., O.Liguory, C.Sarfati, F.vid-Ouaknine, F.Derouin, J.M.Decazes and J.M.Molina, 2000. Disseminated infection due to *Encephalitozoon cuniculi* in a patient with AIDS: case report and review. HIV. Med 1, 155-161.

Gannon,J., 1980. A survey of *Encephalitozoon cuniculi* in laboratory animal colonies in the United Kingdom. Lab Anim 14, 91-94.

Halanova,M., L.Cislakova, A.Valencakova, P.Balent, J.Adam and M.Travnicek, 2003. Serological screening of occurrence of antibodies to *Encephalitozoon cuniculi* in humans and animals in Eastern Slovakia. Annals of Agricultural and Environmental Medicine 10, 117-120.

Harcourt-Brown,F.M. and H.K.Holloway, 2003. *Encephalitozoon cuniculi* in pet rabbits. Vet. Rec. 152, 427-431.

Hersteinsson,P., E.Gunnarsson, S.Hjartardottir and K.Skirnisson, 1993. Prevalence of *Encephalitozoon cuniculi* antibodies in terrestrial mammals in Iceland, 1986 to 1989. J. Wildl. Dis. 29, 341-344.

Hollister,W.S., E.U.Canning and M.Viney, 1989. Prevalence of antibodies to *Encephalitozoon cuniculi* in stray dogs as determined by an ELISA. Vet. Rec. 124, 332-336.

Hsu,V., D.C.Grant, A.M.Zajac, S.G.Witonsky and D.S.Lindsay, 2011. Prevalence of IgG antibodies to *Encephalitozoon cuniculi* and *Toxoplasma gondii* in cats with and without chronic kidney disease from Virginia. Veterinary Parasitology 176, 23-26.

- Igarashi,M., E.Oohashi, G.Dautu, A.Ueno, T.Kariya and K.Furuya, 2008. High seroprevalence of *Encephalitozoon cuniculi* in pet rabbits in Japan. J Vet. Med Sci. 70, 1301-1304.
- Jordan,C.N., A.M.Zajac, K.S.Snowden and D.S.Lindsay, 2006. Direct agglutination test for *Encephalitozoon cuniculi*. Vet. Parasitol. 135, 235-240.
- Katinka,M.D., S.Duprat, E.Cornillot, G.Metenier, F.Thomarat, G.Prensier, V.Barbe, E.Peyretailade, P.Brottier, P.Wincker, F.Delbac, A.H.El, P.Peyret, W.Saurin, M.Gouy, J.Weissenbach and C.P.Vivares, 2001. Genome sequence and gene compaction of the eukaryote parasite *Encephalitozoon cuniculi*. Nature 414, 450-453.
- Keeble,E.J. and D.J.Shaw, 2006. Seroprevalence of antibodies to *Encephalitozoon cuniculi* in domestic rabbits in the United Kingdom. Vet. Rec. 158, 539-544.
- Kodjikian,L., J.G.Garweg, M.Nguyen, T.Schaffner, P.Deplazese and S.Zimmerli, 2005. Intraocular microsporidiosis due to *Encephalitozoon cuniculi* in a patient with idiopathic CD4+ T-lymphocytopenia. Int J Med Microbiol 294, 529-533.
- Künzel,F. and A.Joachim, 2010. Encephalitozoonosis in rabbits. Parasitol. Res. 106, 299-309.
- Li,X., R.Palmer, J.M.Trout and R.Fayer, 2003. Infectivity of microsporidia spores stored in water at environmental temperatures. J Parasitol. 89, 185-188.
- Mathis,A., M.Michel, H.Kuster, C.Muller, R.Weber and P.Deplazes, 1997. Two *Encephalitozoon cuniculi* strains of human origin are infectious to rabbits. Parasitology 114 (Pt 1), 29-35.
- Mathis,A., R.Weber and P.Deplazes, 2005. Zoonotic potential of the microsporidia. Clin. Microbiol Rev. 18, 423-445.
- Mohn,S.F., 1982. Experimental encephalitozoonosis in the blue fox. Clinical and serological examinations of affected pups. Acta Vet. Scand. 23, 503-514.
- Müller,M., 1997. What are the microsporidia? Parasitol. Today 13, 455-456.
- Müller-Doblies,U.U., K.Herzog, I.Tanner, A.Mathis and P.Deplazes, 2002. First isolation and characterisation of *Encephalitozoon cuniculi* from a free-ranging rat (*Rattus norvegicus*). Vet. Parasitol. 107,279-285.

- Murphy, T.M., J. Walochnik, A. Hassl, J. Moriarty, J. Mooney, D. Toolan, C. Sanchez-Miguel, A. O'Loughlin and A. McAuliffe, 2007. 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. *Vet. Parasitol.* 146, 227-234.
- Okewole, E.A., 2008. Seroprevalence of antibodies to *Encephalitozoon cuniculi* in domestic rabbits in Nigeria. *Onderstepoort J Vet. Res.* 75, 33-38.
- Rossi, P., R.G. La, A. Ludovisi, A. Tamburrini, M.A. Gomez Morales and E. Pozio, 1998. Identification of a human isolate of *Encephalitozoon cuniculi* type I from Italy. *Int J Parasitol.* 28, 1361-1366.
- Sak, B., M. Kvac, D. Kvetonova, T. Albrecht and J. Pialek, 2011. The first report on natural *Enterocytozoon bieneusi* and *Encephalitozoon* spp. infections in wild East-European House Mice (*Mus musculus musculus*) and West-European House Mice (*M. m. domesticus*) in a hybrid zone across the Czech Republic-Germany border. *Vet. Parasitol.* 178, 246-250.
- Santaniello, A., L. Dipineto, L. Rinaldi, L.F. Menna, G. Cringoli and A. Fioretti, 2009. Serological survey of *Encephalitozoon cuniculi* in farm rabbits in Italy. *Res. Vet. Sci.* 87, 67-69.
- Schwartz, D.A., R.T. Bryan, R. Weber and G.S. Visvesvara, 1994. Microsporidiosis in HIV positive patients: current methods for diagnosis using biopsy, cytologic, ultrastructural, immunological, and tissue culture techniques. *Folia Parasitol. (Praha)* 41, 101-109.
- Snowden, K., K. Logan and E.S. Didier, 1999. *Encephalitozoon cuniculi* strain III is a cause of encephalitozoonosis in both humans and dogs. *J Infect. Dis* 180, 2086-2088.
- Teachey, D.T., P. Russo, J.M. Orenstein, E.S. Didier, C. Bowers and N. Bunin, 2004. Pulmonary infection with microsporidia after allogeneic bone marrow transplantation. *Bone Marrow Transplant.* 33, 299-302.
- Thomas, C., M. Finn, L. Twigg, P. Deplazes and R.C. Thompson, 1997. Microsporidia (*Encephalitozoon cuniculi*) in wild rabbits in Australia. *Aust. Vet. J* 75, 808-810.
- van Rensburg, I.B. and J.L. du Plessis, 1971. Nosematosis in a cat: a case report. *Journal of the South African Veterinary Association* 42, 327-331.

Wasson,K. and R.L.Peper, 2000. Mammalian microsporidiosis. Vet. Pathol. 37, 113-128.

Weber,R., P.Deplazes, M.Flepp, A.Mathis, R.Baumann, B.Sauer, H.Kuster and R.Luthy, 1997. Cerebral microsporidiosis due to *Encephalitozoon cuniculi* in a patient with human immunodeficiency virus infection. N. Engl. J Med 336, 474-478.

Wilson,J.M., 1979. *Encephalitozoon cuniculi* in wild European rabbits and a fox. Res. Vet. Sci. 26, 114.

Acknowledgements

This project was funded by Defra project grant no SE01526. Thanks to Forestry Commission, United Utilities and the Balfour Estate for access to trapping sites, and to all those who contributed fox carcasses.

Table 1. Species tested for *E. cuniculi* antibodies in each study area and rodent trapping season with number positive for *E. cuniculi* antibodies and associated seroprevalence (exact binomial 95% confidence intervals)

Species	Area	Rodent trapping season				Total	<i>E. cuniculi</i> results	
		1	2	3	4		Positive	Seroprevalence (95% CI)
Prey								
Bank vole	Pentlands	43	44	12	0	99	10	10.10% (4.95-17.79)
	Borders	5	14	0	0	19	3	15.79% (3.38-39.58)
	Cumbria	11	24	19	6	60	6	10.00% (3.76-20.51)
	Total	59	82	31	6	178	19	10.67% (6.55-16.17)
Field vole	Pentlands	21	55	26	0	102	4	3.92% (1.08-9.74)
	Borders	47	46	8	0	101	7	6.93% (2.83-13.76)
	Cumbria	26	43	19	21	109	7	6.42% (2.62-12.78)
	Total	94	144	53	21	312	18	5.77% (3.45-8.96)
Wood mouse	Pentlands	79	34	37	0	150	0	0.00% (0.00-2.43)
	Borders	20	3	33	0	56	0	0.00% (0.00-6.38)
	Cumbria	40	43	10	4	97	3	3.09% (0.64-8.77)
	Total	139	80	80	4	303	3	0.99% (0.20-2.87)
All	Pentlands	143	133	75	0	351	14	3.99% (2.20-6.60)
	Borders	72	63	41	0	176	10	5.68% (2.76-10.2)
	Cumbria	77	110	48	31	266	16	6.02% (3.48-9.58)
	Total	292	306	164	31	793	40	5.04% (3.63-6.81)
Predator								
Cat	Pentlands					7	0	0.00% (0.00-40.96)
	Borders					11	0	0.00% (0.00-28.49)
	Cumbria					9	0	0.00% (0.00-33.63)
	Total					27	0	0.00% (0.00-12.77)
Fox	Pentlands					15	9	60.00% (32.29-83.66)
	Borders					34	20	58.82% (40.70-75.35)
	Cumbria					47	21	44.68% (30.17-59.88)
	Total					96	50	52.08% (41.64-62.39)
All					123			
Grand total						916		

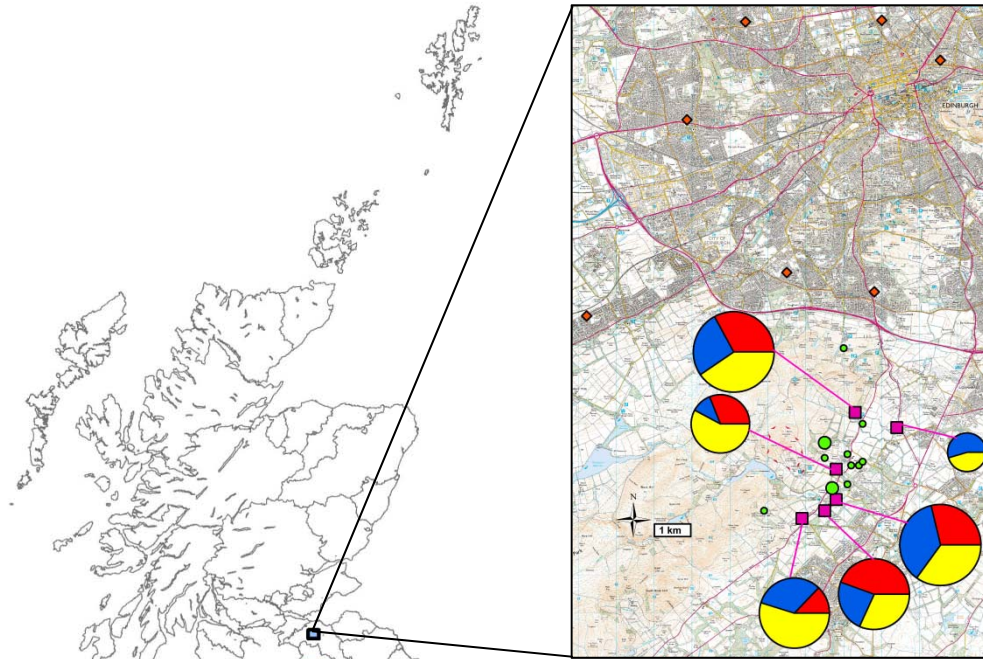
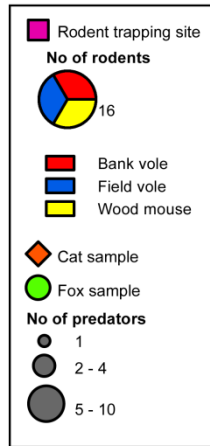
Figure Legends

Figure 1. Summary map of the 3 study areas sampled (Pentlands, Borders and Cumbria). Pie charts for the number of rodents range from 1-80 rodents. For the Borders area a sample was also obtained from one cat where the owner lived 8km north of the area in map (not shown), and for the Cumbria area 2 cat samples were also obtained where the owners lived either lived over 4km north of the area in map (not shown)

Figure 2. Summary of sampling regime for the predator species (Cats and foxes) and prey species (bank vole, field vole and wood mouse) in the three study areas (B = Borders, C = Cumbria, P = Pentlands). Grey polygons indicate the 4 rodent trapping seasons in the overall project. Horizontal lines either indicated the entire period of sampling of that species in that area for cats and foxes, or the trapping period for rodents. Vertical polygons represent the number of animals collected at actual particular time points to test for *E. cuniculi* (range = 1 to 43)

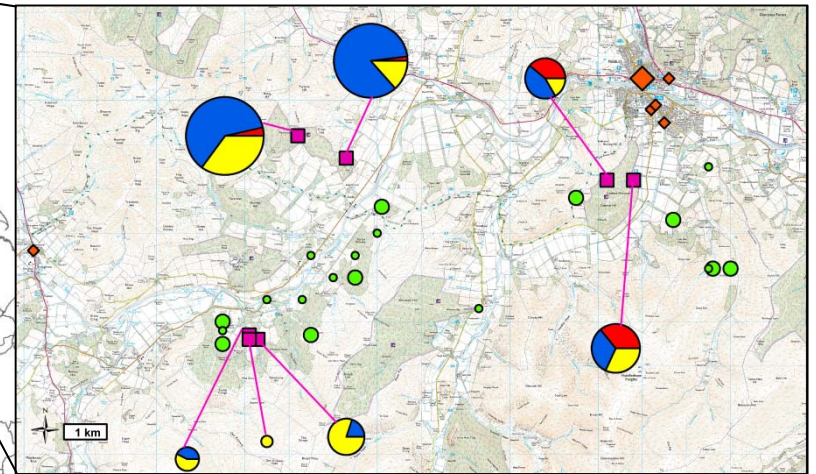
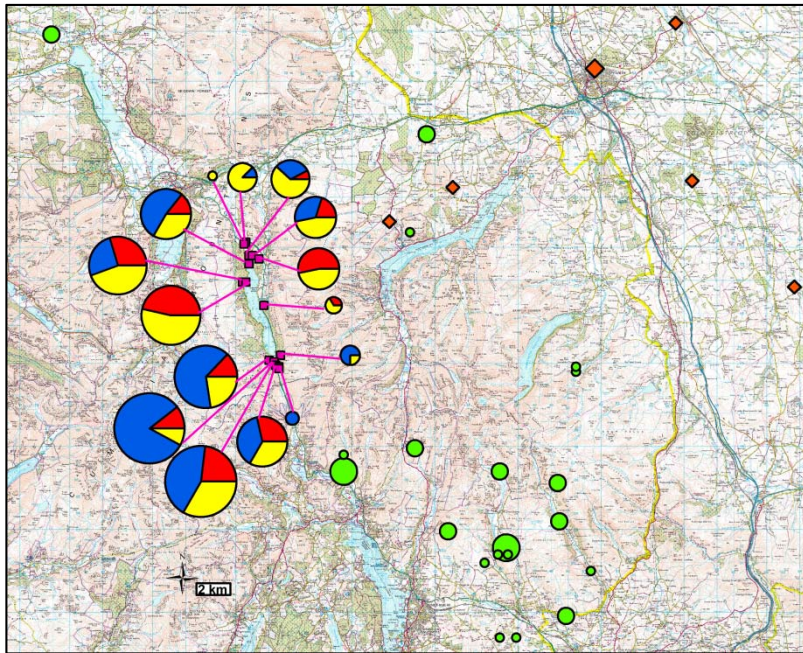
Figure 3. Seroprevalences of the rodent and fox and cat samples for the 3 study areas. The semi-transparent circles under the dark pie charts indicate fox territories of approximately 10km²

Figure 1



Pentlands

Cumbria



Borders

100 km

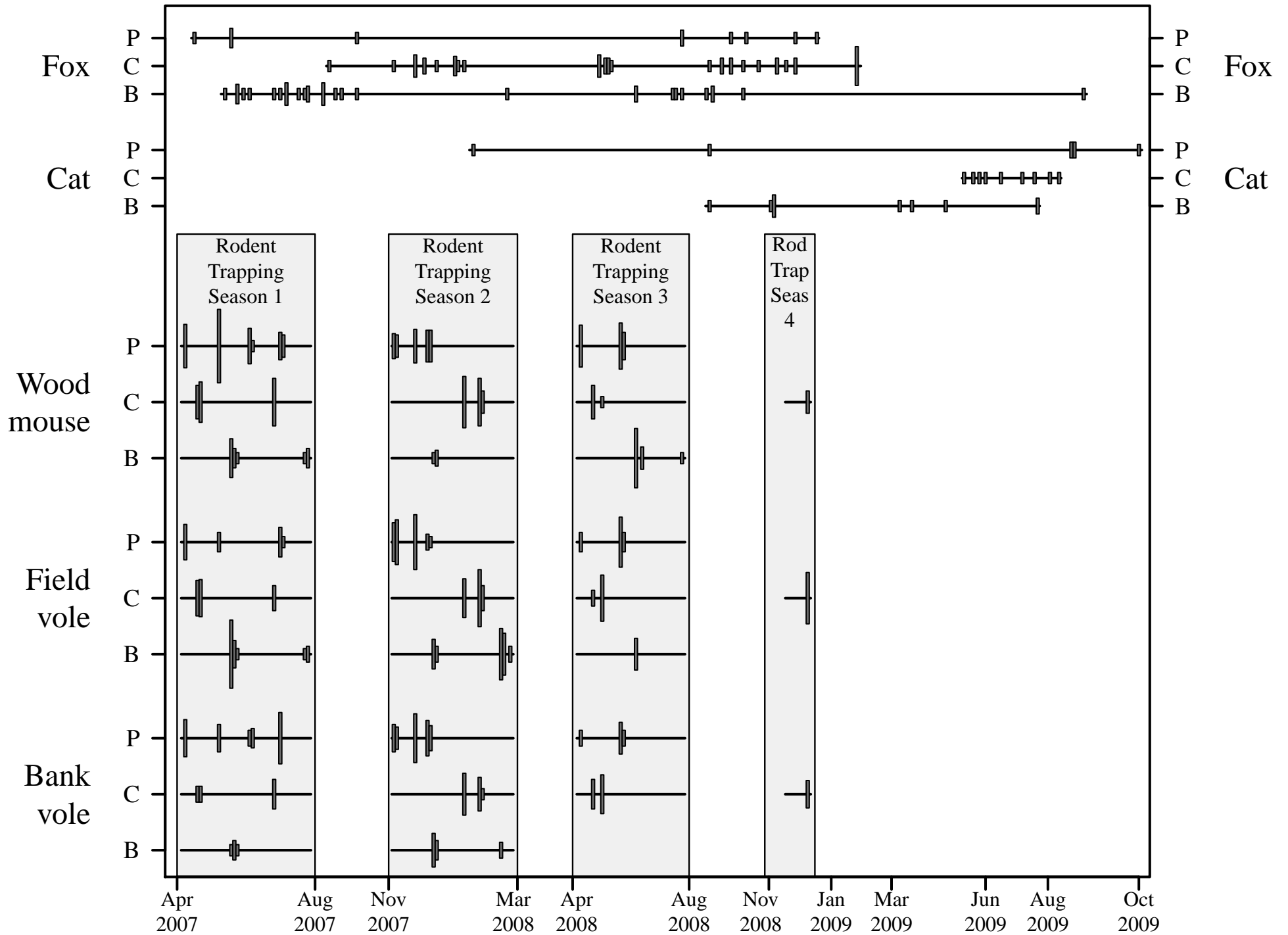
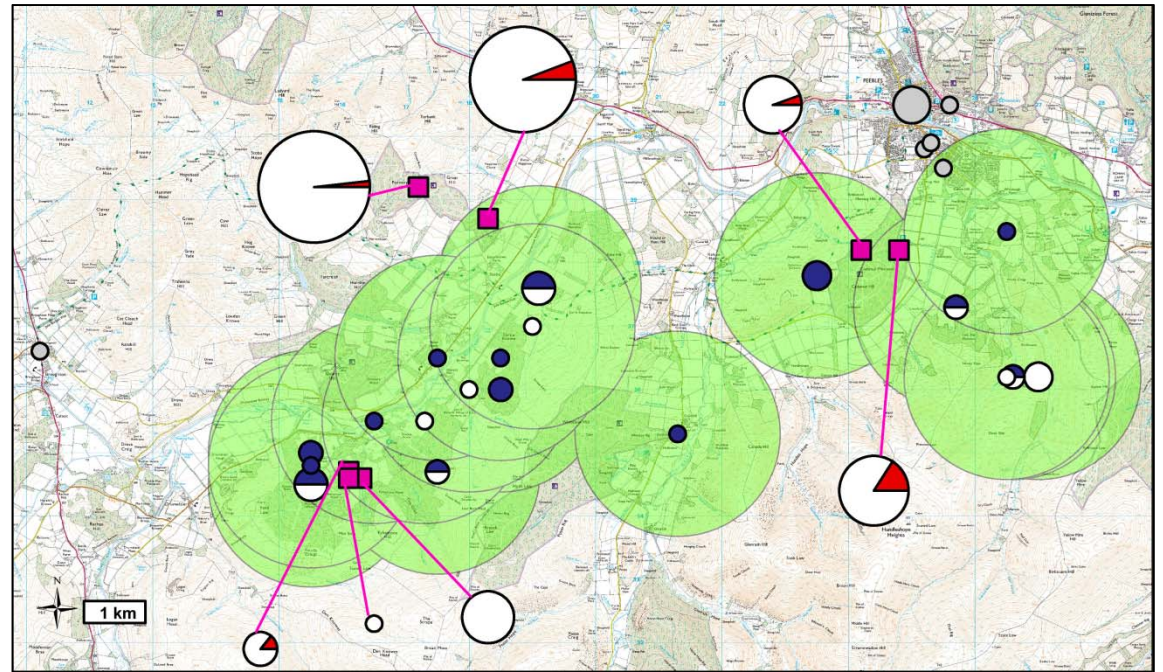
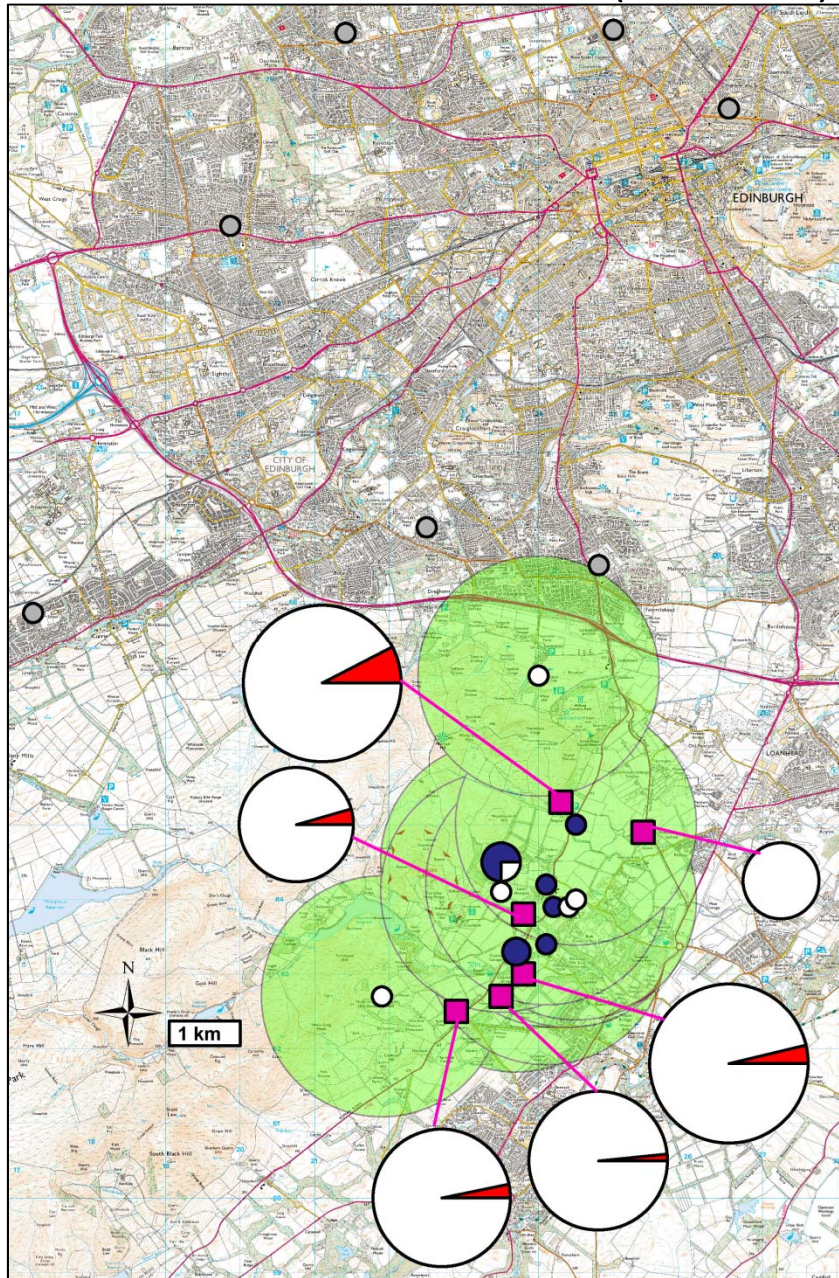


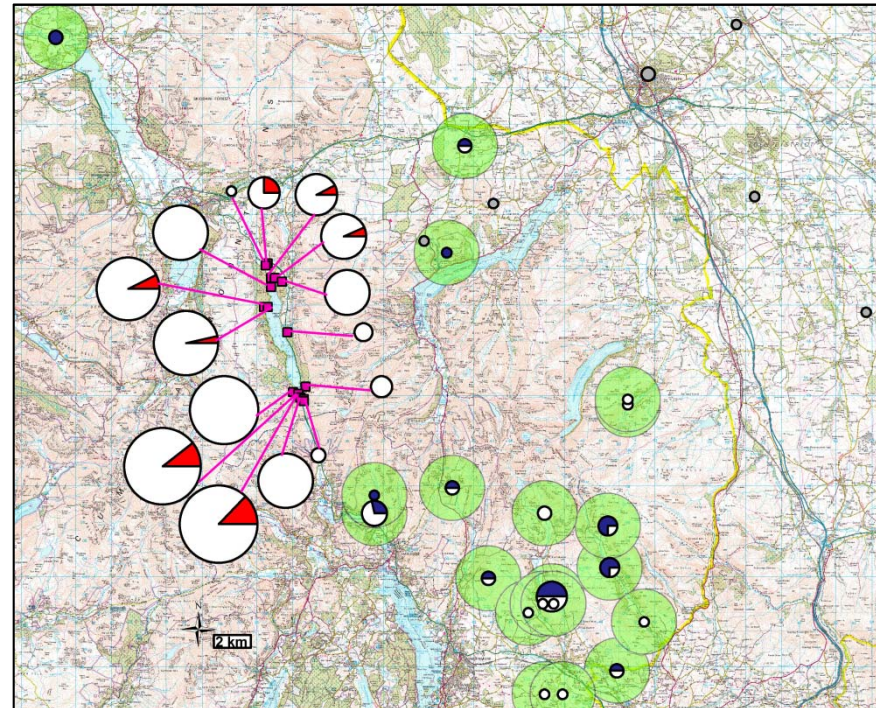
Figure 2

Figure 3

(Pentlands)



(Borders)



(Cumbria)