Surveillance of red fox *Vulpes vulpes* cardiopulmonary parasites in the UK

Submitted by Amelia Jane Brereton to the University of Exeter
as a thesis for the degree of
Master of Science by Research in Biosciences
In October 2011

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Introduction

Wildlife diseases can threaten the agricultural industry, the health of humans, domestic animals, game species, and populations of endangered species, and it is therefore important to manage disease threats (Wobeser 1994). Recent research into the epidemiology and economics of wildlife diseases and outbreaks of new infections in humans and livestock has encouraged the growth in wildlife disease management (Wobeser 2002). The rationale for and process of management has been reviewed regularly, most recently by Delahay, Smith and Hutchings (2009) for mammals. Wildlife can host a wide range of diseases: this review will focus on infectious animal diseases as these pose the most significant and well-researched threats to health and industry (Delahay *et al.* 2009), whereas diseases that are non-infectious are unlikely to pose a threat to human health or wildlife and are therefore not commonly managed (Wobeser 1994). Mammals are the most well studied taxon in this area but other groups such as insect vectors or amphibians endangered by disease are also important and will be included in this review. Firstly, disease and wildlife will be defined and the rationale for management discussed, followed by some of the unique difficulties of managing wildlife diseases. Disease management can take the form of prevention, control or eradication and is carried out by targeting the host population, pathogen or environment in which disease is occurring. These methods will be described, followed by the importance of assessing the outcomes of management for the future.

What is wildlife disease and why is it managed?

Disease is most commonly defined as an impairment of the normal functioning of an organism due to a disease agent, and can affect a range of processes including growth, fecundity, metabolic requirements or behaviour, resulting in morbidity or mortality (Delahay *et al.* 2009). The nature of a disease depends largely on the agent causing this impairment, which can be infectious (e.g. pathogens (biological agents causing disease such as bacteria, viruses, helminths and protozoa) or prions and infectious cancers) or non-infectious (e.g. toxic chemicals or developmental problems such as chromosome disorders or non-infectious cancers). Disease can be acute (short-term) or chronic (long-term) within individuals, and be endemic (constant level) or epidemic (outbreaks) within populations. The most important diseases for management are those caused by infectious pathogens with multiple hosts, which are less closely co-evolved to each host species and therefore likely to be more virulent (Woolhouse 2002).

Wild animal species can act as definitive hosts (in which pathogens reproduce), intermediate hosts (harbouring pathogen for a short period of lifecycle), reservoir hosts (populations in which a pathogen is maintained and can be transmitted to defined target population) or vectors (carries and transmits disease without infection) of diseases. Wildlife can be defined as organisms that grow without human care (Wobeser 1994). However, all wildlife is in some way affected by human interaction and no disease event can be viewed as entirely 'natural' (Buxton 2006). Pathogens are natural in ecosystems and are thought to contribute to 'ecosystem health', increasing resilience through greater connectedness and nestedness of ecosystems (Hudson *et al.* 2006). However, management of the agents of disease is increasingly important as human population density, resource use, agricultural intensification, climate change and global travel increase. This leads to further encroachment into and degradation of wildlife habitat; increased movement of animals and pathogens between regions; an increase in the burden of pathogens in wild animals and increased contact rates between wildlife, humans or domestic populations (Daszak, Cunningham and Hyatt 2000; Bengis *et al.* 2004; Delahay *et al.* 2009).

These factors mean that 'spillover' of diseases from reservoir or definitive wildlife hosts is occurring increasingly often. Spillover from wild sources can cause costly epidemics when a disease is controlled in the human, livestock or endangered population (Artois *et al.* 2001; Gortázar *et al.* 2007). Disease management aims to counteract this effect by reducing contact rates and pathogen densities by a number of methods, to reduce the increasing threat of emerging diseases from wildlife sources. Therefore management is justified primarily as a way to solve problems created by humans, for the benefit of the human population (Wobeser 2002).

Negative impacts of wildlife disease

A number of recent reviews focus on the role of wildlife disease in human health, and specifically the recent increase in emerging infectious diseases (EIDs) in humans (e.g. Taylor, Latham and Woolhouse 2001; Anonymous 2004; Bengis *et al.* 2004; Jones *et al.* 2008). Wildlife diseases are a significant source of EIDs: the most recent estimates put the proportion that are zoonotic (transmitted between humans and animals) at over 60%, with wildlife implicated in over 70% of these, which are often also of high public interest (e.g. pandemics like HIV/AIDS) (Jones *et al.* 2008). Wildlife can either act as a source for infection that spreads to humans rarely and is then maintained within the human population (e.g. epidemics such as avian influenza) or as a reservoir

which maintains the disease and periodically spreads it to humans (Bengis *et al.* 2004). Both types of threat can be managed, the former primarily through reactive methods focusing on the human population, and the latter through both proactive and reactive methods with more of an emphasis on the wildlife population. For example, in Europe, bovine tuberculosis (bTB) and rabies were once threats to human populations but are now a low risk as they are well managed (bTB preventively through pasteurisation of milk, rabies through control or eradication in domestic species and foxes), proving that human health threats can be managed successfully (Gortázar *et al.* 2007).

Similarly, wildlife diseases can be a concern for wild game species (e.g. red legged partridge threatened by avian pox in Spain; Buenestado *et al.* 2004) and livestock (e.g. UK cattle can be infected by bTB from the badger reservoir; Donnelly *et al.* 2007), threatening animal welfare, profitability of these industries in some cases creating high compensation, testing and research costs for governments (e.g. £108.4 million spent on bTB by Defra in 2008-9 (Defra, accessed 06/12/2010)) threatening these industries and also causing concern for animal welfare. Although there are many studies and reviews of certain wildlife disease threats to livestock (e.g. bTB and rabies in Europe are particularly well studied), game species are less well covered, and both have been less frequently reviewed than the threat to human health (though note recent reviews by Gortázar *et al.* (2007) and Simpson (2002) specifically on the threat to livestock).

Wildlife disease is also managed to prevent biodiversity loss by extinctions of small populations of endangered species. This has been considered a low risk in the past because theory suggests pathogens should always go extinct before hosts due to reductions in contact rate, however, research has shown that disease has caused extinctions before (e.g. local and species extinctions of amphibians due to chytridiomycosis; Daszak, Cunningham and Hyatt 2003) and modelling suggests it is a higher risk than previously thought (Castro and Bolker 2005). Disease can infect endangered animals from a reservoir (e.g. domestic dogs infecting African wild dogs with rabies; Lembo *et al.* 2008), through infected prey (e.g. bTB from carcasses or prey to lynx) or indirectly (e.g. lack of prey for lynx and Spanish Imperial eagle due to myxomatosis and rabbit haemorrhagic disease in wild rabbits; Moreno *et al.* 2004) (Gortázar *et al.* 2007). Parasitism can also increase success of invasive species if they introduce new parasites to native hosts with no immunity, or act as a reservoir for native hosts' diseases (Tompkins *et al.* 2010). Management of disease in endangered species has been controversial in the past due to potential negative effects such as stress (e.g. African wild

dogs; De Villiers *et al.* 1995), although this has been contested (Woodroffe 2001), and due to lack of knowledge of the ecology of rare species (Knobel *et al.* 2008).

Why is wildlife disease management difficult?

Managing wildlife diseases presents many unique challenges, compared to livestock and human populations, mainly because wild animal movements are not directly in human control (Wobeser 1994). The particular challenges presented by wild species include: finding infected wild animals; diagnosing disease without obvious signs or specific diagnostic tests available; estimating rates of infection without a high level of sampling; the wide variety of diseases in wildlife and possible interspecific transfer of infections, and the intractable nature of wild animals during management (Wobeser 1994; Artois *et al.* 2001). While livestock is easily traced and treated on an individual basis, it is difficult to identify and recapture wild individuals, meaning management must often tackle disease at a population level. This different approach can require development of novel and specific methods for wildlife disease management compared to livestock management. From a socio-political point of view, wild animal management is also a problem because: wildlife is often used as a resource; public attitudes of affection or perception that wildlife can take care of itself; lack of funding for wild diseases compared to livestock and lack of public or private ownership of wildlife (Wobeser 1994; Artois *et al.* 2001). Most importantly, few countries have a network for surveillance of disease or sharing information about management and this has been a low priority until recently (Gortázar *et al.* 2007).

Justifying management

The difficulties in detecting wildlife disease and gaining public support and funding mean that a strong case has to be made in order to justify action. Many infections are generally considered not worth managing because of the low risk of spread to valuable species or humans, and the small effect on productivity or mortality (Gortázar *et al.* 2007). For example, while the cardiopulmonary nematode *Angiostrongylus vasorum*, which can be passed from the wild foxes to pet dogs, is considered worthy of surveillance due to its potential to harm companion animals, other fox cardiopulmonary parasite species which are infrequently found to cause harm in pets would not be considered such a threat (Morgan *et al.* 2005).

The case for management is debated even in well known and controversial diseases such as bTB in

British cattle. This poses a low health risk to humans since pasteurisation (two bTB cases confirmed from UK cattle) but involves high management costs involved (Torgerson and Torgerson 2008). However, others cite the need to protect international trade as sufficient reason to continue to manage bTB (Gordon 2008).

Discovery that disease prevalence is low in wild species could prove that management is more likely to succeed and be cost effective, encouraging action (Wobeser 1994). On the other hand, in many cases we do not have enough information on the wildlife ecology associated with diseases making management difficult – for example, the virus, vector and role of wild ruminants is speculated but not well known for bluetongue (Gortázar *et al.* 2007).

Management objectives

According to Wobeser (2002), choosing the objective should be the first step in any management plan. The aims of management are to: reduce the reproductive rate of pathogens; reduce host density to reduce the number of susceptible or infected individuals and the contact rate between them; or to alter the environment to reduce contact rate. Essentially, the aim is to reduce spread of the pathogen below a threshold level at which the disease cannot be maintained in the population i.e. where each individual infects less than one other individual (Anderson 1991). There is some debate over whether such thresholds exist or can be useful to management: Lloyd-Smith *et al.* (2005) suggest that thresholds are not abrupt and are also difficult to identify due to the quality of datasets, but that any reduction in density may affect contact rate, decreasing disease. Thresholds therefore remain a widely used concept in management strategies, and are useful as aspirational targets, but should not been seen as set figures.

Management strategies are commonly classed into three areas: prevention, control and eradication, and can also be split into proactive and reactive management (Wobeser 1994). Prevention requires elimination of the risk factors for the introduction of disease (Wobeser 2002). When the disease agent is exotic and host populations have a lower level of immunity to the disease, a disease poses a greater threat, making translocation of wild animals a particularly high-risk activity (Wobeser 2002). According to Wobeser (2002), restricting translocations of wildlife is the most important management method for reducing wildlife disease overall, particularly as conservation efforts and global travel increases. Preventative measures could also include

adhering to biosecurity measures to prevent spread of pathogens by humans during travel, veterinary work and animal husbandry, reduction in movement of domestic animals (Daszak, Cunningham and Hyatt 2000) and prevention of overcrowding caused by supplementary feeding of wild reservoir species (e.g. white-tailed deer in Michigan; Miller *et al.* 2003).

After a disease has emerged in a population, the management objectives are control or eradication. Control is more costly than prevention as it aims to reduce disease burden in individuals or populations to a level that can be maintained in perpetuity, and should therefore be implemented as soon as possible after an outbreak occurs (Artois *et al.* 2001; Wobeser 2002). In order to eradicate a disease either the pathogen or the host must be completely eliminated. In wildlife diseases this is practically extremely difficult. Pathogens can be eradicated through treatment, or by lowering the rate of contact so the pathogen runs out of hosts. Where the host is a native species, eradication is generally considered to be ethically unacceptable, meaning pathogen removal is the only option. Wildlife reservoir hosts are considered for eradication when they are exotic, such as the brushtail possum in New Zealand, primarily managed to reduce damage to forest ecosystems, but which is also a reservoir for bTB (Brown and Sherley 2002). However, this is expensive and has so far never been done solely to prevent disease.

Disease ecology and wildlife disease management have expanded rapidly as disciplines over the past 30 years (Hudson *et al.* 2002). Management has often been reactive and based on expert opinion or methods applied to controlling livestock disease during this time (Artois *et al.* 2001; Delahay *et al.* 2009). Others have commented that wildlife management is based on political (short-term) rather than scientific (long-term) goals (Tompkins and Wilson 1998). In 1994 Wobeser wrote that most wildlife management methods were 'untested'. Management is now increasingly based on detailed research (e.g. the randomised badger culling trial (RBCT); Donnelly *et al.* 2007) but more work is still required as it is now accepted that in order to effectively manage a disease it is important to understand the ecology of the disease and host species, taking a multi-disciplinary approach including ecology, veterinary science and politics.

Management methods

There are many different methods for managing wildlife, which will be reviewed according to the target of the method: pathogen, host, vectors and environment. Growing ideas of integration of

management methods will also be discussed.

a) Pathogen

There are two methods that target the pathogen: vaccination (reduces infected individuals and therefore the total number of pathogenic organisms) or treatment (reduces infected individuals or the number of pathogenic organisms within each individual). Vaccination and treatment are both key methods for endangered, small populations as they are less likely to affect social structure or cause local population extinction. However, they are increasingly employed to tackle agricultural or human health threats as well and are generally seen as less controversial than lethal host manipulation methods (Delahay *et al.* 2009).

Treatment using antibiotics and anthelmintics is common in humans and domestic animals but is costly and practically difficult in wild species.   Although drugs have been used as a reactive method to reduce the burden of macroparasites (helminths and protozoa) in wild individuals with success (e.g. anthelmintics treat *Echinococcus multilocularis* in foxes*;* Eckert and Deplazes 2004) others have concluded that the need to repeat treatment and the variation in results between individuals makes this method unsustainable and impractical in the long-term for wildlife disease management (Murray, Keith and Cary 1996; Wobeser 2002). Treatment also leads to worries over evolution of drug resistance and persistence of the drug in the environment, affecting non-target species (Wobeser 2002). Pathogens can be targeted in the environment by disinfection when they are localised, but this may not always be feasible (e.g. disinfection to remove anthrax in water in South Africa; Berry 1993). Theoretically the numbers of a pathogen could be reduced by disposal of infected carcasses, although these methods are of low priority in wildlife disease management as their effectiveness is questioned and they are difficult to implement at large scale or where carcass locations are unknown (Berry 1993; Gortázar *et al.* 2007).

Vaccination is used to reduce the proportion of individuals in a population that are susceptible to an infectious disease. This makes it less likely that an infected individual will contact a susceptible individual and spread a pathogen. While any reduction is useful, vaccination campaigns aim to reach a level of 'herd immunity' where the rate of pathogen spread is too low to maintain the disease (Bailey 1957). Vaccination is used to manage diseases caused by microparasites (e.g. viruses and bacteria) that can be prevented by immunity, and is an effective method when

diseases are maintained in multiple host species and if animals become infected later in life (Wobeser 2002).

Vaccination has been used for over 2000 years in the human population (Lombard, Pastoret and Moulin 2007), but has not been so widespread in wildlife disease management due to difficulties in capturing animals and administering the vaccine, and has been thought by some to have 'limited application' (Wobeser 1994). However, over the last 15 years it has gained interest and been tested in a range of species, via injection, bait, ballistics, the nasal and conjunctival routes and most recently through viruses in both endangered and widespread species (Cross, Buddle and Aldwell 2007). Vaccination has recently been used successfully to nearly eradicate fox rabies from Europe, and was considered the cheapest and easiest method to deploy in this case (Artois *et al.* 2001). However, there has been a recent re-emergence of rabies in Europe due to relaxation of vaccination and increase in foxes and new hosts such as raccoon dog, suggesting that vaccination must be continuously reapplied to prevent re-emergence (Holmala and Kauhala 2006). Vaccination also contributed to the successful eradication of classical swine fever (CSF) in wild boar in an epidemic outbreak in the Rhineland-Palatinate state of Germany (Von Rüden *et al.* 2008), and lowered prevalence rates in other trials in Baden-Württemburg, although there was insufficient uptake by piglets (e.g. Kaden *et al.* 2000), which has since been addressed through increased vaccination and hunting effort (Kaden *et al.* 2005).

Research into vaccination for CSF and rabies has led to further research into this method, such as management of bTB in badgers in the UK and wild boar in Spain (Ballesteros *et al.* 2009; Delahay *et al.* 2009). Currently, oral vaccination has the best potential for large scale vaccination, although practical difficulties remain, such as preserving the viability of live vaccines after deployment, and the requirement for efficacy and safety testing (Wobeser 2002; Cross, Buddle and Aldwell 2007).

Vaccination is also used reactively to protect endangered species. For example, Ethiopian wolves were vaccinated against rabies during an outbreak in 2003 (Knobel *et al.* 2008). Wolves were targeted at the periphery of the outbreak area to prevent spread, which successfully protected all wolves injected after one month, and halted the outbreak, preventing spread to other nearby packs. However, like medical treatment, vaccination also has negative aspects, such as prevention of evolution of natural resistance to a pathogen, potential for handling to increase stress (see

above), potential for live vaccines to become virulent or otherwise harm host species, or associated increase in reservoir host density by prevention of mortality which could counteract the benefit of vaccination (Wobeser 1994).

b) Host

Host population density can be reduced to lower the contact rate of susceptible and infected individuals, and has been the favoured management method in the past (Wobeser 1994). Theory suggests that host population reduction should be most effective when a disease requires a dense population to persist and when the disease is only maintained in one species (Wobeser 2002). These methods are therefore appropriate for overcrowded rather than endangered populations, so is used in the context of human health and agricultural threats rather than biodiversity threats (Delahay *et al.* 2009). There are three main ways in which host density can be reduced: dispersal, culling and fertility control. Dispersal is not commonly used to manage infectious diseases as it risks spreading infection to areas with susceptible individuals and has little application in this area, but culling and fertility control have both been tested and previously used with success, and continue to be potential management options despite controversies regarding animal rights and negative effects of these methods (Delahay *et al.* 2009).

Selective culling ('test and slaughter') is common in livestock epidemics, such as foot and mouth disease in the UK, but impractical in wildlife. General culling reduces both the number of infected individuals (and therefore the number of infectious organisms) and susceptible individuals (and therefore the rate of pathogen spread) and has been used in wildlife management (e.g. badger culling to manage bTB; Donnelly *et al.* 2007). This method is most applicable where management can take place in a small area and with short duration (Wobeser 1994). Culling is permanent and immediate, and a variety of different culling strategies including targeting of infected setts on breakdown farms and all neighbouring infected setts ('clean ring strategy'), reactive culling at setts on land grazed by reactor cattle ('interim strategy'), and experimental proactive culling have been used in the past (Delahay *et al.* 2009).

However, although culling may be seen as a 'tried and tested' method, it has rarely been assessed for its efficacy and has a poor public perception (Delahay *et al.* 2009). Additionally, culling has caused unpredictable ecological consequences: if birth rates are density dependent, culling can

trigger compensatory reproduction and an increase in newborn, infection-susceptible individuals to the population (Holmala and Kauhala 2006). Behaviour can also be changed, for example, when culling was tested as a method in the RBCT: reactive culling increased the incidence of TB in cattle herd breakdowns by 27% within the culling area, while proactive culling decreased incidence within culling area but increased incidence within a 2km radius (Donnelly *et al.* 2003; Donnelly *et al.* 2006). The hypothesised reason for this effect is disruption of badger social structures and an increase in ranging behaviour (Carter *et al.* 2007). Culling has also been shown to affect other species in the ecosystem, for example, in the RBCT reduction in badger density also caused increased fox density (Trewby *et al.* 2008). It has been difficult to eradicate wildlife diseases through culling in the past, with the exception of removal of TB in the Asian water buffalo in Australia which took 27 years (Cousins and Roberts 2001). In general, culling may be more useful for preventing disease spread (as in livestock) rather than controlling a disease which is already established (Wobeser 2002).

Fertility control, through immunocontraceptive vaccines, implants or abortifacients chemicals, reduces host density like culling, but in the longer-term, and can be permanent or temporary. This method requires much more research before it could be deployed effectively, and is considered impractical in the field by some authors (e.g. Artois *et al.* 2001). Although immunocontraceptives have been trialled for reducing density of several species including deer and wild boar, this method has only been applied to a few diseases, such as brucellosis in bison in the US, which is spread through aborted foetuses and infected milk, so could be particularly suited to this method (Miller, Rhyan and Drew 2004). Fertility control has the advantage of preventing compensatory reproduction as the population falls, and so could be considered more long-lasting and less disrupting to population structures, although reducing the number of breeding females could alter dominance structures (Smith and Cheeseman 2002). Fertility control also increases female condition by reducing reproduction, which is likely to decrease susceptibility to disease, though unpredictable negative behavioural or physiological changes may require further research, such as the effects of extended breeding season and increased lifespan, or more vulnerable secondary sexual characteristics such as antlers (Lincoln, Fraser and Fletcher 1982).

c) Vector

Vectors (organisms which transmit infection between hosts), most commonly ticks, flies and

mosquitoes, can cause emergence of wildlife disease, for example when vectors expand to areas with susceptible individuals through climate (Lindgren, Talleklint and Polfeldt 2000). Reducing the expansion of vectors or their density will therefore reduce the contact rate with susceptible hosts. Vectors of human diseases have been targeted by insecticides in the past (e.g. DDT to reduce mosquitoes spreading malaria) but was generally considered unsuccessful due to evolution of resistance and side-effects on non-target species (Wobeser 2002). Both manipulation of environment (such as burning forest to reduce tick density) and chemicals (e.g. insecticides to control fleas transmitting *Yersinia pestis* between rodents) have been used to target vectors of wildlife disease (Wobeser 2002). However, targeting vectors only applies to specific wildlife diseases, alteration of vector habitat could affect other species utilising the same habitats and evolution of disease resistance has been observed (Wobeser 2002).

d) Environment

The 'environment' is the biotic and abiotic conditions in which an organism exists, such as the physical environment and interactions with other species. Environmental change is a key factor in causing wildlife disease issues, so is key to prevention of disease. Additionally, changing the environment of a host species can be used as a long-term method to control disease prevalence. In theory for example, the environment could be used to manage disease spread between different populations by manipulating connectivity of host populations within metapopulation structures and to create barriers to disease spread (Delahay *et al.* 2009). In practice the environment is not usually the primary route to manage diseases, although there are a number of examples of different environmental targets.

The environment can be manipulated directly to reduce disease agents, for example by application of herbicides or burning vegetation in order to reduce helminths (Boggs *et al.* 1991). Host density can be influenced indirectly by changing the carrying capacity of the environment, for example by reduction or dispersal of supplementary feeding to prevent congregation and disease spread (Miller *et al.* 2003), or reduction in scrub vegetation or refuse to reduce rat density (e.g. White, Horskins and Wilson 1998). However, reducing populations through manipulation of the environment could also cause malnutrition, particularly in sedentary species, and associated disease susceptibility or increased dispersal. Disease carriers and hosts can be excluded from an area through environmental methods, for example, scaring with noise or dogs, or fencing cattle

pastures to reduce contact between deer transmitting bTB to cattle in the United States (Vercauteren, Shivik and Lavalle 2005; Vercauteren, Lavalle and Hygnstrom 2006). Similarly, exclusion of badgers from areas of pasture and agricultural buildings is being examined in the UK to reduce bTB transmission rates by using environmental manipulation to alter host behaviour (Courtenay *et al.* 2006; Ward, Judge and Delahay 2008). Human behaviour can also be classed as environmental management: for example, in order to manage bTB through husbandry methods it would be important to convince farmers to implement them, as they currently do not see them as cost effective (Ward, Judge and Delahay 2008). Public education can also be used to reduce contact between people and wildlife in order to reduce risks to public health (Wobeser 1994).

e) Integrated management

Recent advances in ecological modelling have shown that using multiple management methods can produce a synergistic effect (e.g. Suppo *et al.* 2000). Application of more than one management method is known as 'integrated management' and is increasingly recommended by researchers when mass treatments fail to manage disease, mirroring the move to integrated management in human disease ecology seen since the failure of DDT for malaria treatment. Integrated management can be simultaneous or sequential, and recommendations commonly involve combinations of vaccination, culling and fertility control (Smith and Cheeseman 2002). Additionally, public education should be included if possible in integrated management plans (Wobeser 1994).

Vaccination reduces mortality rates, which increases host density. This can lead to malnutrition and associated loss of condition in the population, and can cause other problems such as crop damage associated with increased population size (Wobeser 1994). Larger populations may also lead to a higher birth rate and therefore increasing proportion of susceptibles (i.e. individuals born post-vaccination). Vaccination programmes may therefore be improved by simultaneous fertility control or culling after vaccination to prevent increase in density. Fox densities have increased in western Europe since eradication of rabies, so contraceptives could now be used to reduce density, reducing the chance of reinfection from eastern Europe (Smith and Cheeseman 2002). Simultaneous vaccination and contraceptive bait could be applied to make a barrier to re-infection (Smith and Cheeseman 2002).

Vaccination is thought to be more effective than culling where birth, death and disease propagation rates are low (Delahay *et al.* 2009). Aubert (1999) showed that vaccination led to rabies elimination while culling only caused lulls in prevalence. However, if these rates are high it may be more effective to combine vaccination with culling, or vaccination with fertility control. For example, Kaden *et al.* (2000) showed that the oral CSF vaccine used in Germany was only ingested by 50% of young boar, so intensive hunting was also recommended for eradication of the disease. Other papers suggest that an inner core area of disease may be best treated through culling while a buffer area is vaccinated to reduce disease spread, or that the culling zone could be simultaneously fenced in to prevent dispersal (Wobeser 2002). Modelling results vary: Smith and Cheeseman (2002) concluded that acute diseases such as rabies can be equally well managed through vaccination, culling, or vaccination combined with fertility control (Smith and Cheeseman 2002), while Suppo *et al.* (2000) concluded that vaccination and fertility control work better in combination. For chronic diseases, culling is theoretically more effective than vaccination in an isolated population, due to reduction in host density, but fertility control combined with vaccination can be as effective (Smith and Cheeseman 2002). Overall, more modelling and experimental work is required to assess combinations of management strategies for different diseases and species, but the combination of fertility control and vaccination should be considered when culling is not publicly accepted or creates social disruption and perturbation.

Assessment of management

Assessment of disease management after application of the above methods is an important part of the management process due to high costs and limited inputs of funding and time. According to Wobeser in his 1994 review, management methods remained "of unproven and untested efficacy". Although this is not now entirely the case, Wobeser later reiterated that most methods are of unknown effectiveness as they are based on livestock or human disease (2002). Assessment should involve several stages, beginning with choosing which parameters to assess, then data collection and analysis. Success of management strategies can be judged by a number of different measures depending on the method, including uptake of baits (treatment and vaccine), immunological response in individuals and populations, and consequences of disease in the human or livestock populations (Wobeser 1994). Calculating cost-benefit analysis is an essential part of modern management to calculate the optimum management strategies, as is mathematical modelling in order to predict future outbreaks and plan the most effective management (Delahay

et al. 2009). Results of assessment should be collated and published for further evidence-based management in other countries, and for use in further management models (Wobeser 1994). Although assessment and publishing is improving, reviews still call for greater emphasis on this, particularly risk analysis and planning for future disease events (Artois *et al.* 2001; Gortázar *et al.* 2007).

Conclusion

The growth of public and academic interest in wildlife disease management in recent years, and related emergence of zoonotic infectious diseases threatening humans and valuable species has led to an increase in research in this area. This has shown that wildlife often acts as a reservoir host for diseases that spillover to infect important populations, particularly in areas affected by human disturbance such as habitat destruction and climate change. However, as wildlife management is a relatively new focus for efforts, many methods are based on those used to treat human and livestock: methods based on targeting the pathogen (treatment and vaccination) are very effective at treating these groups but are more difficult to implement in wildlife due to their freedom of movement. Treatment and dispersal methods are probably the least useful for treating wildlife disease. A growing interest in longer-term solutions such as vaccination and fertility control, and continued use of culling are the most common management methods found in the literature, and may be best used in combination together. Disease can also be managed through environmental manipulation to alter host densities or remove pathogens, however these are mainly in the early stages of development. Many methods remain unproven for use in wildlife, and each wildlife disease and species will be best managed through different methods depending on the specific circumstances, therefore a great deal more research is required before all wildlife diseases are manageable through manipulation of environment and wildlife reservoirs. Diseases which are currently the focus of management and research include bovine TB, rabies and classical swine fever – reviewers recommend more research on other diseases such as bluetongue where the role of wildlife and possible management is less certain (Gortázar *et al.* 2007). A combination of mathematical modelling to identify knowledge gaps, indoor trials of vaccines and immunocontraceptives, and experimental field trials are required to test methods, with less emphasis on observational studies (Gortázar *et al.* 2007). Previous reviewers have stressed the importance of having a defined threat and objectives for management – these should include prevention, control or eradication of disease, and proactive prevention of disease is the least costly

objective if possible. The need for multidisciplinary teams to control disease and investment in monitoring and sharing systems for the results of management are also key recommendations in the literature (Daszak *et al.* 2000; Gortázar *et al.* 2007).

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2. Research Project:

Surveillance of red fox *Vulpes vulpes* cardiopulmonary parasites in the UK

Summary

1. *Angiostrongylus vasorum*, *Crenosoma vulpis* and *Eucoleus aerophilus* are nematode parasites which all cause respiratory distress in domestic dogs and are maintained in wild fox populations. Although they can be treated by anthelmintic drugs they can be difficult to diagnose and treatment regimes remain under-evaluated, making it important for veterinarians and pet owners to be aware of the potential for infection with these species. The recent emergence of these species has seen a number of survey studies in Europe and Canada, with varying results for prevalence and associations with fox age, sex, body condition, seasonality and co-infections.

2. While *C. vulpis* and *E. aerophilus* are endemic in foxes across the UK, *A. vasorum* is hypothesised to be spreading from current foci in the south of the country, warranting repeated surveys of the fox population to monitor changes in distribution and prevalence.

3. In this study we aimed to assess the change in range of *A. vasorum* across the UK since previous study which used foxes from 2005-2006. We also aimed to analyse any changes in prevalence or associations with co-infection, fox condition and other factors since previous study using general linear modelling. Hearts and lungs of 103 foxes from four regions of the UK were examined for nematode parasites. 23 foxes were from the English-Scottish border region where *A. vasorum* has not previously been found.

4. *A. vasorum* was not detected in the borders region, and had not significantly increased in prevalence in known foci. *E. aerophilus* was still the most common species found and UK-wide prevalence was 62.5% higher in the present study than in previously study although it remains within the range of other European studies. There were significant relationships between fox body condition and *E. aerophilus* burden and *A. vasorum* presence, and between season and *E. aerophilus* burden. Presence of *E. aerophilus* was significantly associated with decreased *A. vasorum* burden. These results differ from previous work from the UK.

5. Veterinarians should be aware of the potential for northwards spread *A. vasorum*, and the risks of infection from all three species elsewhere in the country. *E. aerophilus* may be emerging in the UK fox population, but future studies should confirm this using a standardised methodology. Detailed study of fox density in different regions, and better sampling of urban foxes would also benefit future studies, while sampling of foxes from the border region should be repeated to monitor spread.

Introduction

Wild animal populations can act as reservoirs for disease, permanently maintaining pathogens which are transmitted to target populations (Haydon *et al.* 2002). Many of these wildlife diseases adversely affect humans and animals valued as livestock, game, pets or for conservation. Considerable effort and money is invested in researching and monitoring some of these disease systems, such bovine tuberculosis in the UK or avian influenza. The wild red fox *Vulpes vulpes* population of Europe has been targeted for management as a reservoir for rabies in recent years (Artois *et al.* 2001) and as reservoirs for *Echinococcus multilocularis,* but management of other parasites has not been prioritised. In particular, the fox is though to act as a reservoir for three cardiopulmonary nematode species: *Angiostrongylus vasorum* (Baillet, 1866), *Crenosoma vulpis* (Dujardin, 1845) and *E. aerophilus* (Creplin, 1839, syn. *Capillaria aerophila*). These parasites are an emerging disease threat to companion animals across Europe and it is therefore important to continually monitor their prevalence and raise awareness of them in the veterinary profession (Traversa, Cesare & Conboy 2010). They can cause a range of respiratory symptoms such as coughing and wheezing, with *A. vasorum* also causing heart damage, bleeding disorders and related neurological problems (Traversa *et al.* 2010). The wide range of non-specific symptoms, as well as sub-clinical infections can make diagnosis of cardiopulmonary infections in domestic animals difficult. Although they can all be treated with anthelmintic drugs which have been tested for safety and efficacy, large scale studies and comparisons of treatment programmes are lacking (Traversa *et al.* 2010).

Despite their growing prevalence across Europe, the literature on cardiopulmonary parasite infection consists mainly of case reports from companion animals (e.g. Yamakawa *et al.* 2009), with fewer studies into the potential wildlife reservoirs in different countries (Table 1). However, reports on prevalence and spread in wildlife, and other factors influencing infection in the reservoir such as seasonality may be important in raising awareness of the threat posed by these parasites. Published studies show that the prevalences of all three parasite species are inconsistent between and within countries. It is therefore even more important that each affected region has its own monitoring programme as it is likely that these differences could be caused by regional differences in climate, seasonal weather patterns, fox density and the sex, age and body condition of foxes, which also need to be researched further to be fully understood.

Table 1. Summary of recent studies of cardiopulmonary nematode prevalence in red foxes (note that recovery techniques are not standardised between studies).

Location	Study	Prevalence (%)		
		A. vasorum	*E. aerophilus*	*C. vulpis*
Austria	Lassnig *et al.* 1998	0	49.7	24.9
Catalonia	Mañas *et al.* 2005	22.7	59	33.9
Denmark	Saeed *et al.* 2006	48.6*	74.1	17.4
Ebro valley	Gortázar *et al.* 1998	20.7	34.8	2.5
Hungary	Sréter *et al.* 2003	5	66	24
Murcia	Martínez-Carrasco *et al.* 2007	1.8	5.4	0
Newfoundland	Jeffery *et al.* 2004	56*	0	87
Norway	Davidson *et al.* 2006	0	88	58
Prince Edward Island	Nevárez *et al.* 2005	0	68.6	78.4

*prevalence in endemic area only

A. vasorum is the focus of many fox parasite studies due to the severity of its effects on companion animals, as well as recent emergence in new countries (e.g. the UK, Denmark and Italy), while *C. vulpis* and *E. aerophilus* are more often considered less of a threat to pets and reported incidentally (Taubert *et al.* 2009). *A. vasorum* infects both canids and felids in many locations around the world, causing respiratory and bleeding disorders which can be initially difficult to diagnose (Koch and Willesen 2009, Traversa *et al.* 2010). It has recently been suggested that *A. vasorum* could also cause larva migrans in humans (Saeed *et al.* 2006). In general *A. vasorum* is found in contained foci within affected countries, with sporadic cases in other areas (Morgan *et al.* 2005). However, it is increasingly being found outside these endemic foci globally (Morgan *et al.* 2005). This emergence could be due to increasing awareness and improved detection, but reviewers suggest that this does not fully explain the trend observed, highlighting the need for further research and monitoring work (Morgan *et al.* 2005, Koch and Willesen 2009, Jeffries *et al.* 2010, Traversa *et al.* 2010).

The probable lifecycle of *A. vasorum* is described in detail elsewhere (e.g. Morgan *et al.* 2005), but the main hypothesis is that foxes and dogs acquire *A. vasorum* by ingesting infected gastropod or amphibian hosts (species unknown), or food contaminated with infected secretions, then pass out larvae in their faeces, which can then reinfect these intermediate hosts (Borovkova 1947). There

are therefore a number of ways in which *A. vasorum* may be emerging worldwide. Movement of pets between and within countries could spread the parasite over long distances, while climate change could allow spread of the intermediate slug host to new areas or changes in density (Jefferies *et al.* 2010). Additionally, *A. vasorum* could spread through the presumed reservoir, the red fox, which can travel long distances while infected with parasites or carrying intermediate hosts on the fur (Simpson 1996). Fox rehabilitation may also spread infection across countries (Simpson 1996). Changes in fox density, diet or behaviour could account for emergence of infection in dogs, through increased contact rate particularly in urban areas, where increasing fox population densities raise the risk of disease spread to domestic animals (Saeed *et al.* 2006, Morgan *et al.* 2008). Despite the interest in foxes as a reservoir for *A. vasorum*, *E. aerophilus* and *C. vulpis* across Europe, the actual rate and mechanism of transfer between foxes and pets is unknown, and these reasons for emergence remain speculative (Koch and Willesen 2009). However, evidence for *A. vasorum* in particular suggests that where infection is detected in dogs it is also found in wild foxes (Morgan *et al.* 2005), and spread of infection between foxes and dogs has been demonstrated experimentally (Bolt *et al.* 1992). Dog populations do not maintain high levels of infection of *A. vasorum* and infection in foxes is thought to be much more common by comparison (Morgan *et al.* 2010). Fox surveys therefore provide a better picture of distribution than case reporting in dogs and are used in many countries (Helm *et al.* 2010, Table 1).

A. vasorum has only recently arrived and spread across the UK. The parasite was first detected in dogs nearly 20 years ago (Simpson and Neal 1982), followed by detection in foxes in this area some years later (Simpson 1996). South Wales and south-east England are now also considered endemic foci of infection in dogs (Morgan *et al.* 2008), with dog case reports from more northerly areas (Hayes and Rowlands 2004, Helm *et al.* 2009, Yamakawa *et al.* 2009, Yates 2009). In foxes, Morgan *et al.* (2008) recently detected infection in known endemic areas and as far north as the Midlands, while a case of infection has been reported in a Chesire fox (Routh 2009), suggesting that *A. vasorum* is spreading northwards through the fox population. Climate envelope modelling has shown that this is likely to occur as climate across the UK is suitable, and this puts a high number of pets at risk of infection (Morgan *et al.* 2009). Morgan *et al.* gave a snapshot picture of disease potential at the time of study (January 2005 - April 2006), but continued survey work is necessary as the parasite spreads. We hypothesised that *A. vasorum* would have spread outside its previous geographical range in a northwards direction in line with case reports, and also into new areas in

the south of the country in our samples from May 2006 onwards. We also hypothesised that in known endemic areas *A. vasorum* prevalence would have increased, as higher prevalences are recorded in other countries' endemic regions (Table 1).

Unlike *A. vasorum*, the other known cardiopulmonary nematodes of foxes *C. vulpis* and *E. aerophilus* are endemic throughout the UK, and *E. aerophilus* has also been detected in UK dogs (Morgan *et al.* 2008, Traversa *et al.* 2010). Both are classed as 'emerging' in Europe due to increasing reports in recent years (Traversa *et al.* 2009, Traversa *et al.* 2010). *C. vulpis* is not known to cause mortality in dogs, but has been neglected in the past and is becoming more widely recognised as a cause of respiratory disease in several European countries (Traversa *et al.* 2010). Adults are found in the bronchi of canids and mustelids, and as with *A. vasorum* the lifecycle may involve a number of suitable gastropod species as intermediate hosts and amphibian paratenic hosts which have ingested infected gastropods (Wetzel and Mueller 1935, Anderson 2000). *E. aerophilus* has been documented as a cause of mortality in dogs, although infection is mainly sporadic and sub-clincal (Traversa *et al.* 2010). This species infects canids, felids and mustelids, which either ingest eggs directly from the environment (direct life cycle), or infected earthworms (indirect life cycle) (Borovkova 1947, Anderson 2000, Traversa *et al.* 2009). The distribution of both species in the UK fox population is not known in detail, and varies widely between countries, although *E. aerophilus* is generally detected at higher prevalences than *C. vulpis* (Table 1). Clinical effects in foxes are also unknown (Morgan *et al.* 2008), although both are thought to cause pneumonia in wild carnivores (Nevárez *et al.* 2005), and *C. vulpis* has been suspected of causing poor fur quality and mortality in young ranched silver foxes previously (Ershov 1956). *E. aerophilus* has also been recorded in one human case (Lalosević *et al.* 2008). Lastly, the nematode *Dirofilaria immititis* can also be recovered from fox cardiopulmonary samples, and is important in Europe as a cause of mortality in dogs with potential to infect humans, although it has never been found so far in the UK (Traversa *et al.* 2010). We hypothesised that surveillance of red foxes for *E. aerophilus* and *C. vulpis* would reveal no changes in distribution or prevalence since previous study, while the likelihood of detecting *D. immititis* would be low.

Little is known about the factors influencing likelihood of individual infection with *A. vasorum* or other cardiopulmonary nematodes within the UK fox population, such as sex, age, body condition or co-infection. Continued study improves knowledge of what factors may increase risk of disease

transmission and explain differences in risk to companion animals between endemic regions. Few other studies of fox parasites have included this type of analysis, studies have not been repeated within the same regions, and inconsistent patterns in these associations have been found in different areas (Jeffery *et al.* 2004, Davidson *et al.* 2006, Saeed *et al.* 2006, Morgan *et al.* 2008). In this case, it was expected that associations between parasite prevalence and fox age, sex, body condition, season and co-infection were likely to have remained constant since study by Morgan *et al.* (2008). We therefore hypothesised that *A. vasorum* would be seasonal, and *E. aerophilus* would be significantly affected by sex, while associations with age and condition would not be significant, and there would be no associations between infection with different nematode species.

In order to test these hypotheses, 103 red foxes collected from 2005-2010 were used, with the aim of surveying the UK fox population for prevalence, burden and distribution of all three nematode species, in order to assess changes since previous study by Morgan *et al.* (2008) to analyse patterns in prevalence related to fox age, sex, body condition and seasonality. We aimed to use this information to assess the levels of risk posed to companion animals and humans from the wildlife reservoir in different regions of the UK.

Materials and Methods

Study materials

Fox carcasses are collected after culling by landowners and pest controllers by the Food and Environment Agency for *Trichinella* surveillance work, after which remaining organs are stored at -18°C. The sex, age (juvenile, young adult or aged adult, classified using incisor wear and condition) and body condition (good, fair or poor, based on lumbar and retroperitoneal fat thickness) of the animals are also recorded. For the present study 103 foxes were selected from storage: 23 foxes were available from the northern area of the UK where *A. vasorum* had not previously been found from 2005 to 2009, and 80 foxes were sampled from southern areas from 2007 to 2010 (Figure 1).
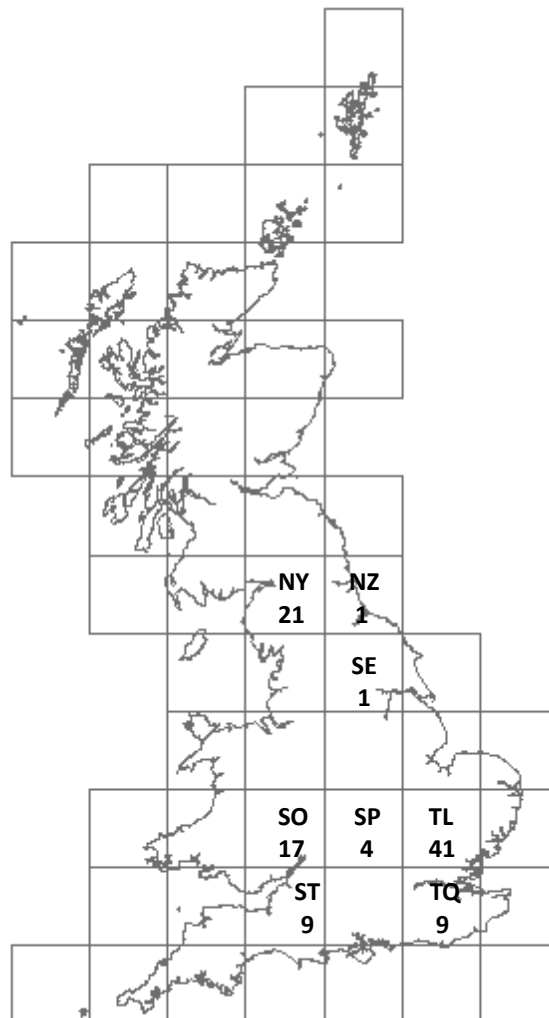

Figure 1: Distribution of samples from the UK, showing number of samples collected from each National Grid 100km square included in this study.

Parasite recovery and identification

Hearts and lungs were defrosted in a cooler at 4°C for at least 16 hours prior to parasite recovery. Samples were labelled with a unique four digit code written on the sample bag, which allowed the origin of the sample to remain unknown during parasite recovery and identification, but was later linked to individual sample data. Samples were also given a sequential number. One or more lung lobes were damaged in 17 foxes where the animal had been shot, and in this case the number of lobes that could not be effectively flushed was recorded. Fifteen foxes did not have a trachea that could be sampled as this had been removed during previous *post mortem*, and this was also recorded.

The outside of each sample bag was rinsed before opening to remove material from other sources. Organs were removed from sample bags, which were then washed out into a sieve. The heart and lungs were then separated by making an incision through the major vessels between them. The trachea was incised longitudinally in order to expose the interior surface, inspected visually for adult nematodes, which were placed in a storage pot, and a scalpel used to scrape a sample of mucous from the surface. This was transferred to a labelled slide. The eggs of *E. aerophilus* and the larvae of *A. vasorum* and *C. vulpis* were identified based on morphological characteristics at 100x magnification (McGarry and Morgan 2009, Traversa *et al.* 2010) and presence or absence recorded. The pericardium was removed from the heart, rinsed and inspected visually for parasites. The heart was incised traversely between the base and apex, and the lower portion also washed and visually inspected. All heart chambers and the pulmonary arterial trunk were opened, washed and visually inspected and the washings were then passed through a 150μm sieve.

Parasites were recovered from the lungs following the method used previously by Morgan *et al.* (2008)*,* which is based on a technique modified from Oakley (1980). Lungs were flushed then dissected as this combination of methods was previously found to be most effective (Morgan *et al.* 2008). The lungs were placed in the sieve, and each lobe was flushed by inserting a syringe tip attached to a tap with rubber tubing into a major blood vessel. Water was then flushed into the lungs until the lobe was a pale cream colour. Where the lung remained dark in colour or was partially damaged the syringe was inserted directly into the lung. After flushing the major blood vessels and airways of the lungs were dissected and visually inspected for nematodes which were placed in a petri dish. The sieve residue was rinsed into this petri dish, and all adult nematodes

extracted using a dissecting microscope, and stored in 70% ethanol to be later identified by mounting on a microscope slide and viewed at 10x magnification. The nematode species recovered from fox hearts and lungs can be identified using morphological characteristics (McGarry and Morgan 2009, Traversa *et al.* 2010).

Statistical analysis

Statistical tests were performed in R version 2.12.1 (R Core Development Team 2010) and the significance level was set at α = 0.05 for all tests. In this study 'burden' was defined as the count of adult nematodes per individual of each parasite species. The following definitions of prevalence and intensity were used, following Margolis *et al.* (1982):

Prevalence = number of infected hosts / total number of hosts examined
Mean Intensity = total number of adult nematodes / number of infected hosts

Prevalence and mean intensity, and intensity variance were calculated for each species overall, and prevalence was also calculated for each species by region in order to compare to previous study. To do this the same regions were used as in Morgan *et al.* (2008): North, Midlands, South, East and South-east (see Table 4 for corresponding OS grid squares). 95% confidence intervals for prevalence were calculated using the exact binomial method.

Initial statistical tests were performed to confirm that recovery rate of adult nematodes did not improve significantly over the course of the study, nor was affected significantly by lung damage. To test whether recovery of worms improved with experience, a GLM with negative binomial error structure was fitted with dissection order as the independent variable and *E. aerophilus* adults recovered as the dependent variable, as this was the most frequently found species. Number of adults recovered was not significantly affected by order of dissection (X^2_1 = 0.493, p = 0.483). The effect of lung damage on recovery was also tested by fitting a GLM with negative binomial errors with lung damage ('one or more lobes damaged' or 'undamaged') as the independent variable and total number of worms recovered as the dependent variable: in this case all species were included in the dependent variable as each is found in a different area of the lung. Lung lobe damage did not have a significant effect on overall worm recovery (X^2_1 = 0.212, p = 0.645).

Detection of infection with each species of nematode varied between different methods (tracheal

scrape for eggs and larvae, lung flush for adults, and dissection after flushing for adults, Table 2). No adult worms were recovered using dissection after flushing, in contrast to Morgan *et al.* (2008). No *A. vasorum* larvae were identified using tracheal scrapes, and prevalence was also lower for *C. vulpis* and *E. aerophilus* using tracheal scrapes compared to flushing (Table 2). Detection of nematodes was higher when tracheal scrapes and lung flush data were combined, suggesting that it is important to use both methods. Therefore, data for presence-absence of adults and eggs were combined for each individual, and this new variable was used for further models of presence-absence.

Table 2. Detection of infection using tracheal scrape and lung flush techniques for each nematode species.

Species	Tracheal scrape only (where trachea present, *n* = 88)		Lung flush only (*n* = 103)		Combined tracheal scrape and lung flush (where trachea present, *n* = 88)	
	Number infected	Percetage infected	Number infected	Percetage infected	Number infected	Percetage infected
A. vasorum	0	0	12	11.7	11	12.5
C. vulpis	4	4.5	5	4.9	6	6.8
E. aerophilus	24	27.3	64	62.1	58	65.9

The change in regional parasite prevalence between the samples used by Morgan *et al.* (2008) from January 2005 to April 2006, and samples from after April 2006 analysed in this study was tested using paired t-tests for each species: for *E. aerophilus* this was done between all regions, and for *A. vasorum* between all regions except North. For this comparison the same region categories were used as in previous study. However in all other models region was reclassified into fewer categories, with Midlands and South areas from previous study being merged into one 'Midlands/South' category, as the actual geographical locations of these foxes was clustered together. Study years ranged from 2005 – 2010, but with only one sample from both 2005 and 2008. In models where year was included as a random effect, GLMMs were fitted twice, with all the data and with data from 2006-7 and 2009-10 only, however in all cases there was no difference in the order effects were removed from the model, or to which effects remained significant, so the results reported here are from the models with all data included. Additionally, year and region were confounded as samples from the northern region were older than southern samples, so year was not fitted as a fixed effect. Presence-absence rather than adult burdens of parasite species

were used as independent variables in models, as this should be a more reliable measure. Foxes were classed into season culled following the same classification as Morgan *et al.* (2008) (Winter = December to February, Spring = March to May, Summer = June to August, Autumn = September to November). Coat condition was not included in any models as only three individuals were recorded with fur loss. Similarly, presence-absence and prevalence of *C. vulpis* were not used as independent or dependent variables in models as so few individuals were infected. Interactions between fixed effects were only fitted in models with enough degrees of freedom.

Factors affecting the presence-absence of both *E. aerophilus* and *A. vasorum* were modelled using GLMMs with binomial error structure and year fitted as a random effect. In one model *A. vasorum* was fitted as the independent variable with region, condition, sex, season, age and *E. aerophilus* presence-absence as independent variables. In another model *E. aerophilus* was the dependent variable and region, sex, age, condition, season, *A. vasorum* presence-absence and the presence-absence of trachea were independent variables. Tracheal presence was included in order to test whether lacking this part of the lung sample altered recovery of *E. aerophilus*. Models were also fitted with adult nematode burdens from infected indivduals (adults or eggs found) as the dependent variable, using negative binomial error structure. This made it impossible to include year as a random effect in these models due to current limitations of the lmer function in R. For *E. aerophilus* the following fixed effects were included in the full model: *A. vasorum* presence-absence, age, sex, trachea presence-absence and interactions between condition and season, and region and season. For *A. vasorum* the following effects were included in the full model: region, *E. aerophilus* presence-absence, sex, condition and season. Full models were fitted as described, significance of fixed effects tested and the least significant effects eliminated stepwise until a minimum adequate model (MAM) was reached containing only significant effects. MAMs were then used to predict adult burden and probabilities of occurrence based on the different levels of each significant effect, by back-transformation. The association between body condition and season was also tested using a chi-square goodness of fit test.

Results

Overall prevalence and intensity of infection

Parasites were found in 70.9% of foxes collected in 2005 – 2010. In total 11.7% were infected with

A. vasorum and *E. aerophilus* was the most frequently found species (Table 3). Between 1 and 40

adult nematodes of each species were recovered from individual foxes, with the mean intensity

similar in *A. vasorum* and *E. aerophilus* (5.3 and 5.9 nematodes respectively) but higher in *C. vulpis*.

In each species the variance in intensity greatly exceeded the mean intensity, meaning that

distribution among hosts is overdispersed (Saeed *et al.* 2006). No *D. immititis* adults were

recovered.

Table 3. Comparison of prevalence and intensity of infection of each parasite species between
previous study by Morgan *et al.* (2008) and the present study, including all samples from 2005 -
2010. 95% confidence intervals for prevalence are shown in brackets.

Species	Overall Prevalence		Adult Intensity (where present)					
			Mean		Variance		Range	
	Current Study	Morgan *et al.*	Current Study	Morgan *et al.*	Current Study	Morgan *et al.*	Current Study	Morgan *et al.*
A. vasorum	11.7 (6.2-19.5)	7.1 (5.2-9.7)	5.3	6.7	54.8	88.1	1-26	1-59
C. vulpis	6.8 (2.8-13.5)	2 (1-3)	13.0	5.6	193.5	54.0	2-31	1-22
E. aerophilus	65.0 (55.0-74.2)	40 (36-44)	5.9	4.4	52.2	40.5	1-40	1-51

Regional prevalences

A. vasorum was found in the South-east (four foxes from nine samples) and Midlands (eight foxes

from 21 samples), but not in the northern region, and was therefore not detected in any new OS

grid squares (Table 4). No *A. vasorum* was detected in the East region where it had been

previously found. *E. aerophilus* was more evenly spread across regions than *A. vasorum* as in

previous study, while *C. vulpis* was only found in the North, Midlands and South-east. Paired t-

tests showed that there was a significant difference in regional prevalences between the previous

and current study for *E. aerophilus* (t_4 =4.37, p = 0.0120, Figure 2), but not for *A. vasorum* (t_4 =

2.21, p = 0.0914, Figure 3).

Table 4. Prevalence of each parasite by geographical region, 95% confidence intervals for prevalence are shown in brackets.

| Region	OS National Grid Squares		n		Prevalence (%)		
	May 2006 – May 2010	Morgan *et al.* (2008)	May 2006 – May 2010	Morgan *et al.* (2008)	*A. vasorum*	*E. aerophilus*	*C. vulpis*
North	NY, NZ, SE	NJ-NY, SD-SJ	23	138	0 (0-28.3)	72.7 (39.0-94.0)	18.2 (2.3-51.8)
Midlands	SO, SP	SK, SO, SP	21	126	28.6 (11.3-52.2)	61.9 (38.4-81.9)	14.3 (3.0-36.3)
South	ST	ST, SY	9	29	22.2 (2.8-60.0)	77.8 (40.0-97.2)	0 (0-33.6)
East	TL	TA-TM	41	128	0 (0-8.6)	68.3 (51.9-81.9)	0 (0-8.6)
South-east	TQ	SU, SZ, TQ-TV	9	125	44.4 (13.7-78.8)	33.3 (7.5-70.1)	11.1 (0.3-48.3)
Total			103	546	13.2 (7.0-21.9)	64.8 (54.1-74.6)	6.6 (2.5-13.8)
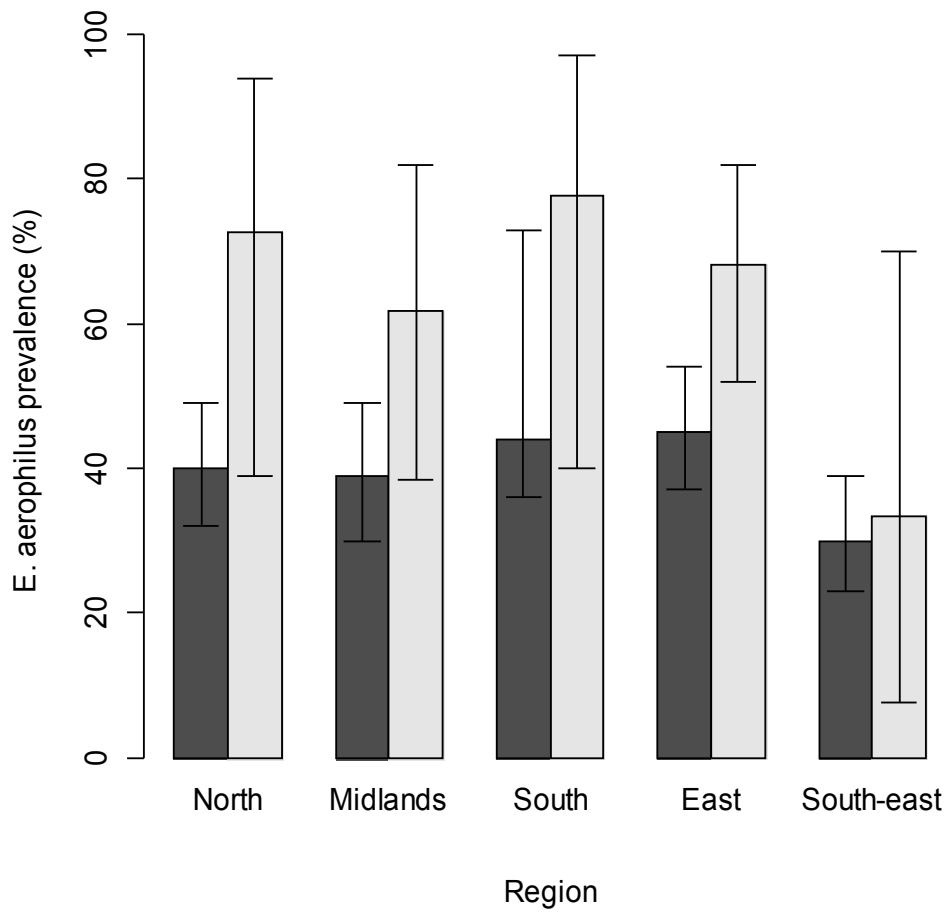
Figure 2. Regional prevalences of *E. aerophilus* from the previously published study (dark grey bars), and the current study samples from May 2006 – May 2010 only (light grey bars), bars show means with 95% confidence intervals.
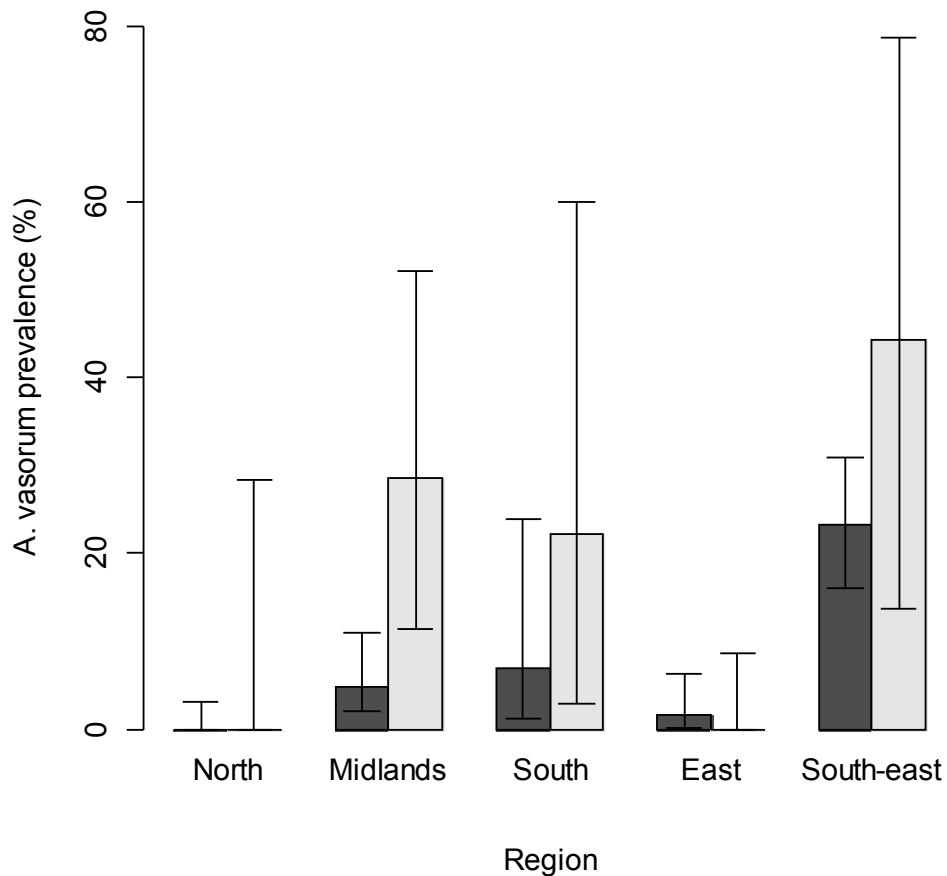
Figure 3. Regional prevalences of *A. vasorum* from the previously published study (dark grey bars), and the current study samples from May 2006 – May 2010 only (light grey bars), bars show means with 95% confidence intervals.

Eucoleus aerophilus presence-absence and burden

Presence-absence of trachea did not have a significant effect on recovery of *E. aerophilus*, measured either by presence-absence or burdens (Presence: $X^2_3 = 0.112$, p = 0.738 , burdens: $X^2_1 = 0.0339$, p = 0.854). Presence-absence of *E. aerophilus* was not significantly affected by any of the other independent variables fitted (Table 6). However, *E. aerophilus* burden from infected individuals was significantly associated with body condition ($X^2_2 = 7.72$, p<0.05), with lowest burdens in foxes in good condition, and season ($X^2_3 = 10.6$, p<0.05), with the greatest difference in burden between winter and spring (Table 7 and Figure 4). However there was no significant interaction between season and body condition ($X^2_4 = 7.24$, p = 0.124), and a chi-square test of goodness of fit between season and body condition showed that body condition was not significantly different between seasons ($X^2_6 = 10.6$, p = 0.103).
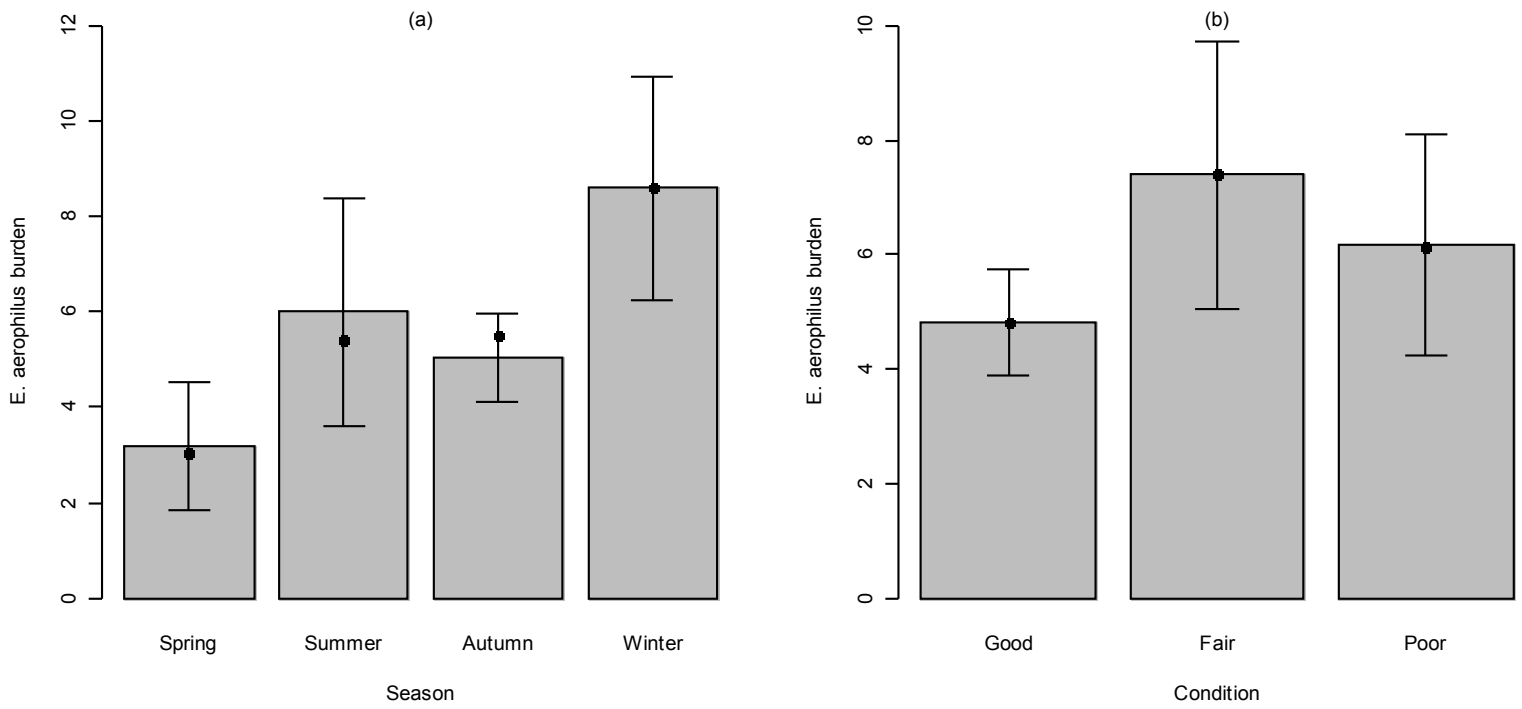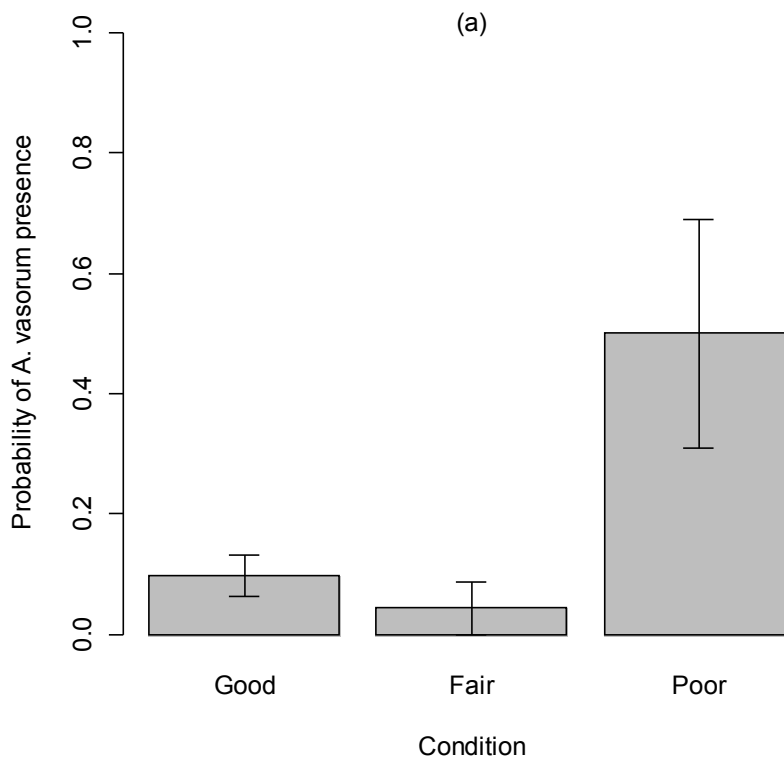
Figure 4. The effect of (a) season and (b) condition on adult burdens of *E. aerophilus* from infected foxes only. Bars show means and standard errors from raw data, points show predicted values for *E. aerophilus* burden from GLM.
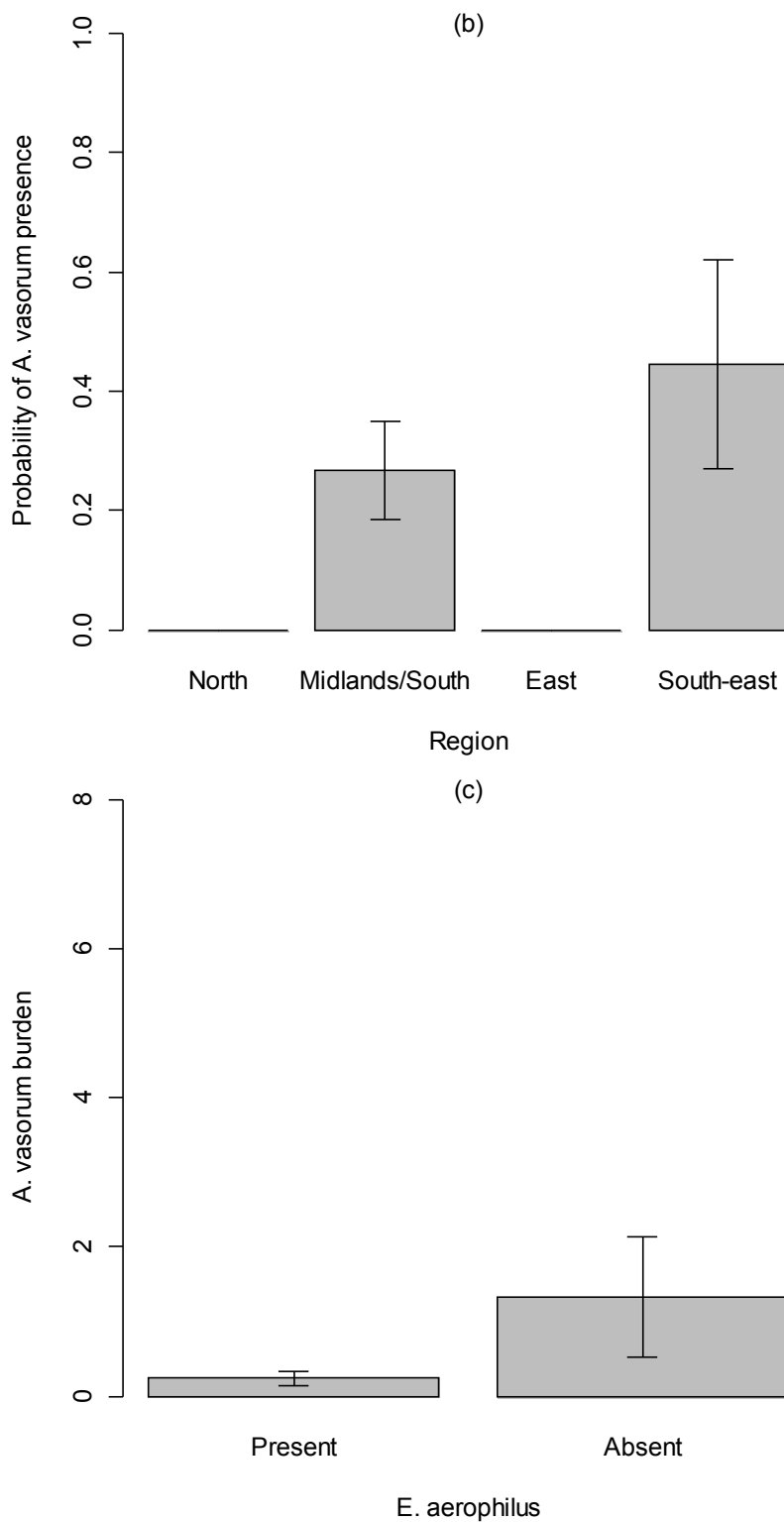
Figure 5. Plots of the likelihood of presence of *A. vasorum* by (a) condition and (b) region, and (c) the effect of *E. aerophilus* presence-absence on *A. vasorum* burden, with standard error bars, using raw data.

A. vasorum presence-absence and burden

Presence-absence of *A. vasorum* was significantly affected by region (X^2_3 = 24.8, p<0.0001, being present only in Midlands and South-east, Figure 5 and Table 8) and condition (X^2_2 = 7.23, p<0.05, increased presence with poor condition, Figure 5 and Table 8). Burden of *A. vasorum* was

significantly associated with presence-absence of *E. aerophilus*, with lower burden where *E. aerophilus* was present (Table 9 and Figure 5).

Discussion

a) Key findings

A. vasorum was not detected in the borders region where we hypothesised it may have spread since previous study by Morgan *et al.* (2008). Additionally, the prevalence of *A. vasorum* had not significantly increased in known foci in the south of the UK, and infection was not detected in new areas in the south of the country. However, veterinarians should still be aware of potential for spread northwards in the UK, and future studies should use a larger sample size from this region to increase the power of detection. Our results suggest that *E. aerophilus* has significantly increased in prevalence since previous study across the UK, and future studies should include analysis of this parasite to confirm this trend using a standardised recovery method.

Significant relationships were found between poor fox body condition and increased *A. vasorum* presence, and good body condition and lower *E. aerophilus* burden*. Presence of *E. aerophilus* was significantly associated with decreased *A. vasorum* burden, and *E. aerophilus* burden changed significantly with season. These results differ from previous studies and highlight the need for further research in order to understand changing patterns of disease ecology within and between countries. This also shows that despite Morgan *et al.*'s (2008) recommendation to use presence-absence data rather than burden data as it is quicker to obtain and shows the same patterns, we found different results when using the different types of data, so in future work using burden data would still be justified.

Parasite ecology and evolution should be considered not only across geographic scales but also within and between individuals in a population. Patterns in *A. vasorum* and *E. aeorphilus* infection prevalence and burden have been found at the level of individual foxes, regional populations and countries (Table 1). The above key findings and possible implications will now be discussed within this framework.

b) Causes of variation in infection among individual foxes: co-infection, sex, age, health and seasonality

Co-infection

Co-infection has been defined as simultaneous infection of a host with two or more parasite species (Graham *et al.* 2007), and has been investigated in many disease systems in the field and laboratory, including that of multiple nematode infections in wild mammals (Behnke *et al.* 2001, Cattadori, Boag & Hudson 2008). In their previous fox parasite survey, Morgan *et al.* (2008) and Jeffery *et al.* (2004) found no significant associations between likelihood of infection with multiple nematode species, and I hypothesised that this would still be the case in the UK. Table 5 shows that rates of co-infection were relatively low, with only 15 individuals with dual or triple infections, compared to other studies with higher co-infection rates (e.g. 75% for *E. aerophilus* and *C. vulpis* in Nevárez *et al.* 2005).

Counter to predictions, *A. vasorum* burden was significantly lower when *E. aerophilus* was present within foxes (Figure 5). This does not fit with previous results from Saeed *et al.* (2006), which revealed a positive relationship between *E. aerophilus* and *A. vasorum* burdens. However, results for associations between *A. vasorum* and *C. vulpis* in other studies also vary (Saeed *et al.* 2006, Jeffery *et al.* 2004). It should also be noted that different studies use different methods to evaluate co-infection, depending on sample size and range of species, which may explain variation in results: for example, Morgan *et al.* (2008) used Spearman rank correlation on burdens of species, while Saeed *et al.* (2006) used a pairwise correlation test. Our models only included the two most common species found, *A. vasorum* and *E. aerophilus*, and used only presence-absence as an independent variable in GLM and GLMMs, as presence-absence should be a more reliable indicator of infection, and includes both adults and eggs. Additionally in this study only eight foxes were infected with both *A. vasorum* and *E. aerophilus*, so this result should be treated cautiously.

However, there are theoretical reasons why *A. vasorum* and *E. aerophilus* could be negatively correlated in this way. Parasites could compete directly for food or space, or indirectly through host immune response to one parasite influencing a second parasite, in this case negatively known as cross-immunity (see Cox 2001 for a review of potential immunological mechanisms). If these parasite species do affect each other within hosts, influencing species burdens, aggregation within

populations or seasonality as in other systems (Cattadori *et al.* 2008) it may be important to continue to monitor *E. aerophilus* prevalence as well as *A. vasorum* due to its potential effects on the more threatening species within foxes, and to further understand this relationship. A much larger sample size would clearly be useful for detecting more cases of co-infection as it occurs at low frequencies (Table 5).

Table 5. Frequency of infection with one, two or three cardiopulmonary nematodes within individual foxes.

Species	Frequency	Percentage of foxes
A. vasorum	2	Single infections: 60.2%
C. vulpis	2	
E. aerophilus	58	
A. vasorum and *C. vulpis*	2	Dual infections: 8.7%
A. vasorum and *E. aerophilus*	6	
C. vulpis and *E. aerophilus*	1	
A. vasorum, *C. vulpis* and *E. aerophilus*	2	Triple infections: 1.9%
No infection	30	29.1%

Sex

We hypothesised that *E. aerophilus* prevalence would be significantly higher in males than females, consistent with Morgan *et al.* (2008) and the immune handicap hypothesis that predicts that testosterone supresses the immune system, leading to higher parasite burden in males (Zuk 1990). However, despite this being a reasonably strong effect in previous study, there was no significant difference between sexes for any measure of parasitism in this study. Sex is not always a significant predictor of infection: for example, Jeffery *et al.* (2004) found no significant effects of sex, Saeed *et al.* (2006) found significance for *A. vasorum* only, while Morgan *et al.* (2008) found no significance for *A. vasorum*. This may be due to local conditions altering infection risk between the sexes in different study locations and during different years. Changing relationships between sex and disease prevalence and burden make it difficult to say how this may impact infection of pets, however.

Age

As in Morgan *et al.* (2008), and Jeffery *et al.* (2004), age was not a significant factor in *A. vasorum* or *E. aerophilus* prevalence or burden where tested. However, as with other factors it should be

noted that age is sometimes a significant factor in parasite distribution: Saeed *et al.* (2006) found lower prevalence of *A. vasorum* and *E. aerophilus* in cubs compared to adults, Jeffery *et al.* (2004) found juveniles had higher burdens of *C. vulpis,* Davidson *et al.* (2006) that juveniles had higher prevalence of this species, while Helm *et al.* (2010) found that younger dogs have greater infection with both *A. vasorum* and *C. vulpis*. These results are generally explained by differences in behaviour and diet between age classes. In these cases it is juveniles that are significantly different to adults (both young and aged). In the present study there were very few juvenile foxes, so it would be useful to have a more representative sample in future study.

Fox health

Fox health is not the primary focus of research into canine parasites, but will affect population dynamics, density and behaviour, which could have a significant effect on disease transmission to companion animals, so has been incorporated into previous fox surveys by Jeffery *et al.* (2004), Saeed *et al.* (2006) and Morgan *et al.* (2008). We hypothesised that fox body condition would not significantly affect prevalence or burden of any parasite species as in Morgan *et al.* (2008), as well as a similar study by Jeffery *et al.* (2004) in Newfoundland. However GLMM showed that parasite presence and burden were significantly associated with body condition: poor body condition with increased probability of *A. vasorum* infection (Figure 5), and good body condition with lower burdens of *E. aerophilus* adults, although the greatest difference in burden was between good and fair body condition, not good and poor body condition, making the nature of this effect unclear (Figure 4). The causal nature of these relationships is also uncertain, although susceptibility to infection resulting from body condition, or body condition influenced by infection are both plausible. Few previous studies have included body condition in analyses of parasite prevalence and burden, making conclusions harder to draw (Richards, Harris & Lewis 1995, Saeed *et al.* 2006, Morgan *et al.* 2008). Morgan *et al.* did not include body condition in linear regression models due to lack of variation in this effect within the sample, although it was described has having no association with parasites in preliminary analysis. Significance in this study may result from better variation in this sample despite reduced size or to observed increases in parasite burden and prevalence. This result again suggests that future studies of parasitism in the fox reservoir should be carried out, as relationships and distributions can change, which may influence transmission to dogs. Other measures of body condition could clarify the relationships found here: for example, lumbar and retroperitoneal fat thickness were used here and have previously been suggested as

the best indicators of condition (Winstanley, Saunders & Buttemer 1998, Jeffery *et al.* 2004), but the data used in this study had been classed into three body condition groupings rather than given as original measurements, which would be a more precise measure of body condition.

Previous studies have shown that infection with *A. vasorum* is associated with thickening of the right ventricle in some studies (Poli *et al.* 1984, Morgan *et al.* 2008, although not in Jeffery *et al.* 2004), and lower heart mass to body mass ratio (Jeffery *et al.* 2004). Nevárez *et al.* (2005) also found significant changes in the lungs due to *C. vulpis* and *E. aerophilus* infection in agreement with older studies, although the clincal effects of this on foxes is not known, while Jeffery *et al.* 2004 found no visible changes due to *C. vulpis* infection. Coat condition data was also available for the foxes collected for this study, but only three individuals had 'fur loss' rather than a 'full coat' recorded, so this variable was not used in analyses. However, two of the individuals with fur loss were also infected with *A. vasorum*, while Morgan *et al. (*2008) note a possible link between parasite infection and sacroptic mange, citing evidence from Italy (Balestrieri *et al.* 2006), Copenhagen (Willingham *et al.* 1996) and the Iberian Peninsula (Segovia, Torres & Miquel 2004). Other aspects of the relationship between fox health measures and parasitism are poorly studied and in general the effect of cardiopulmonary nematodes on fox health is uncertain (Morgan *et al.* 2008). This could be further investigated in future research, although as the impact of this on pets is unclear this is not likely to be a priority.

Seasonality

We hypothesised that infection with *A. vasorum* would be seasonal as in Morgan *et al.* (2008) where prevalence was higher in foxes collected in summer and autumn. However, no seasonality was found in *A. vasorum* infection in this study. Seasonality in *A. vasorum* infection does vary between different studies: for example, Saeed *et al.* (2006) found the opposite pattern to Morgan *et al.* (2008) with significantly lower prevalence of *A. vasorum* in foxes killed in the summer in Denmark. This may be a result of different weather patterns between countries, as well as variation in European weather patterns between years. Additionally, in most studies carcass collection is concentrated during periods of hunting (Morgan *et al.* 2008) which leads to biased samples, with foxes not represented across the whole year. This study is a good example of this, with all winter samples coming from the North and East regions where there is very low prevalence of *A. vasorum,* and consequently a very non-representative sample for this species.

Additionally there may be some confounding between season and age of foxes, given that there are more young foxes in the spring, and age could not be tested in all of our models.

Season had a significant effect on *E. aerophilus* burden, with lowest adult burdens in spring, and highest in winter (Figure 4), but no effect on overall *E. aerophilus* presence-absence. This fits with results from Morgan *et al.* (2008), who found no seasonality in *E. aerophilus* prevalence, while they did not present results for seasonality in burden. Their result was interpreted as a lack of seasonality in parasite transmission, or other effects such as density dependence cancelling out seasonality. However, Saeed *et al.* (2006) did find significantly higher prevalences of *E. aerophilus* in spring and winter. As with *A. vasorum*, seasonality of *E. aerophilus* infection is inconsistent between studies, but this may result from differences in climate or year of study. Overall our results suggest that prevalence in foxes is not seasonal, but that intensity of infection once infected does vary, possibly due to changes in susceptibility due to weather conditions and diet in different seasons, or accumulation of *E. aerophilus* adults during the year (the adult lifespan in foxes is 10-11 months (Borovkova 1947)). Although body condition and season both significantly influence burden of *E. aerophilus*, there was no significant interaction between body condition and season in models, and further analysis showed no significant association between body condition and season. As dogs are likely to become infected by ingesting earthworms in the same way as foxes, it is likely that infection risk follows the same patterns, and is not seasonal.

c) Causes of variation in infection among fox populations: changing distribution and regional prevalences

Angiostrongylus vasorum
The primary aim of this study was to determine whether *A. vasorum* in the wild red fox reservoir had spread from its previously known geographic range in the south of the UK into the region classed as 'North' (the English-Scottish borders, and one sample from Yorkshire), where no infected foxes had been found previously (Morgan *et al.* 2008). However, in the present study no foxes from the North were infected with *A. vasorum* (Table 4). Spread to this region is expected due to suitable climatic conditions (the January isotherm does not fall below -4°C and relative humidity allows the intermediate hosts to exist (Jeffery *et al.* 2004, Morgan *et al.* 2009)), and possible

mechanisms include transportation of pets and rehabilitated foxes, natural fox dispersal and changes in density, or spread of intermediate hosts (Simpson 1996, Morgan *et al.* 2008, Jefferies *et al.* 2010). Lastly, similar geographic expansions have been observed in countries where *A. vasorum* has been recently introduced, such as Denmark, where the parasite was first found in dogs which had travelled to France, and more recently in the fox reservoir (Finnerup 1983, Willingham *et al.* 1996, Bolt *et al.* 2006, Saeed *et al.* 2006).

A possible reason for the lack of detection of *A. vasorum* in this region is the small sample size (23 of foxes) available from 2005-2009. If the parasite has spread to this region in the fox reservoir it is likely to be at a low prevalence of infection and aggregated in a small number of individuals, so a sample size this small would have a very low chance of detection. This is supported by our results showing that *A. vasorum* is over-dispersed in the fox population, aggregated in a few foxes with a negative binomial distribution, as in Morgan *et al.* (2008) and Saeed *et al.* (2006), a typical distribution for macro-parasites (Shaw, Grenfell & Dobson 1998). This study also aimed to discover whether *A. vasorum* prevalence or intensity of infection had increased in areas where it was already known to exist, and whether the parasite had spread from existing foci to cover whole areas of the country as predicted by Morgan *et al.* (2008). Statistical analysis showed region was a significant predictor of *A. vasorum* presence-absence (Figure 5), as the parasite is present in the South-east and Midlands regions but not in the East. In the previous study, *A. vasorum* was found for the first time in the East region, and following the above predictions prevalence here would have been expected to rise to levels similar to the South, Midlands and South-east regions. The lack of infected individuals found in the East region despite it being the largest sample (41 foxes) suggests that at least in this area foci are not breaking down to cover whole areas of the country. Additionally, in the other regions average prevalence was higher than in previous study (Figure 3), but paired t-tests showed that the change in regional prevalences was not significant between studies. The mean intensity of infection was slightly lower in this study (5.3) compared to Morgan *et al.* (2008) (6.7) (Table 3), and so was the range of adult burdens, suggesting that intensity of infection is not increasing where infection is already prevalent in the southern areas of the UK. Overall these results suggest that the distribution of *A. vasorum* in known endemic areas in the south of the UK are not changing rapidly, although the limited sample size of this survey necessitates continued study in the future to monitor further change, and the reasons for the continuance of patchy distribution remain unknown (Morgan *et al.* 2008).

Eucoleus aerophilus, Crenosoma vulpis and Dirofilaria immitits

No *D. immititis* adults were recovered from the fox hearts and lungs, although this does not prove its absence from the UK. This is important because *D. Immititis* is a zoonotic disease threat in Europe, infecting dogs and cats, and may spread when the ranges of suitable vector species change with changing climate (Traversa *et al.* 2010). As expected, *E. aerophilus* presence-absence did not vary significantly between regions, underlining its known endemic presence in the UK fox population. This is also in accordance with results from other countries where presence does not vary between geographic regions (e.g. Denmark, Saeed *et al.* 2006). Burden also did not vary between regions, although this has been significant in similar studies previously (Saeed *et al.* 2006). *C. vulpis* was not as evenly spread as *E. aerophilus*, present only in the North, Midlands and South-east, however as only seven cases of infection were found we cannot be sure whether there are significant differences between regions.

The distributions of both *C. vulpis* and *E. aerophilus* were overdispersed like *A. vasorum*, with the intensity variance greater than the mean intensity, with a few individuals having high burdens of parasites (Table 3). This is common in parasite distribution and means in this case that a few individuals are responsible for shedding most eggs into the environment (Shaw & Dobson 1995, Shaw, Grenfell & Dobson 1998). In light of the evidence that parasite prevalences can change within countries within a few years (e.g. present study, Saeed *et al.* 2006, Willingham *et al.* 1996), it has been suggested that regular surveys must be carried out in order to monitor change (Saeed *et al.* 2006). Although both *E. aerophilus* and *A. vasorum* parasites were previously known in the UK, parasite aggregation, changes in regional and overall prevalence and small available sample sizes in this and likely future studies mean that surveillance work should be done regularly in order to detect changes in disease threat.

d) Causes of variation among studies: comparisons between the current study, Europe and Canada.

Across Europe and Canada's Atlantic coast there have been many studies of fox cardiopulmonary parasites, and the most recent are summarised in Table 1 (see Introduction). In this study the average prevalence of *A. vasorum* where it is known to occur in the UK fits within the range found in other countries (Table 3), and was not significantly different to previous study by Morgan *et al.*

(2008) although European results suggest that it could be maintained at much higher prevalences if conditions are suitable. This suggests that *A. vasorum* prevalence should be monitored in current foci as well as in the border region where it may spread in the future. Prevalences of *E. aerophilus* and *C. vulpis* in this study also fit within the range of previous results. We hypothesised that the distribution of *E. aerophilus* and *C. vulpis* would have remained the same since previous study as these species are endemic across the whole country. *C. vulpis* prevalence remained very low, making analyses unreliable, however, paired t-tests showed that there was a significant change in *E. aerophilus* prevalence between studies, with Figure 2 suggesting that mean prevalence was higher in all regions. This would fit within the pattern observed across Europe for cardiopulmonary nematode emergence (Traversa *et al.* 2010). On the other hand, higher prevalence could be due to differing recovery technique: in the previous study it was noted that *A. vasorum* recovery was prioritised over other species, while in this study I attempted to recover all species, which is a possible explanation for such a change. In this study the average intensity of adult infection was higher for both *E. aerophilus* and *C. vulpis*, but lower for *A. vasorum*, which supports that idea that adult recovery was more evenly focused between species rather than universally more effective (Table 3). However, as the observed changes in prevalence, average adult intensity and range of adult intensity between this and previous study were not unidirectional, this suggests that difference in recovery technique between studies was not the cause of these changes.

Comparison of the studies in Table 1 shows both that prevalences vary considerably between different areas for all species, and that species prevalences vary relative to to each other in different studies. For example, *E. aerophilus* is the most highly prevalent species in European studies, but is absent from Newfoundland, and on Prince Edward Island *E. aerophilus* is less prevalent than *C. vulpis.* The prevalence of *E. aerophilus* ranges from 0-88%, and *C. vulpis* from 0–87%. Differences in the prevalence of these species, and between *A. vasorum* prevalence at different endemic foci, is most likely to relate to environmental conditions (e.g. temperature, rainfall, habitat type), range and density of intermediate host species available or fox population density. Prevalence will also relate to the history of the dispersal of the parasite: for example, it is thought that *A. vasorum* only recently arrived in Newfoundland from Europe (Jefferies *et al.* 2010). Although parasite recovery methods vary between the studies summarised in Table 1 – a range of techniques can be used, including faecal flotation, lung flushing, tracheal scrapes and dissection –

it is unlikely that such wide ranging prevalences could result solely from these differences in method or prioritisation of *A. vasorum* recovery over other species.

The high variation and inconsistency of results between studies has been a source of differing opinions over the role of foxes as a reservoir for *A. vasorum* (Mañas *et al.* 2005), although the general consensus is that foxes do play an important role in the epidemiology of this species.   It would be useful if future studies of this kind used a standardised recovery technique and statistical analyses in order to compare results and analyse whether any of the above explanatory factors have consistent effects on infection, or to peform a meta-analysis of studies to determine the effect of different techniques on results. While large-scale parasite surveys are valuable for raising awareness of spread, the mechanisms of transmission between species, range of intermediate host species and causes of emergence remain uncertain (Koch and Willesen 2009). Therefore more experimental infection studies would be useful to understand the epidemiology of cardiopulmonary nematode infection and methods for infection control (Mañas *et al.* 2005, Morgan *et al.* 2010).

Conclusion and Future Study

This study is one of a number of similar surveys of fox parasites from Europe and elsewhere in the world (Table 1) that have been used to understand the spread of cardiopulmonary nematodes which may affect dogs, cats and humans. These studies have yielded different results for parasite prevalences, and for the significance of factors such as fox age, sex, body condition and co-infection. At the populations level, this study showed no evidence of *A. vasorum* presence in the English-Scottish border region or for an increase in prevalence in previously known areas in the south of the country. However, further work should be done to understand the spread of infection between the Midlands and the North, and to look in particular at mechanisms of spread. *E. aerophilus* remained the most prevalent species, and appears to have increased in prevalence across all regions, although recovery methods should be standardised in future to confirm this trend for emergence. At the individual level, fox body condition, season and co-infection were found to significantly affect aspects of infection with *E. aerophilus* and *A. vasorum* counter to expectations based on previous work by Morgan *et al.* (2008) and other studies.

A key limitation of the present study was sample size and selection. For example, where previous

studies have been larger, researchers have chosen to analyse only parasites infecting more than 30 individuals for association with other factors like sex, season and age (Saeed *et al.* 2006), but this would have limited this study as only *E. aerophilus* infection reached this threshold. The results of this study have not always been in line with those from the previous larger study by Morgan *et al.* (2008). The reasons for this are unclear but may be due to differences in sampling areas available and weather conditions between the years foxes were collected affecting gastropod hosts, fox condition and density. Statistical analyses were also approached differently in the current study, with GLMs incorporating negative binomial and binomial error structures in order to deal with parasite aggregation and presence-absence data respectively, whereas the previous statistical methodology does not indicate use of these error structures. This should make any significant results from the current study more reliable than contradictory results from previous investigation. However, there is a risk with such a small sample size of falsely accepting the null hypothesis when there could in fact be significant associations between factors, such as parasite presence and age or sex, which we concluded were non-significant in this study. Because *A. vasorum* and *C. vulpis* in particular are rare in the UK fox population analyses would be more robust with a much larger sample size than was available for analysis here.

As discussed in Morgan *et al.* (2008), sampling for these surveys is opportunistic as foxes are mainly collected by culling by rural game keepers, so the fox habitats sampled are not representative of the entire UK population. Additionally, sampling may be biased due to characteristics of the foxes making them more likely to be shot which may also influence parasite infection, such as body condition or individual foraging behaviour. Ideally future studies should sample more foxes from the northern region, and the area between the Midlands and the English-Scottish borders if surveying for *A. vasorum*, and more equally between regions and seasons if surveying for general parasite trends. Additionally, sample selection could be improved to reduce the confounding of year and regions. Combining data across studies and countries could also allow better analysis of seasonality, climate and weather patterns.

In future it would also be beneficial to sample more foxes from urban areas as well as rural foxes. Urban foxes are an important concern for health of pets and humans due to increased likelihood of contact and environmental contamination, for example, recent study suggested that *Echinococcus multilocularis* transmission risk is higher in the 'urban periphery' and surrounding recreational

areas than in rural areas (Deplazes *et al.* 2004). Urban foxes were initially thought to be a British phenomenon but many European cities have now also been colonised since rabies in rural foxes has been targeted with a vaccination programme, with studies showing there is likely to be movement and gene flow between urban and rural areas (Wandeler *et al.* 2003). Estimates of fox populations in cities in the UK also suggest higher population densities than in rural areas, although detailed recent figures of population density and rates of disease spread are not readily available (Harris & Rayner 1986). In fox parasite studies with urban samples (e.g. Saeed *et al.* 2006 for Copenhagen), it is urban individuals that have higher prevalences of *E. aerophilus*. In this study, only three foxes came from the centre of urban areas (in this case London), and two were infected with *A. vasorum*, which shows the potential of urban foxes to transmit important diseases to urban dogs. In the future, it would be beneficial to survey foxes from RTAs and pest controllers, or urban faecal samples to improve the representation of urban foxes.

In general it would be informative to have improved fox density data from across the UK in order to analyse effects on fox disease and health. Fox density could interact with disease prevalence in a number of ways, including density dependence of disease prevalence and regulation of popluation size. While Webbon, Baker and Harris (2004) support the idea that foxes in the UK exist at higher density in certain habitat types, such as arable and pasture, compared to others such as upland, other authors have disagreed with this (Heydon, Reynolds & Short 2006). If density does vary with habitat however it could influence disease prevalence and spread between regions if these were characterised by different habitats and associated factors such as prey abundances. Density has been incorporated in Denmark, where Saeed *et al.* (2006) showed that higher fox density was associated with higher *E. aerophilus* abundance and increased frequency of co-infection with two or more parasite species. Disease spread may also depend on the distribution of intermediate host species, but the distribution (and identity) of these species is also not well studied and is likely to be sensitive to future changes in climate (Traversa *et al.* 2010).

Monitoring spread of nematodes in reservoir populations is important to raise awareness of possible causes of disease in pets. Symptoms caused by all three nematodes found in the UK are not always obvious and may be misidentified or go unreported (Helm *et al.* 2009, Yamakawa *et al.* 2009). Therefore, routine surveys of foxes could be a better method of monitoring the parasites involved compared to dog screening (Yamakawa *et al.* 2009), although it has been suggested that

faecal flotation should be used to test pets for disease more frequently (Willingham *et al.* 1996). Saeed *et al.* (2006) recommended regular helminth surveys of foxes (e.g. 5-10 years), especially because parasites are aggregated within populations in certain individuals, which may be missed in small samples. It is therefore recommended that a similar survey study is carried out on UK foxes in the future, which would also be useful for detecting arrival of *D. immititis* into the UK. As all three species found can be effectively prevented and treated using regular doses of anthelmintic drugs (reviewed in Traversa *et al.* 2010), it is very important that pet owners are made aware of the risks of infection and necessity of treatment, as well as raising awareness in the veterinary profession of potential diagnoses.

Acknowledgements

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Appendix: GLM and GLMM results
For each model tables show order fixed effects were removed, degrees of freedom, X^2 and *p*-values of effects removed or remaining significant in the MAM. Table titles give dependent variables.

Table 6. *E. aerophilus* presence-absence

Fixed Effects	Order removed from model	d.f.	X^2	*p*
Sex	1	1	0.0012	0.9724
Trachea	2	1	0.1120	0.7380
Season	3	3	1.3218	0.7240
Age	4	2	0.9243	0.6299
A. vasorum presence	5	1	0.8614	0.3533
Region	6	3	6.7099	0.0817

Condition	7	2	3.1561	0.2064

Table 7. *E. aerophilus* adult nematode burden (infected individuals)

Fixed Effects and Interactions	Order removed from model	d.f.	X^2	*p*
Trachea	1	1	0.1493	0.6992
Region:Season	2	6	6.4224	0.3776
Sex	3	1	0.8054	0.3695
Age	4	2	3.0494	0.2177
Region	5	3	4.3153	0.2294
Condition:Season	6	4	7.2401	0.1237
A. vasorum presence	7	1	3.1545	0.0757
Condition	**Remains in MAM**	**2**	**7.7168**	**0.0211**
Season	**Remains in MAM**	**3**	**10.6313**	**0.0139**

Table 8. *Angiostrongylus vasorum* presence-absence

Fixed Effects	Order removed from model	d.f.	X^2	*p*
Season	1	3	0.9815	0.8057
Age	2	2	0.9416	0.6245
Sex	3	1	0.2761	0.5993
E. aerophilus presence	4	1	0.8605	0.3536
Region	**Remains in MAM**	**3**	**24.7620**	**0.0000**
Condition	**Remains in MAM**	**2**	**7.2257**	**0.0270**

Table 9. *A. vasorum* adult nematode burden (infected individuals)

Fixed Effects	Order removed from model	d.f.	X^2	*p*
Sex	1	1	0.0556	0.8136
Condition	2	2	0.8305	0.6602
Season	2	3	0.6250	0.7316
Region	4	1	0.3138	0.5754
***E. aerophilus* presence**	**Remains in MAM**	**1**	**10.2460**	**0.0014**

Word count (excluding references and tables): 9796

Number of tables and figures: 13

Number of references: 55

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