The helminth parasites of Irish badgers: an untold story

Rachel Louise Byrne

A thesis submitted in fulfilment of the Degree of Master of Science to the University of Dublin, Trinity College

May 2020

Declaration

I declare that this thesis has not been submitted as an exercise for a degree at this or any other university and it is entirely my own work.

I agree to deposit this thesis in the University's open access institutional repository or allow the library to do so on my behalf, subject to Irish Copyright Legislation and Trinity College Library conditions of use and acknowledgement.

Rachel Louise Byrne
May 2020

Summary

The European badger (*Meles meles*) is a member of the Mustelid Family and Ireland's largest terrestrial carnivore. Since the identification of badgers as wildlife reservoirs of bovine tuberculosis (bTB), extensive research has been conducted in the field of badger ecology in the ROI and badgers have become the focus of a reactive culling strategy. As badgers are a protected species in the ROI, culling has only been used as an interim control in the absence of a viable, effective vaccine. Research has now shown, in both controlled and field studies, that a low dose of liquid encapsulated Bacillus Calmette-Guérin (BCG) vaccine is effective at promoting a powerful immune response by triggering the T-helper 1 (Th1) arm of the adaptive immune system. A strong immune response is thereby essential for both the host's ability to control pathogenesis of bTB and the efficacy of the BCG.

One factor that has been identified as disrupting the immune response to tuberculosis infection is an underlying helminth infection. Helminths are a diverse group of large, multicellular parasitic worms. Some species have been found to live for many years - even decades - within a living host. To enable them to survive, many use highly effective mechanisms of immune subversion. This not only allows helminths to evade expulsion by the immune system but has also been shown to result in 'spill over suppression' of routine vaccinations and reduced control of mycobacterial infections.

To date, only 5 studies across the badgers' Eurasian range have described the helminth community of badgers, none of which occur in the ROI. To fill this knowledge gap, a total of 289 badgers were collected from western and eastern counties and examined for the presence of helminth parasites. Infection was diagnosed using a combination of gross organ dissection and faecal egg/larval counts. Access to data from both methods of detection is rare and this offered the unique opportunity to evaluate the efficacy of coprological analysis in comparison to adult worm burden. My results show that for hookworm (*Uncinaria criniformis*), an individual was more likely to be diagnosed incorrectly than correctly for a helminth infection. In contrast, for lungworm (*Aelurostrongylus falciformis*), neither adult worm burden nor faecal larval

counts were sensitive for diagnosing infection. However, when infection was present, coprological analysis was an effective indicator of intensity of infection.
The helminth community consisted of 8 nematodes and included 4 identified to species, 1 identified to genus and 3 unknown - *Aelurostrongylus falciformis; Crenosoma melesi.; Eucoleus aerophilus; Uncinaria criniformis; '*Species A'; *Strongyloides spp.* and two unidentifiable but morphologically distinct nematodes *(*nematode 1 and 2*)*. *U. criniformis* infection was found to be endemic throughout the badger population and *A. falciformis* and *Strongyloides spp.* were common. Interestingly, all helminths described here belong to the Nematoda group and the majority of the known species exhibit direct life cycles.

It is hoped the results from this study will serve as a baseline of badger health surveillance, as well as illuminating the helminth community, in the Irish badger, as an important comparator for other countries across the badgers' range. Additionally, as the ROI is now moving towards a vaccination-led strategy for the control of bTB, it is imperative that work is continued to investigate the effect of underlying helminth infection on both bTB pathology and vaccination efficacy, in light of the helminth endemicity reported here and the well documented immunological effects of helminth infection.

Table of contents

List of figures

List of tables

Chapter 1

Introduction

1.1. Irish badgers: Behaviour, ecology and bovine tuberculosis

The European badger (*Meles meles*), as shown in figure 1.1, is a member of the Mustelid Family and Ireland's largest terrestrial carnivore (Smal, 1995). Badgers are common in the Republic of Ireland (ROI), present in all 26 counties (Smal, 1995) with a reported medium population density (Gaughran *et al.*, 2018). Extensive research has been conducted in the field of badger ecology in the ROI, the Western limit of their Eurasian distribution (Byrne *et al.*, 2012). Badgers live in underground burrow systems called setts. Each sett typically houses 3-6 adults (O'corry-Crowe *et al.*, 1996; Sleeman *et al.*, 2009) commonly known as a clan, cete or social group. Badgers are fossorial feeders, scavenging for food in and on top of soil (Cleary *et al.*, 2009). They exhibit both crepuscular and nocturnal life cycles (Neal, E., Cheeseman, 1996) and as such are rarely seen by humans (Roper, 2010), even in the United Kingdom (UK) where they have a high population density. Since 1974, badgers have been identified as wildlife reservoirs of bovine tuberculosis (bTB). Reservoirs of the disease are individuals who can transmit *Mycobacterium bovis*, the pathogen responsible for bTB infection, without succumbing to the disease and who often remain asymptomatic. This allows such individuals to go undetected in a population and potentially aids in disease spread (Delahay *et al.*, 2001). As a result, badgers have become a focus of bTB control programmes.

Figure 1.1: The European badger (*Meles meles*). Photo taken by RB in County Wicklow, Republic of Ireland.

Bovine TB is a disease of significant economic and agricultural importance due to its ability to spread rapidly through cattle herds (Zinsstag *et al.,* 2006). Identification of badgers as potential sources of bTB outbreak in the ROI led to focused reactive badger culling within a 1km radius of a bTB outbreak in cattle, wherever the cause could not be attributed to cattle to cattle transmission (Bielby *et al.*, 2016). Inevitably, culling has led to a reduction of badger population size in ROI (Byrne, 2013). However, as badgers are a protected species in the ROI under the 1974 Wildlife Act, culling has only been used as an interim control in the absence of a viable, effective vaccine. Controlled studies on both laboratory-infected and wild badgers have shown that a low dose of liquid encapsulated Bacillus Calmette-Guérin (BCG) vaccine is effective at producing a powerful immune response, by triggering the T-helper 1 (Th1) adaptive immune response (Aznar *et al.*, 2011; Gormley *et al.*, 2017; More and Collins, 2016). A strong immune response is essential for both the host's bTB control and the efficacy of the BCG vaccine (Moliva *et al.*, 2015).

1.2. TB and the immune system

Once the bacterium responsible for tuberculosis, *Mycobacterium spp.*, enters the host it migrates to the alveolus of the lung (Bloom, 1994). This triggers the host to elicit a Th1 response (Havlir *et al.*, 1991; Horsnell, 2014). Macrophages, large phagocytic cells, engulf the bacterium (Gonzalez-Juarrero *et al.*, 2001) which is contained, not destroyed, within granulomas consisting of numerous layers of Th1 immune cells. In normal bacterial infections, after the bacterium is engulfed, the complex would bind with lysosymes to release reactive oxygen and nitrogen and inevitably to destroy the pathogen (Forrellad *et al.*, 2013). *Mycobacterium spp.* can avoid this due to their ability to produce numerous virulence factors (Schluger *et al.*, 2012). These virulence factors inhibit the fusing of the phagocyte-lysosome complex and allow the *Mycobacterium spp.* to exist within macrophages in a dormant state. In most cases, the host immune system can contain the *Mycobacterium spp.* inside granulomas and suppress TB replication. Notably, in a small number of individuals, immediately or even years later, the bacterium is able to re-activate from the dormant state, replicate and spread to the lungs and other organs (Smith, 2003). The mechanism by which

bacteria can do this is still unknown. However the occurrence has been significantly linked to a loss of immune function (Flynn and Chan, 2001).

1.3. Co-infection of helminths and TB

One factor that has been identified as disrupting the immune response to *Mycobacterium spp.* infection is an underlying helminth infection (Salgame *et al.*, 2013). Helminths are a diverse group of large, multicellular parasitic worms and some species can live for many years – even decades – within a living host (Mueller, 1965). Often these infections are dismissed as benign, as clinically they do not exhibit symptoms. Recently, however, immunologists have demonstrated that helminths use highly effective mechanisms of immune subversion (Maizels and Yazdanbakhsh, 2003) by immunodulation of the host's immune system. Such mechanisms not only allow helminths to evade expulsion by the immune system but also result in 'spill over suppression' of routine vaccinations and reduced control of mycobacterial infections (Maizels and Yazdanbakhsh, 2003; Sabin *et al.*, 1996). This is because hosts react differently to helminth infection than to mycobacteria (Salgame *et al.*, 2013). In contrast to the Th1 response induced by TB and other bacterial infections, helminths elicit a strong Th2 response by activation of mechanisms that aid in parasite resistance and host tolerance of helminth infection (Naranjo Lucena *et al.*, 2017). Th1 and Th2 subsets of the immune system are described as reciprocally cross-inhibitory (Maizels *et al.*, 1993) and thereby one type will typically gain at the expense of the other. This cross-inhibition within the immune system has the ability to reduce resistance to both helminth and bacterial infection.

The phenomenon of co-infection between TB and helminths is a topic of considerable interest and importance (Li and Zhou, 2013; Abate *et al.*, 2015). This is not only because of the potential risk to human health, biodiversity and agricultural production (Bengis *et al.*, 2002) but also because of the influence helminth co-infection has upon the pathology of microparasitic infection (Elias *et al.*, 2006) by dampening the host's ability to illicit a strong Th1 response (Maizels and Yazdanbakhsh, 2003). Furthermore, helminth infection's ability to interfere with control therapies, including vaccination strategies, is of particular public health significance (Bobat and Cunningham, 2014).

1.4. Parasite communities of badgers

The helminth parasites of the European badger has been documented across its Eurasian distribution. Hancox (1980) was the first and only study to systematically review all published parasite records of the badger, *M. meles*. The review concluded that the available data were diffuse and restricted to certain regions across the badger's distribution. Despite being published over three decades ago this still pertains, with numerous data from across Continental Europe and two reports from Great Britain but no comprehensive reports from the ROI, as shown in Table 1.1. This is highlighted by Sleeman and Kelly (1997) in their report of parasites and diseases of Irish badgers, which stated that no attempt had yet been made to document the helminth parasite community of this important wildlife species. Subsequently one study assessed the parasites of wild red foxes, pine martens and badgers in ROI through the inspection of faeces (Stuart *et al.*, 2013). Researchers found eggs from two nematode species; *Uncinaria criniformis* (40%) and *Eucoleous aerophilus* (6%), with only prevalence being reported in only a small number of hosts (n=50). It is important that there be an independent review of the helminth parasite community of Irish badgers and results are not extrapolated from the UK or continental Europe, as both groups exhibit different population densities and foraging behaviour to that of the Irish badger. In continental Europe and the UK, badgers are found in low (Roper, 2010) and high density populations (Judge *et al.,* 2015) respectively. As demonstrated by Arneberg *et al.,* (1998) in higher host density populations the prevalence, abundance and intensity of infection are higher than those in lower densities. Additionally, Irish badgers have been shown to exhibit seasonal variation in their diet compared to that of UK badger populations (Cleary *et al.,* 2009). As many species of helminths are transmitted through the ingestion of contaminated soil (Bethony *et al.*, 2006), diet is central to helminth infection.

Since 1980, 38 helminths have been reported in the badger host including 27 nematodes, 6 cestodes and 5 trematodes. Of these *Uncinaria spp.* and *Aelurostrongylus spp.* are the most common. The most prevalent helminth appears to be *Strongyloides spp.* (Rosalino *et al.,*2006). However, comparison of prevalence is

difficult with sample sizes below 100 in 14 of the 16 published studies (Table 1.1). Additionally, reporting of intensity is rare (n=4), despite agreement within the parasitological community that intensity of infection is a critical parameter for understanding macroparasite infection (Poulin, 2018).

Diagnosis of helminth infection was mostly consistent, with adult worm burden being used for many of the published studies. Adult worm burden is deemed the gold standard for diagnosing helminth infection and remains one of the key elements to develop our understanding of the population dynamics and impact of macroparasite infections (Seivwright *et al.*, 2004; Tchuem Tchuenté, 2011). However, obtaining data on adult worm counts is often logistically or ethically difficult and as such faecal egg or larval counts are often used as an indirect measure of intensity of infection. Despite the widespread use of coprological analysis for diagnosis, the reliability of faecal egg/larval counts as an assessment of adult worm burden is rarely evaluated, especially in wild hosts (Gassó *et al.,*2015). Consequently, there is a need for an extensive study into the relationship between faecal egg/larval counts and adult worm burden as well as the sensitivity of coprological analysis for the diagnosis of helminth infection.

The aim of this study was therefore threefold: (i) to describe the helminth parasite community of Irish badgers, reporting prevalence, abundance, intensity and aggregation; (ii) to explore the relationship between adult worm burden and egg/larval counts; and (iii) to evaluate the sensitivity and specificity of coprological analysis as an effective diagnostic tool for hookworm and lungworm. It is hoped the results from this study will serve as a baseline of badger health surveillance, as well as illuminating the helminth community in the Irish badger as an important comparator for other countries across the badgers' range.

Table 1.1: The helminths of European badgers (*Meles meles*), From 1980- 2018

Publication author	Country	sample size (n)	Parasite Class	*Parasite Species*	Location in host	Prevalence %	Intensity	method of diagnosis
Jones *et al.*, 1980	United Kingdom	118	Nematoda	*Capillaria sp.*	Pulmonary	5.1	N/A	Worm burden
				Uncinaria stenocephala	Intestine	22.9	N/A	Worm burden
				Molineus patens	Intestine	27.1	N/A	Worm burden
				Strongyloides spp.	Intestine	0.8	N/A	Worm burden
				Aelurostrongylus falciformis	Pulmonary	0.8	N/A	Worm burden
			Cestoda	*Mesocestoides lineatus*	Intestine	1.6	N/A	Worm burden
				Dilepis undula	Intestine	0.8	N/A	Worm burden
			Trematoda	*Ityogonimus lorum*	Intestine	0.8	N/A	Worm burden
		80		*Capillaria erincacei*	Intestine	25	N/A	Worm burden
Loos-Frank and Zeyhle, 1982	Germany	84	Nematoda	*Uncinaria criniformis*	intestine	4	N/A	Worm burden
			Cestoda	*Atriotaenia incisa*	intestine	15.5	N/A	Worm burden
				Taenia martis	intestine	2	N/A	Worm burden
Priemer and Lux, 1994	Germany	11	Cestoda	*Atriotaenia incisa*	intestine	36.3	N/A	Worm burden
Magi *et al.*, 1999	Italy	19	Nematoda	*Aelurostrongylus falciformis*	Pulmonary	21.2	N/A	Worm burden
				Capillaria sp.	Pulmonary	31.6	N/A	Worm burden
				Crenosoma melesi	Pulmonary	21.1	N/A	Worm burden
				Mesocestoides	Intestine	21.1	N/A	Worm burden
				Molineus patens	Intestine	21.1	N/A	Worm burden
				Uncinaria criniformis	Intestine	84.2	N/A	Worm burden
Torres *et al.*, 2001	Spain	85	Nematoda	*Aelurostrongylus pridhami*	Pulmonary	6.4	1.3	Worm burden
				Aelurostrongylus vasorum	Pulmonary	6.4	2.7	Worm burden
				Aonchotheca putorii	Gastric mucus	29.6	36.1	Worm burden
				Crenosoma melesi	Pulmonary	6.4	2.3	Worm burden
				Mastophorus muris	Stomach	14.3	1.3	Worm burden
				Molineus patens	Intestine	38.3	10.06	Worm burden
				Pearsonema plica	Bladder	2.1	1	Worm burden
				Physaloptera sibrica	Stomach	23.1	1.3	Worm burden

Table 1 continued

Publication author	Country	sample size (n)	Parasite Class	*Parasite Species*	Location in host	Prevalence %	Intensity	method of diagnosis
Torres *et al.*, 2001 continued				*Strongyloides sp.*	Intestine	30.9	21.2	Worm burden
				Trichinella sp.	Intestine	2.1	N/A	Worm burden
				Uncinaria criniformis	Intestine	51.6	52.5	Worm burden
				Vigisospirura potekhina	Stomach	7.3	4.7	Worm burden
			Cestoda	*Atriotaenia incisa*	Intestine	12.4	107.2	Worm burden
				Mesocestoides sp.	Intestine	14.3	3	Worm burden
			Trematoda	*Brachylaima sp.*	Intestine	66.6	1.1	Worm burden
				Euparyphium melis	Intestine	7.7	2	Worm burden
				Euryhelmis squamula	Intestine	9.5	74	Worm burden
Millian *et al.*, 2004	Spain	26	Nematoda	*Aonchotheca putorii*	Gastric mucus	0.46	224.6	Worm burden
				Physaloptera sp.	Stomach	0.19	12.23	Worm burden
				Molineus patens	Intestine	0.39	265	Worm burden
				Uncinaria criniformis	Intestine	0.35	55.4	Worm burden
				Strongyloides sp.	Intestine	0.61	2068	Worm burden
				Angiostrongylus sp.	Pulmonary	0.42	65.7	Worm burden
				Crenosoma sp.	Pulmonary	0.04	50	Egg/larval count
			Cestoda	*Atriotaenia incisa*	Intestine	0.69	3484	Worm burden
				Mesocestoides sp.	Intestine	0.04	500	Worm burden
				Taenia sp.	Intestine	0.04	1	Worm burden
			Trematoda	*Euryhelmis squamula*	Intestine	0.12	1708	Worm burden
				Brachylaima sp.	Intestine	0.04	1642	Worm burden
Davidson *et al.*, 2006	Norway	9	Nematoda	*Aelurostrongylus falciformis*	Pulmonary	55.5	N/A	Worm burden & faecal
				Crenosoma melesi	Pulmonary	22.2	N/A	Worm burden & egg/larval count
Gorski *et al.*, 2006	Poland	17	Nematoda	*Uncinaria sp.*	Intestine	5.9	N/A	Egg/larval count
				Capillaria sp.	Pulmonary	11.8	N/A	Egg/larval count
			Trematoda	*Opisthorchis sp. Or Metorchis sp.*	Gall bladder	11.8	N/A	Egg/larval count

Table 1 continued

Publication author	Country	sample size (n)	Parasite Class	*Parasite Species*	Location in host	Prevalence %	intensity	method of diagnosis
Rosalino *et al.*, 2006	Portugal	163	Nematoda	*Mastophorus muris*	Stomach	11.7	N/A	Egg/larval count
				Molineus patens	Intestine	8.6	N/A	Egg/larval count
				Uncinaria criniformis	Intestine	2.5	N/A	Egg/larval count
				Strongyloides sp.	Intestine	43.6	N/A	Egg/larval count
			Cestoda	*Atriotaenia incisa*	Intestine	16.6	N/A	Egg/larval count
Magi *et al.*, 2009	Italy	6	Nematoda	*Angiostrongylus vasorum*	Pulmonary	16.6	N/A	Worm burden
Popiolek *et al.*, 2009	Poland	1	Nematoda	*Crenosoma vulpis*	Pulmonary	100	3	Worm burden
Gerrikagoitia *et al.*, 2010	Spain	50	Nematoda	*Angiostrongylus daskalovi*	Pulmonary	24	N/A	Worm burden
Ribas *et al.*, 2011	Catalonia	44	Nematoda	*Troglotrema acutum*	Nasal sinuses	2.27	8	Worm burden
Moskwa *et al.*, 2012	Poland	7	Nematoda	*Trichinella britovi*	Intestine	28.5	N/A	Worm burden
Stuart *et al.*, 2013	Republic of Ireland	50	Nematoda	*Eucoleus aerophilus*	Pulmonary	6	N/A	Egg/larval count
				Uncinaria criniformis	Intestine	40	N/A	Egg/larval count
Demiaszkiewicz *et al.*, 2017	Poland	9	Nematoda	*Aelurostrongylus falciformis*	Pulmonary	22.2	N/A	Worm burden

[a]N/A= Not available

Chapter 2

A comparison of helminth infections as assessed through coprological analysis and adult worm burdens in a wild host[1]

2.1 Introduction

An accurate assessment of adult worm burden in helminth infections remains one of the key elements that develop our understanding of the population dynamics and impact of macroparasite infections (Seivwright *et al.*, 2004; Walker *et al.,* 2013). However, this remains challenging because the collection of comprehensive data, based upon worms collected at post-mortem or expelled worm numbers after chemotherapy, is often logistically or ethically impossible. In the absence of worm burden data, egg or larval counts continue to be used as indirect measures of the extent of infection in the diagnosis of helminths of human, veterinary and wildlife significance (Gulland and Fox, 1992; Guyatt and Bundy, 1993; Irvine *et al.,* 2001; Seivwright *et al.,* 2004). Despite the widespread use of coprological analysis for helminth infection diagnosis, the reliability of faecal egg/larval counts as an assessment of adult worm burden is rarely evaluated, especially in wild hosts, with the majority of studies conducted in small ruminants (Gassó *et al.,* 2015). There is, however, a consensus, irrespective of host species, that the accuracy of faecal egg and larval counts for diagnosing helminth infection is restricted because of several factors that include, but are not limited to, their absence when only male worms are present, variation in the methodologies employed in diagnostic procedure including faecal sample collection (Seivwright *et al.,* 2004), variation in the abundance of eggs or larvae produced by the worms (Hudson, 1986; Shaw and Moss, 1989) and the influence of

[1] The majority of the contents of chapter 2 have been accepted for publication pending suitable minor revisions in Byrne, Fogarty, Marples and Holland (2018)

density-dependent factors (Keymer and Slater, 1987; Schad and Anderson, 1985; Tompkins and Hudson, 1999). In a recent analysis, utilizing both chemo-expelled *Ascaris* adult worms collected from children and egg production assessed by the formyl-ether concentration method (Holland *et al.,* 1989), we were able to model the effects of improved diagnostic sensitivity and how that might impact upon the assessment of the success of large-scale control programmes for soil-transmitted helminths (Medley *et al.,* 2016). In contrast, the present study utilises similar data from a wild host, the European badger (*Meles meles*), providing support for the wider applicability of scientific findings from wildlife health surveillance as an important component of the one health concept (Daszak *et.al.,* 2000; Akdesir *et al.,* 2018).

Hookworm infection by *Uncinaria spp.* (Jones *et.al.,* 1980; Loos-Frank and Zeyhle, 1982; Torres *et.al.,* 2001; Rosalino *et.al.,* 2006; and see Hancox, 1980, for a review) and lungworm infection by *Aelurostrongylus falciformis* (Stubbe, 1965; Jones *et al.,* 1980; Magi *et al.*, 1999; Davidson *et al.,* 2006) have been described in badgers from across their Eurasian distribution (Neal and Cheeseman, 1996), including the Republic of Ireland (ROI) (Stuart *et al.*, 2013; Ursula Fogarty unpublished observations). However, despite numerous published studies on hookworm infection and several on lungworm in badgers, there is a paucity of information on population dynamics of these helminth infections. Only one study to date has reported the intensity of hookworm infection as measured by worm burden (Torres *et al.*, 2001) with the remainder only recording prevalence data (Table 1.1) . Additionally, the frequency distribution of any helminth worm burdens across the badger population has not yet been described. For all studies, infection was diagnosed by either faecal egg/larval counts or post-mortem examination and consequently the relationship between coprological analysis and adult worm burden has not been evaluated.

We were provided with the unique opportunity to access both the burden of hookworm (*Uncinaria criniformis*) derived from the intestine and of lungworm (*Aelurostrongylus falciformis*) derived from the lungs during post-mortem examination of badgers, together with faecal samples collected from each animal. This allowed the identification of both hookworm eggs and lungworm larvae as well as the evaluation of coprological analysis as an effective diagnostic tool for helminth infection.

The aims of this study were therefore threefold: (i) to determine the prevalence, abundance and intensity of hookworm and lungworm based upon both adult worm burdens and faecal egg/larval counts, (ii) to explore the relationship between adult worm burden and egg/larval counts, and (iii) to evaluate the sensitivity and specificity of coprological analysis as an effective diagnostic tool for hookworm and lungworm.

2.2 Material and Methods

2.2.1 Study animals

In total, 289 badgers were examined for helminth parasites. Badgers were provided by the Irish Department of Agriculture, Food and the Marine (DAFM) from the strategic culling programme conducted in bovine tuberculosis endemic regions by DAFM, under a National Parks and Wildlife Services license. No animals were killed specifically for the purposes of this study.

Badgers were transported from their site of culling to the Irish Equine Centre (IEC) within 1-3 days and dissected on the day of arrival. All organs were removed from the badgers, and blood samples taken by an experienced veterinary surgeon (UF) and labelled with unique DAFM issued badger identification codes. Samples were chosen at random from Western counties and Eastern counties across one sample year. Laboratory work at Trinity College Dublin was approved by the School of Natural Sciences Research Ethics Committee (code:2016-28).

2.2.2 Collection of samples

Hearts, lungs and gastrointestinal tracts were collected and frozen without preservative, at -20^{o}C until examination. Faecal samples were collected from the rectum of gastrointestinal tracts and fixed in 10% formalin.

2.2.3 Gross organ dissection for the identification of adult helminth worms in situ

Organs were dissected over 3-12 months and were defrosted overnight at 5^{o}C and inspected within 8 hours. Hearts and lungs were processed and examined by a modified flush and dissection technique (Mccarthy *et al.,* 2018; Morgan *et al.,* 2008). The heart was removed from the pericardial tissue and a small incision was made in the right ventricle. The aorta and pulmonary artery were clamped using forceps and water was pumped through the incision in the right ventricle until the heart and lungs were fully inflated and white in colour. Fluid was still able to leave the trachea with all washings being collected in a shallow tray. Blood clots were broken up by manual pressure. The clamps were then removed and the heart cut open. All cardiac chambers, arteries and veins were cut lengthways and inspected visually for parasites. The heart was then removed from the lungs by transecting the major vessels as closely as possible to the lungs. An incision was then made lengthways down the trachea and along both main bronchi. Using fine scissors, all visible bronchi were opened and scrutinised for parasites. The hearts and lungs were then submerged in water, together with the bags the organs were stored in, and the washings were collected in a tray.

The GI tracts were cut open lengthways, flushed using 0.9% saline and scrutinised for helminth parasites.

For all organs, the washings were inspected visually for parasites before being passed through sieves of apertures 150 and 64μm. Sieves were then washed with water with all runoff being caught in a tray and scrutinised for helminth parasites. Whenever they were found, helminth parasites were removed, counted and preserved in 70% ethanol. Species identification was confirmed by Eileen Harris, Senior Curator, Parasite worms from the Natural History Museum, London.

2.2.4 Microscopic identification of helminth ova and larvae in the faeces

A modified formol-ether concentration technique (Allen and Ridley, 1970) was used to detect helminth parasite eggs and larvae. Samples were processed blind with two slides being examined for each individual badger and the egg per gram count (e.p.g) and larvae per gram (l.p.g) calculated for each individual.

2.2.5 Data analysis

All results were calculated from the recorded data, and were performed using the statistical analysis software package, R Studio 1.0.153 for Mac (R Core Team 2017).

2.2.5.1 Prevalence

The prevalence for a given parasite species was defined as the number of hosts infected with one or more individuals of a particular parasite species divided by the number of hosts examined for that parasite species (Bush *et al.,* 1997).
Confidence intervals (*CI*) around prevalence values were calculated using the formula;

$$CI = \sqrt{(n)(p)(q)}$$

Where:
n= sample size
p= mean prevalence
and q = 1-p.

2.2.5.2 Abundance, intensity and aggregation

Mean abundance and intensity values were calculated for both adult worm burden and faecal egg or larva counts. Mean abundance is defined as the total number of individuals of a particular parasite in a sample of a particular host species, divided by the total number of hosts examined for that species (including zero counts). Mean intensity is the average number of a particular parasite species among only the infected members of a particular host species (Bush *et al.,* 1997).

As helminth parasites tend to have an aggregated distribution, with a large number of parasites being found in only a small number of individuals of the population, the variance to mean ratio ($s^2/\bar{x}$) was calculated as a measure of aggregation (Holland *et al.,* 1989). Any ratio with a value greater than one was deemed to be aggregated. Abundance and intensity values were transformed using the $\log_{10}(x+1)$ to normalise skewed data and to account for the large number of negative samples.

2.2.5.3 Sensitivity and specificity

To evaluate egg counts as a diagnostic tool, the sensitivity and specificity were calculated. The sensitivity is the probability that a helminth positive individual will be diagnosed as positive, termed a true positive (TP), and the specificity is the probability that a helminth negative individual will be diagnosed as negative, termed a true negative (TN). False negatives (FN) occur when infection is missed by the test. If the test indicates infection when none is present, this is termed a false positive (FP). Sensitivity is equal to $TP/(TP + FN)$ and specificity is equal to $TN/(FP + TN)$ (Altman and Bland, 1994). As faecal egg and larval counts are being evaluated as a diagnostic test, these will be termed index tests and adult worm burden as the diagnostic gold standard.

2.3 Results

Within the GI tract, the prevalence of hookworm *Uncinaria criniformis* adults was 59.2% (CI 95% 53.3%- 64.9%), and in comparison, the prevalence of hookworm eggs in the faeces was 24.6% (CI 95% 19.7%-29.9%), as shown in Table 2.1. In lung samples, the prevalence of lungworm *Aelurostrongylus falciformis* was 20.8% (CI 95% 16.2% - 25.9%) and within the faeces, the prevalence of lungworm larvae was 18.3% (CI95% 14.0%-23.3%). As shown in Tables 2.1 and 2.2, the mean abundance and intensity was higher in hookworm, for both adult worm burden and faecal egg count.

Table 2.1: Prevalence and abundance for adult hookworm (*Uncinaria criniformis*) and adult lungworm (*Aelurostrongylus falciformis*) worm burden and corresponding faecal egg/larval counts (e.p.g/l.p.g)(n = 289). The parasite location within the host is stated next to the parasite name as gastrointestinal tract (**GI**) and lungs (**L**).

	Prevalence		Abundance			
	Adult worm	Eggs/larvae	Adult worm		Egg/larvae	
Helminth species	% (CI)	% (CI)	Mean(±SE)	Min-max	Mean(±SE)	Min-max
U. criniformis. (GI)	59.2 (53.3-64.9)	24.6 (19.7-29.9)	22.5±3.2	0-500	43.6±20.1	0-330
A. falciformis (L)	20.8 (16.2-25.9)	18.3 (14.0-23.3)	2.29±0.4	0-53	14.0±23.3	0-2120

Both hookworm and lungworm had an aggregated distribution, as shown in Table 2.2. However, the aggregation of both species of helminths differed relative to the method of diagnosis. For adult worm burden, hookworm had a higher value for aggregation (127.8) than lungworm (23.6), the frequency distribution can be seen in Figure 2.1.

However, for faecal counts, the l.p.g. of lungworm had a much higher value for aggregation (921.8) than that of hookworm e.p.g. (23.6).

Table 2.2: Intensity and aggregation of adult hookworm (*Uncinaria criniformis*) and adult lungworm (*A. falciformis*) worm burden and corresponding faecal egg/larval counts (n = 289).

Helminth species	Intensity				Aggregation ($v : \bar{x}$)	
	Adult worm		Eggs/larvae			
	Mean(±SE)	Min-Max	Mean(±SE)	Min-Max	Adult worm	Eggs/larvae
U. criniformis (GI)	38.1±5.0	1-500	177.3±80.2	10-330	127.8	20.9
A. falciformis (L)	11.1±1.6	1-53	154.2±48.5	10-2120	23.6	921.8

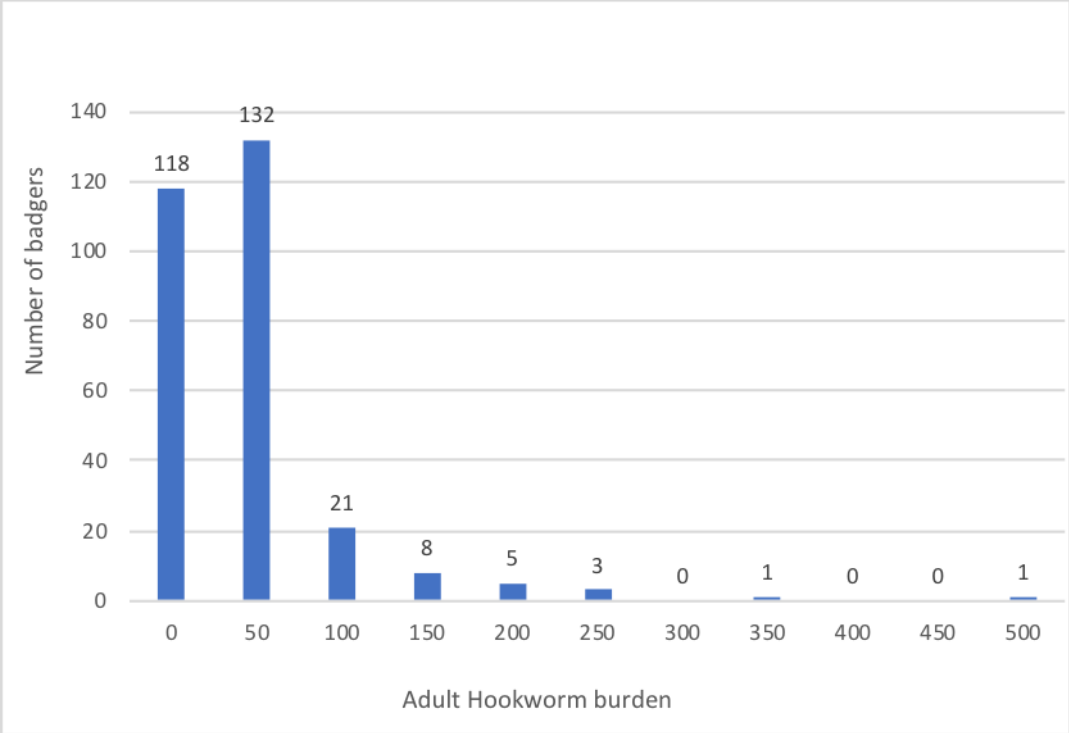
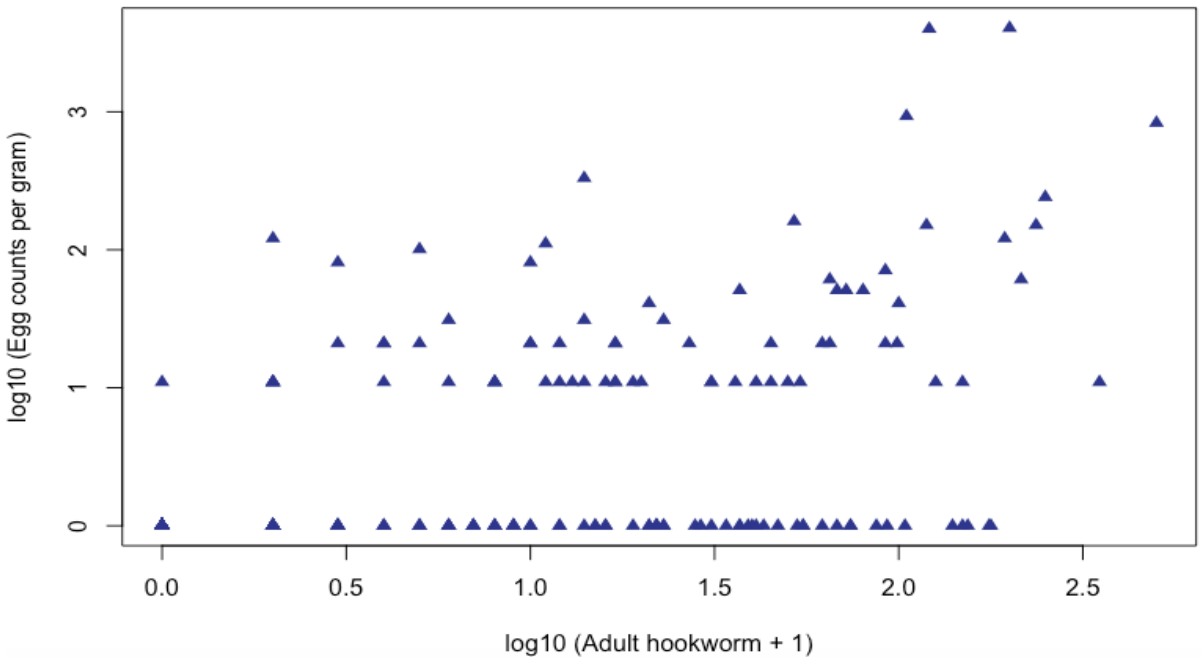
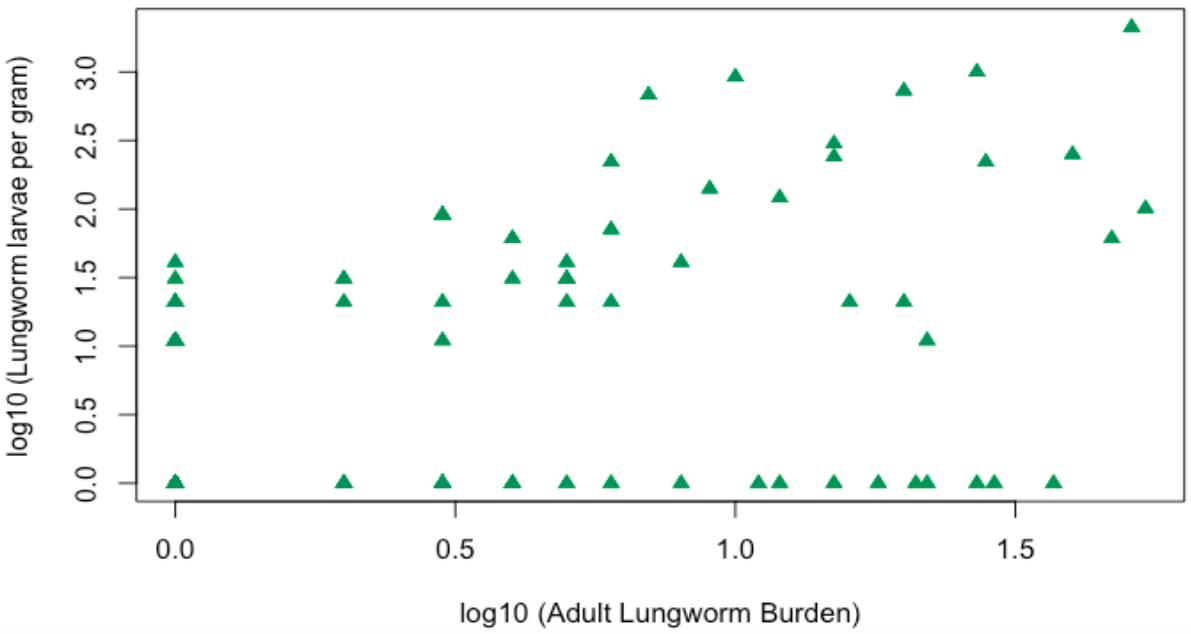


Figure 2.1. The frequency distribution of adult hookworm (*Uncinaria criniformis)* in Irish badgers (n=289). Numbers on the top of each bar represent number of hosts for each category of worm burden.

Using abundance data, the relationship between adult worm burden and e.p.g./l.p.g was explored for each helminth species (Figures 2.2 and 2.3). For both species of



helminths, the relationship between worm burden and egg or larval production was statistically significant with the numbers of eggs or larvae produced increasing as



worm burden increased (hookworm. R^2=0.29, t=11.12, p<0.01; lungworm=R^2=0.38, t=13.38, p<0.01). Additionally, for both helminth species, there were many instances that individuals had a high helminth worm burden but no eggs (n=100) or larvae (n=54) were detected in the faeces.

Figure 2.2: The relationship between adult hookworm (*Uncinaria criniformis*) burden and faecal egg counts. (e.p.g) in Irish badgers (n=289).

Figure 2.3: The relationship between adult lungworm (*A. falciformis*) burden and number of larvae in Irish badgers (n=289).

Finally, the efficacy of coprological analyses as an effective diagnostic tool was evaluated. The evaluation of hookworm e.p.g. compared to hookworm adults present in the GI tract resulted in a sensitivity of 41.5% (CI95% 34.05%-49.29%) and a specificity of 100% (CI95% 96.92% - 100%)(see Table 2.3). There were no individuals positive for eggs but negative for worms.

Table 2.3: A comparison of faecal egg count and adult hookworm (*Uncinaria criniforms*) worm burden as a diagnostic tool. **TP**= True positive, **TN**= True negative, **FP**= False positive, **FN**= False negative.

Diagnostic gold standard- adult hookworm burden			
Index test Faecal egg count		Positive	Negative
	Positive	**TP** 71	**FP** 0
	Negative	**FN** 100	**TN** 118
	Total	171	118

v

Lungworm *A. falciformis* l.p.g. as a diagnostic test were also evaluated compared to *A. falciformis* adults found in the lungs, this resulted in a sensitivity of 10% (CI 95% 3.76%-20.51%) and a specificity of 79.48% (CI 95% 73.66% - 84.51%)(Table 2.4). There were only 6 instances were both adult worms and larvae in the faeces were detected. In contrast to hookworm diagnosis, there were instances (n=47) of larvae identified in the faeces but adult worms were not detected in the lungs.

Table 2.4: A comparison of larval count and adult lungworm (*A. falciformis*) burden as a diagnostic Tool. **TP**= True positive, **TN**= True negative, **FP**= False positive, **FN**= False negative.

Diagnostic gold standard- adult lungworm burden			
Index test Larval count		Positive	Negative
	Positive	**TP** 6	**FP** 47
	Negative	**FN** 54	**TN** 182
	Total	60	229

For each helminth species (*Uncinaria criniformis* and *A. falciformis*) when using e.p.g./l.p.g for diagnosis there were a large numbers of false negatives (FN), a result of infection being missed due to the inability of coprological analyses to detect all infections.

2.4 Discussion

Within our sample of 289 badgers, the prevalence of both hookworm (*Uncinaria criniformis*) and lungworm (*Aelurostrongylus falciformis*) was consistently under-reported by faecal egg/larval counts when compared to adult worm burden. Despite the high prevalence of adult hookworm infection reported, the prevalence was considerably lower when using only faecal egg count data. In contrast, the prevalence of lungworm burden (20.8%) and larval counts (18.3%) was similar although still higher for adult worm burden, suggesting that larval count might be a suitable indicator of lungworm infection. Our findings are similar to those of Gassó *et. al.,* (2015) who described low sensitivity of faecal egg counts in wild boar infected with a range of helminths. Additionally, looking more broadly across studies in domesticated species, our results are congruous with horse necropsy studies which reported that infection was missed by both faecal egg and larval counts for numerous species of intestinal helminth (Nielsen *et al.,* 2010).

A dominant component of the host-macroparasite relationship is the aggregated distribution of helminth parasites within mammal populations (Crofton, 1971; Galvani, 2003), a finding supported by extensive empirical evidence (Wilson *et al.,* 2002). This has given rise to the term 'wormy' individuals (Hotez *et al.,* 2008), i.e. individuals with heavy helminth infections. We found that in our badger sample both helminth species, hookworm and lungworm, had a highly aggregated distribution. The aggregated distribution of helminth infection within hosts has been linked to increased disease transmission (Bradley and May, 1978) and as such, it has been suggested that these wormy individuals should be the target of strategic disease control programmes (Lloyd-Smith *et al.,* 2005). Interestingly, we found that adult hookworm demonstrated greater aggregation than adult lungworm. In contrast however, based upon faecal

counts, lungworm larvae demonstrated higher aggregation than hookworm eggs. Results such as these underline potential threats to future control programmes that hope to identify and target 'wormy' individuals by the use of faecal egg or larval counts.

Studies on the relationship between egg/larval counts and adult worm burden are rare due to the challenges, both logistical and ethical, in obtaining samples. Where data have been collected, these are typically analysed using worms expelled from the host after anthelminthic treatment (Elkins *et al.,* 1991; Kim *et al.,* 2011). There are many acknowledged limitations of obtaining adult worms by purgation such as the limited efficiency of anthelminthic drugs on non-gastrointestinal helminths (Shen *et al.,* 2007), worms being expelled before samples could be collected and therefore being excluded from the count (Kim *et al.,* 2011), and worms that sequester in the caecum rather than enter the faeces (Anderson and Schad, 1985). These limitations lead to the conclusion that chemo-expulsion can be used to calculate the minimum burden of infection (Kim *et al.,* 2011), but, depending on the helminth species and notably the location within the host, it may not accurately reflect the worm burden. Here, we present a unique evaluation of the relationship between hookworm eggs and lungworm larvae with their respective adult worm burden as measured by post-mortem examination. This method is likely to be more accurate than counting worms after treatment, considering the given limitations.

We found that within the badger community of the Republic of Ireland, there is a significant relationship between faecal egg or larval counts and adult worm burden for both species of helminths, such that as adult worm burden rose, so did the number of eggs/larvae observed in the faeces. Additionally, there was a statistically significant positive relationship between the log transformed worm burden and hookworm egg and lungworm larval counts suggesting that both techniques offer a good assessment of intensity of infection. Consequentially, we suggest that although faecal counts were ineffective at discriminating between positive and negative individuals, when the host was producing eggs/larvae, our data could be used to approximate the number of adult worms present. Also, we suggest that density-dependant suppression of egg or larva production, as described by Anderson & Schad (1985), is weak or non-existent, at

least up to the observed worm intensity of 500 worms in the badger host. A similarly linear relationship was observed in *Trichostrongylus tenuis* infection, with reported worm intensity of 8000 worms, in red grouse (Seivwright *et al.,* 2004). Interestingly, the relationship between worm burden and their reproductive products in badgers, contradicts what is observed in humans where faecal counts do not exhibit a significant relationship with adult worm burden (Anderson and Schad, 1985) and the density-dependant relationship between the two is well documented (Anderson and Schad, 1985; Churcher *et al.,* 2005; Keymer and Slater, 1987).

This is, to our knowledge, the most extensive and robust evaluation of coprological analysis as a diagnostic tool for the presence of helminth infection in a wild host to date. We found that coprological analyses have low sensitivity for diagnosing positive helminth infections compared to adult worm burden. For hookworm infection, a positive host was more likely to be falsely diagnosed as negative than accurately recognised as positive by faecal egg counts. Although this finding is not unexpected, with previous reports in wild boar (Gassó *et al.,* 2015), domesticated horses (Nielsen *et al.,* 2010) and humans infected with *Opisthorchis viverrini* demonstrating low sensitivity of faecal egg counts compared to adult worm burden (Sithithaworn *et al.,* 1991), it is important that our results are not interpreted to suggest that coprological analysis is not a useful diagnostic tool for helminth infection. It has long been acknowledged that there is a lack of consensus for an agreed gold standard diagnostic test for helminth infection (Nikolay *et al.,* 2014). This uncertainty is supported in this study by the large number of labelled "false positives" in lungworm diagnosis, in which adult worms were not observed in the lungs despite an active infection being present. This can be explained to some extent by the small size (48-150μm) and inaccessible niche of the lungworm, *A. falciformis*. As the helminths are found embedded within the lung tissue, they can be difficult to detect despite the most effective dissection and flush technique being used for the harvesting of lungworm (Houpin *et al.,* 2016). Thus, we suggest that a composite reference standard be used for lungworm diagnosis in which, in lieu of a gold standard, the results of two or more tests be pooled together to encourage accurate diagnosis (Naaktgeboren *et al.,* 2013).

Moreover, it is important to note that the output of eggs or larvae can vary (Anderson and Schad, 1985) as a result of genetic (Stear *et al.,* 2007) and environmental factors (Stromberg, 1997) and bacterial co-infection. Little is known about the effect of bacterial co-infection in wild hosts. However, in humans co-infected individuals have been shown to shed more eggs than individuals with single infections (Lass *et al.,* 2013). As badgers are known to be hosts of bovine tuberculosis (Corner *et al.,* 2011) it is possible similar co-infection may be occurring.

In this study, faecal egg and larval counts were shown to have low sensitivity for diagnosing helminth infection in a wild host. Hookworm infection was consistently under-reported in faecal egg count analysis and was found to be endemic within the Irish badger (*Meles meles*) population. Identifying lungworm within the organ proved less accurate with neither larval count nor worm counts offering a sensitive diagnostic test. This study is the first to investigate the frequency distribution of helminth infection and evaluate coprological analysis against worm burden as a diagnostic tool within the badger host.

Chapter 3

Characterisation of the helminth parasite community of European badgers (*Meles meles*) in Ireland reveal Nematode domination.

3.1 Introduction

Little is known about the helminth parasites of the European badger (*Meles meles*) in the Republic of Ireland, the Western limit of its Eurasian distribution (Roper, 2010).

Sleeman and Kelly (1997), in a review of parasites and diseases of Irish badgers which focused on ectoparasites and Tuberculosis, stated that no attempt had yet been made to document the helminth parasite community of this important wildlife species. Subsequently one study assessed the parasites of Red foxes, pine martens and badgers in the ROI through the inspection of faeces (Stuart *et al.,* 2013). Researchers found eggs from two nematode species in a sample of 50 roadkill badgers collected across Ireland and reported a prevalence of 40% for *Uncinaria criniformis* and 6% for *Eucoleous aerophilus*.

A review of the helminth parasite community of Irish badgers is particularly important because the population biology and ecology of the host differs markedly from that of British and continental European badgers (Byrne *et al.,* 2012). In Ireland, the population of badgers exists at a medium density of approximately 1/km^2 (Gaughran, 2018), unlike those in the UK, where badgers are largely found at much high densities (>10/km^2) (Judge *et al.,* 2015), or continental Europe, where badgers live at low densities (<0.1/km^2) (Roper, 2010). Arneberg *et al.* (1998) investigated the effect of a host's population density on helminth infection across 19 mammalian groups, equating to 6670 individual hosts, and demonstrated that at higher host population densities, the prevalence, abundance and intensity of infection tend to be higher than those detected at lower densities.

Since 1980, 16 studies have been published on helminth infections of the European badger. However, only five described the parasite community (Jones *et al.,* 1980; Magi *et al.,* 1999; Torres *et al.,* 2001; Millan *et al.,* 2004; Rosalino *et al.,* 2006;). The remainder focused upon a single species of helminth (Davidson *et al.,* 2006; Gerrikagoitia *et al.,* 2010; Moskwa *et al.,* 2012; Popiołek *et al.,* 2009; Priemer and Lux, 1994; Ribas *et al.,* 2012) or the incidental detection of infection during examination of a range of wild carnivore hosts (Loos-Frank and Zeyhle, 1982; Górski *et al.,* 2006; Magi *et al.,* 2009), where badger helminth infection is reported tangentially and sample sizes are small. This lack of data is surprising given the increased research focus on badger ecology since the identification of badgers as wildlife reservoirs of bovine tuberculosis (bTB) in 1974 (Noonan *et al.,*1975). Additionally, in recent years there has been a growing interest in the 'one health' approach (Akdesir *et al.,* 2018). This is the concept that the findings from wildlife health surveillance have a wider applicability to

emerging and established zoonotic diseases. Thereby, an investigation of the helminth parasite community of such an ecologically important species as the badger (Byrne *et al.,* 2012) would not only enrich our understanding of badger ecology but also the structure and dynamics of macroparasitic infections.

Helminth parasite communities can be divided into two hierarchal levels (Loxton *et al.,* 2017); the infracommunity and component community. The infracommunity is the collection of all parasite individuals of all species within one host (Bush *et al.,* 1997). It is thought that it is the interaction between the different species of parasites within a host that shapes the component community (Poulin, 2001), all parasite individuals of all species associated with a host population. As outlined by Loxton *et al.* (2017), a central goal of host-parasite community studies is the identification of the causes of parasite aggregation, and variation in parasite abundance, within and between host populations. Therefore, it is important that host factors such as sex, location and temporal variability are considered when reporting the dynamics of a helminth community.

The aims of this study were therefore three fold: (i) to describe the helminth community of European badgers in the ROI for the first time, (ii) to report the prevalence, abundance, intensity and aggregation of each helminth infection detected, and (iii) to explore the relationship between infection and factors such as host sex, region and season of sampling.

3.2 Methods

3.2.1 Study animals

In total, 289 badgers were examined for helminth parasites. Badgers were provided by the Irish Department of Agriculture, Food and the Marine (DAFM) from the strategic culling conducted in bovine tuberculosis endemic regions by DAFM, under a National Parks and Wildlife Services license. No animals were killed specifically for the purposes of this study.

Badgers were transported from their site of culling to the post-mortem laboratory within 1-3 days and dissected on the day of arrival. All organs were removed from the

cadavers, and blood samples taken by an experienced veterinary surgeon (UF) and labelled with unique DAFM badger identification codes. Samples were chosen at random from Western counties and Eastern counties across one sample year; Spring (March, April, May), Summer (June, July, August), Autumn (September, October, November) and Winter (December, January, February)(see Table 3.1). The influence of badger age on the parasite community could not be explored because very few first year badgers were caught due to the capture method used by DAFM. Laboratory work at Trinity College Dublin was approved by the School of Natural Sciences Research Ethics Committee (code: 2016-28).

Table 3.1: The sample size of the Badgers by sex, region and season (total n= 289)

		Females		**Males**		Total
		East	West	East	West	
Seasons	**Spring**	8	25	11	28	72
	Summer	18	32	12	24	86
	Autumn	4	26	9	11	50
	Winter	11	21	9	40	81
	Total	41	104	41	103	**289**

3.2.2 Collection of samples

Hearts, lungs, livers and gastrointestinal tracts were collected and frozen without preservative, at -20^{o}C until examination. Faecal samples were extruded from the rectum of gastrointestinal tracts and fixed in 10% formalin.

3.2.3 Gross organ dissection for the identification of adult helminth worms in situ

Organs were dissected over 3-12 months and were defrosted overnight at 5^{o}C and inspected within 8 hours. Hearts and lungs were processed and examined by a modified flush and dissection technique (Morgan *et al.,* 2008; Houpin *et al.,* 2016;) as described in Byrne *et al.* (2018). The GI tracts were cut open lengthways, flushed using 0.9% saline and scrutinised for helminth parasites (Byrne *et al,* 2018). The lobes of the liver were separated and submerged into warm water, the liver was then cut into 2cm^{2}

cubes and wrapped in a nylon/viscose cotton mesh fabric. The cubes were again submerged in warm water with manual pressure being applied by opening and closing the fist in which the samples were held. This process was repeated three times for each sample. The fabric was then unwrapped and visually inspected for parasites as well as were the cubes of liver. The bags the organs in which the organs had been stored were turned inside out and submerged in water. The water was then passed through sieves of aperture 150 and 64μm and scrutinised for parasites.
Whenever they were found, helminth parasites were removed, counted and preserved in 70% ethanol. Species identification was confirmed by Eileen Harris, Senior Curator, Parasitic worms from the Natural History Museum, London.

3.2.4 Microscopic identification of helminth ova and larvae in the faeces

A modified formol-ether concentration technique (Allen and Ridley, 1970) was used to detect helminth parasite eggs and larvae. Samples were processed blind with two slides being examined for each individual badger and the egg per gram count (e.p.g) and larvae per gram (l.p.g) calculated for each sample.

3.2.5 Data analysis

All results were calculated from the recorded data, and were performed using the statistical analysis software package, R Studio 1.0.153 for Mac (R Core Team 2017). All additional tools from statistical packages are cited in the relevant text. The prevalence, abundance, intensity and aggregation of each helminth species was calculated. Where available these were analysed using worm burden data (*Aelurostrongylus falciformis, Crensoma spp., Uncinaria criniformis*) or faecal egg/larval counts (*Eucoleus aerophilus, Strongyloides spp.,* Species A*,* Unknown 1*,* 2). The mean species richness was calculated as a measure of the infracommunity (Loxton *et al.,* 2016). Generalised linear models (GLMs) were used to interrogate the relationship between host factors and helminth infection, as recommended for the analysis of aggregated parasite data (Wilson and Grenfell, 1997; O'Hara and Kotze, 2010). Abundance and intensity were modelled with the modified negative binominal GLM (Loxton *et al.,* 2016) from the MASS package in R (Venables and Ripley, 2002). Full factorial models incorporated measures of season (4 levels: spring, summer, autumn, winter); region (2 levels: East,

West) and sex (2 levels: female, male). The minimal sufficient model was derived by simplifying the model using the step procedure (see Loxton *et al.,* 2016). The residual deviance of the simplified model was used to perform a goodness of fit test for the overall model. Models were deemed to fit reasonably well if the goodness-of-fit χ^2 test was not statistically significant. Level of significance was set at $P < 0.05$.

3.3 Results

Of the 289 badgers examined, 83.2% (CI95% 78.2 – 87.1%) were positive for one or more helminth species. Overall 8 helminth species were recovered from Irish badgers, as shown in Table 3.2. Five species of adult worms were recovered via gross organ dissection, two of which were located in the lungs; 663 were *Aelurostrongylus falciformis* and 236 were *Crenosoma melesi.* The remaining three were from the GI tract, or which 6516 were *Uncinaria criniformis* and 2 were unidentifiable nematodes (nematode 1 and nematode 2). Species identification could not be confirmed due to the condition of specimens. No badgers were only infected with these nematodes, individuals infected with nematodes 1 and 2 were not incorporated in parasite community analysis. Two helminths (one genus and one species) were identified from the faeces: *Eucoleus aerophilus* (eggs) and *Strongyloides spp.* (larvae), as shown in Figure 3.1a and b. In addition, a large number of unknown larvae (Species A), as shown in Figure 3.1c, were recorded but could not be identified as no adult worms were recovered. Seven badgers were infected by only Species A.

Table 3.2: Helminth species recovered from European badgers (*Meles meles*) in the Republic of Ireland by taxon, species name, location in the host, life cycle and method by which infection was diagnosed.

Taxon	Species name	Location in the body	Life cycle	Diagnostic method
Nematoda	*Aelurostrongylus falciformis*	Lungs	Indirect[b]	WB[d]
	Crenosoma melesi	Lungs	Indirect[b,c]	WB
	Eucoleus aerophilus	Lungs	Direct	FEC[e]
	Nematode 1	Gastrointestinal tract	NA	WB

Nematode 2	Gastrointestinal tract	NA	WB
Species A	NA[a]	NA	FLC[f]
Strongyloides	NA	Direct	FLC
Uncinaria criniformis	Gastrointestinal tract	Direct	WB

[a] NA = Not available [b]Intermediate host: Snail; [c]Intermediate host: Slug [d]WB = Worm burden; [e]FEC = Faecal egg count; [f]FLC = Faecal larval count

Figure 3.1: Images of helminth ova and larvae recovered from the faeces of European badgers (*Meles meles*) in the Republic of Ireland. 3.1a. *Strongyloides spp.* larvae; 3.1b. *Eucoleus aerophilus* ova; 3.1c. Unknown large larvae (Species A).

The prevalence, abundance, intensity, range and aggregation of helminth infection based on adult worm burden or faecal egg/larval counts was calculated (Table 3.3). The most prevalent and abundant helminth was *U. criniformis* with 171 badgers (59.2% CI 95% 53.25-64.88%) infected and an average abundance of 22.5 (±3.16). Of the helminths found in the lungs, *A. falciformis* was the most common, infecting 60 individual badgers resulting in a prevalence of 20.7% (CI95% 16.2-25.9%). Despite having the lowest prevalence of 1.03% (CI95% 0.2-3.0%) *Crenosoma melesi* had the highest mean intensity (78.6 ±125.8) compared to all other helminths. All species of helminth exhibited an aggregated distribution throughout the host population. *U. criniformis* was the most aggregated ($S^2/\bar{x}$ =127.8), with 62% of the 6513 *U. criniformis* recovered being found in only 8% (n=28) of the sample.

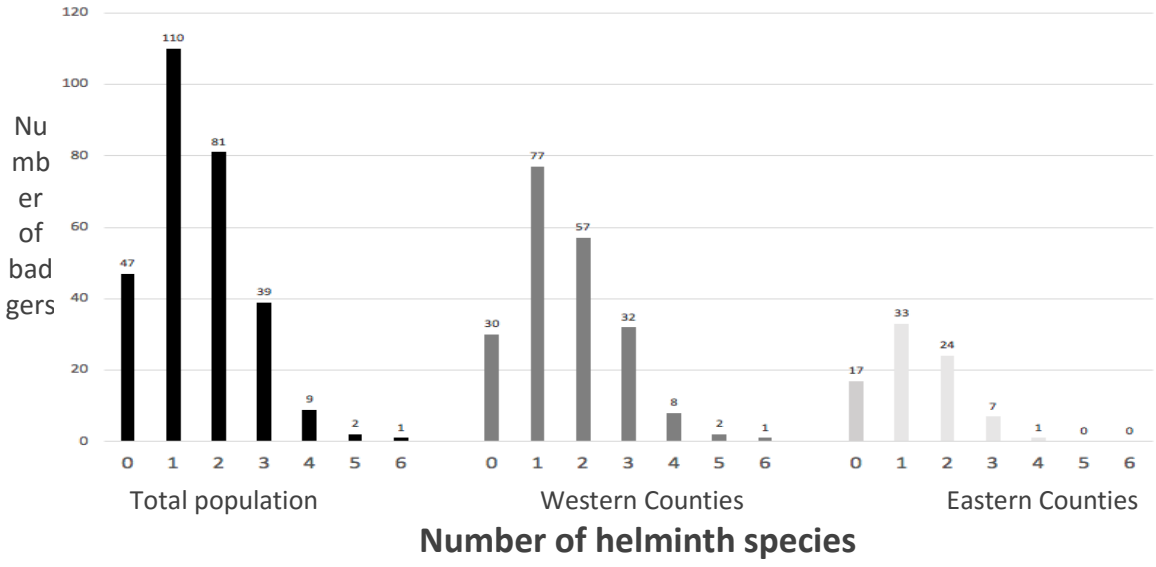
Table 3.3: The prevalence (CI95%), mean abundance (±S.E.), range (minimum-maximum), intensity (±S.E.) and aggregation ($S^2/\bar{x}$) of helminth parasites in European badgers (*Meles meles*) in the Republic of Ireland.

		Prevalence %	CI 95%	Mean Abundance±S.E	Min-max	Mean Intensity±S.E	Aggregation $S^2/\bar{x}$
Adult worm burden	*Uncinaria criniformis*	59.2	53.25-64.88	22.5±3.158	0-500	38.2±65.5	127.8
	Aelurostrongylus falciformis	20.7	16.2-25.9	2.29±0.433	0-53	11.05±12.9	23.6
	Crenosoma melesi	1.03	0.2-3.0	0.82±0.76	0-224	78.6±125.8	16.1
	Unknown 1	1.03	0.2-3.0	0.02±0.19	0-2	2±1.4	NA
	Unknown 2	2.07	0.7-4.4	0.04±0.36	0-1	2.1±0.98	NA
Faecal egg/ larval counts	*Strongyloides spp.*	28.02	22.9-33.58	14.05±2.72	0-450	50.1±76.7	3.29
	Eucoleus aerophilus	8.3	5.9-12.10	1.94±0.55	0-90	23.3±24.3	4.86
	Species A	27.6	22.6-33.2	16.23±2.78	0-460	58.6±74.8	2.91

The relationship between the prevalence, abundance and intensity of each helminth species and host sex, region and season of sampling was explored but none of these factors were found to statistically significantly influence the prevalence, abundance or intensity of any of the helminth species.

All the helminths found in this sample were nematodes (Table 3.2). The mean species richness was 1.43 (± S.E.1.03). Species richness did not differ significantly between the sexes, the region of origin or season of culling. Overall, 110 individuals were infected with a single species of helminth and 131 (54.1%) badgers were co-infected with 2 or more helminth species. The highest level of co-infection was 6, as shown in Figure 3.2. This was seen in only 1 individual, a male badger from a Western county. Similarly, the two individuals that had 5 helminth species reported were also males from Western counties.

Figure 3.2: The frequency distribution of the number of helminth species found in badgers for the total sample (n = 289) (a), the western counties (n = 207) (b) and the eastern counties (n = 82) (c). Numbers



on the top of the bars represent numbers of badgers with each number of species.

3.4 Discussion

This is the first comprehensive report of the helminth parasite community of Irish badgers. Within our sample of 289 badgers, helminth infection was shown to be endemic within the Irish population, with 83.2% of all individuals positive for at least one species of helminth. This contrasts with the Manual of Wildlife Causalities issued by the British Small Animal Veterinary Association (BSAVA) to practising veterinary professionals, both in the UK and ROI, as a guide on how to treat wild animals that are sick or injured. Here, it states that infection by endoparasites (i.e. helminths) of badgers is infrequent and most are accidental infections from other species (Mullineaux *et al.,* 2017). In light of this study, it is our recommendation that this be updated so that: (i) helminth infection is investigated in sick or injured badgers and (ii) such reports are able to be recorded appropriately and the extent of helminth infection is known.

Hancox (1980) was the first and only researcher to systematically review all published parasite records of the European badger, *M. meles*. The review concluded that the available data were diffuse and restricted to certain regions across the badger's distribution. Despite being published over three decades ago, this still pertains, with the majority of data from across Continental Europe, two reports from Great Britain but no comprehensive reports from the ROI, until now (Table 3.4). Since 1980, 38 helminths have been reported in the badger host including 27 nematodes, 6 cestodes and 5 trematodes. Of these, *Uncinaria spp.* and *Aelurostrongylus spp.* are the most common. The most prevalent helminth appears to be *Strongyloides spp.* (Rosalino *et al.,* 2006). However, the majority of host sample sizes fall below 100 in 14 of the 16 published studies (Table 3.4). Additionally, reporting of intensity of infection is very rare (n=4 studies), despite agreement within the parasitological community that intensity of infection is a critical parameter for understanding the epidemiology and population biology of macroparasite infections (Anderson *et al.,* 2014).

With the exception of reports from Spain (Torres *et al.,* 2001; Millan *et al.,* 2004) where collectively 19 different species of helminths have been described, the helminth

community of badgers has been consistently reported as less than 10. This is surprising given that badgers are fossorial feeders, consuming prey items in and on soil (Cleary *et al.,* 2009; Roper, 2010), and a large number of helminths are transmitted through the ingestion of eggs or larvae in soil contaminated with faeces (Jourdan *et al.,* 2018). All helminths described in our study are nematodes, several of which exhibit a direct life cycle (Table 3.2). Interestingly the two most prevalent helminths described here, *Uncinaria criniformis* (59.2%) and *Strongyloides spp.* (28.2%) are both known to be transmitted directly (Rosalino *et al.,* 2006). This higher prevalence is to expected as transmission is not reliant on an intermediate host.

Within this study, *U. criniformis* was the most prevalent species, with a prevalence of 59.2%, the highest compared to all published data to date (see Table 3.3). This result was unexpected because although *Uncinaria spp.* are the most commonly reported helminth of the Eurasian badger (Loos-Frank and Zeyhle, 1982; Torres *et al.,* 2001 Millan *et al.,* 2004; Górski *et al.,* 2006; Rosalino *et al.,* 2006; Stuart *et al.,* 2013), the prevalence is often reported as less than 10% (Davidson *et al.,* 2006; Loos-Frank and Zeyhle, 1982; Millan *et al.,* 2004; Rosalino *et al.,* 2006). However, our high infection rate is consistent with that observed by Stuart *et al.,* (2013) who reported a prevalence of 40% in a sample of 50 Irish badgers. This suggests that *U. criniformis* infection in Irish badgers might be consistently high compared to other continental European populations (see Table 3.4).

Our study represents the first records of *Crenosoma melesi* and *Strongyloides spp.* helminths in a badger host in the Republic of Ireland. We found a high prevalence of *Strongyloides spp.* infection (28%) and given the numerous reports of *Strongyloides westeri* helminths parasitizing horses in the ROI (O'Meara and Mulcahy, 2002), further studies should be conducted to allow the identification of the *Strongyloides spp.* found in badger hosts, to determine if these helminths overlap with those found in equine helminth communities.

It is interesting that no significant relationship between host sex, region or season of sampling was observed for prevalence, abundance, intensity or mean species richness. This contrasts with a study in wild rats which reported that helminth burden was significantly higher in males compared to females as well as describing seasonal

changes in helminth prevalence (Kataranovski *et al.,* 2011). Additionally, Loxton *et al.* (2016) found that in their study of native wood mice and invasive bank voles, site of sampling was the most significant factor influencing mean species richness.

Publication author	Country	sample size (n)	Parasite Taxon	*Parasite Species*	Location in host	Prevalence %	Intensity	Method of diagnosis
Jones *et al.*, 1980	United Kingdom	118	Nematoda	*Capillaria sp.*	Pulmonary	5.1	N/A	Worm burden
				Uncinaria stenocephala	Intestine	22.9	N/A	Worm burden
				Molineus patens	Intestine	27.1	N/A	Worm burden
				Strongyloides spp.	Intestine	0.8	N/A	Worm burden
				Aelurostrongylus falciformis	Pulmonary	0.8	N/A	Worm burden
			Cestoda	*Mesocestoides lineatus*	Intestine	1.6	N/A	Worm burden
				Dilepis undula	Intestine	0.8	N/A	Worm burden
			Trematoda	*Ityogonimus lorum*	Intestine	0.8	N/A	Worm burden
		80		*Capillaria erincacei*	Intestine	25	N/A	Worm burden
Loos-Frank and Zeyhle, 1982	Germany	84	Nematoda	*Uncinaria criniformis*	intestine	4	N/A	Worm burden
			Cestoda	*Atriotaenia incisa*	intestine	15.5	N/A	Worm burden
				Taenia martis	intestine	2	N/A	Worm burden
Priemer and Lux, 1994	Germany	11	Cestoda	*Atriotaenia incisa*	intestine	36.3	N/A	Worm burden
Magi *et al.*, 1999	Italy	19	Nematoda	*Aelurostrongylus falciformis*	Pulmonary	21.2	N/A	Worm burden
				Capillaria sp.	Pulmonary	31.6	N/A	Worm burden
				Crenosoma melesi	Pulmonary	21.1	N/A	Worm burden
				Mesocestoides	Intestine	21.1	N/A	Worm burden
				Molineus patens	Intestine	21.1	N/A	Worm

				Uncinaria criniformis	Intestine	84.2	N/A	burden Worm burden
Torres *et al.*, 2001	Spain	85	Nematoda	*Aelurostrongylus pridhami*	Pulmonary	6.4	1.3	Worm burden
				Aelurostrongylus vasorum	Pulmonary	6.4	2.7	Worm burden
				Aonchotheca putorii	Gastric mucus	29.6	36.1	Worm burden
				Crenosoma melesi	Pulmonary	6.4	2.3	Worm burden
				Mastophorus muris	Stomach	14.3	1.3	Worm burden
				Molineus patens	Intestine	38.3	10.06	Worm burden
				Pearsonema plica	Bladder	2.1	1	Worm burden
				Physaloptera sibrica	Stomach	23.1	1.3	Worm burden

Table 3.4: The helminths of European badgers (*Meles meles*), 1980- 2018

Table 3.4 continued

Publication author	**Country**	**sample size (n)**	**Parasite Class**	***Parasite Species***	**Location in host**	**Prevalence %**	**intensity**	**method of diagnosis**
Torres *et al.*, 2001			Nematoda	*Strongyloides sp.*	Intestine	30.9	21.2	Worm burden
continued				*Trichinella sp.*	Intestine	2.1	N/A	Worm burden
				Uncinaria criniformis	Intestine	51.6	52.5	Worm burden
				Vigisospirura potekhina	Stomach	7.3	4.7	Worm burden
			Cestoda	*Atriotaenia incisa*	Intestine	12.4	107.2	Worm burden
				Mesocestoides sp.	Intestine	14.3	3	Worm

								burden
			Trematoda	*Brachylaima sp.*	Intestine	66.6	1.1	Worm burden
				Euparyphium melis	Intestine	7.7	2	Worm burden
				Euryhelmis squamula	Intestine	9.5	74	Worm burden
Millian *et al.*, 2004	Spain	26	Nematoda	*Aonchotheca putorii*	Gastric mucus	0.46	224.6	Worm burden
				Physaloptera sp.	Stomach	0.19	12.23	Worm burden
				Molineus patens	Intestine	0.39	265	Worm burden
				Uncinaria criniformis	Intestine	0.35	55.4	Worm burden
				Strongyloides sp.	Intestine	0.61	2068	Worm burden
				Angiostrongylus sp.	Pulmonary	0.42	65.7	Worm burden
				Crenosoma sp.	Pulmonary	0.04	50	Egg/larval count
			Cestoda	*Atriotaenia incisa*	Intestine	0.69	3484	Worm burden
				Mesocestoides sp.	Intestine	0.04	500	Worm burden
				Taenia sp.	Intestine	0.04	1	Worm burden
			Trematoda	*Euryhelmis squamula*	Intestine	0.12	1708	Worm burden
				Brachylaima sp.	Intestine	0.04	1642	Worm burden
Davidson *et al.*, 2006	Norway	9	Nematoda	*Aelurostrongylus falciformis*	Pulmonary	55.5	N/A	Worm burden & faecal
				Crenosoma melesi	Pulmonary	22.2	N/A	Worm burden & egg/larval count
Gorski *et al.*, 2006	Poland	17	Nematoda	*Uncinaria sp.*	Intestine	5.9	N/A	Egg/larval count
				Capillaria sp.	Pulmonary	11.8	N/A	Egg/larval count
			Trematoda	*Opisthorchis sp. Or Metorchis sp.*	Gall bladder	11.8	N/A	Egg/larval count

Table 3.4 continued

Publication author	**Country**	**sample size (n)**	**Parasite Class**	***Parasite Species***	**Location in host**	**Prevalence %**	**intensity**	**method of diagnosis**

Rosalino *et al.*, 2006	Portugal	163	Nematoda	*Mastophorus muris*	Stomach	11.7	N/A	Egg/larval count
				Molineus patens	Intestine	8.6	N/A	Egg/larval count
				Uncinaria criniformis	Intestine	2.5	N/A[a]	Egg/larval count
				Strongyloides sp.	Intestine	43.6	N/A	Egg/larval count
			Cestoda	*Atriotaenia incisa*	Intestine	16.6	N/A	Egg/larval count
Magi *et al.*, 2009	Italy	6	Nematoda	*Angiostrongylus vasorum*	Pulmonary	16.6	N/A	Worm burden
Popiolek *et al.*, 2009	Poland	1	Nematoda	*Crenosoma vulpis*	Pulmonary	100	3	Worm burden
Massey *et al.*, 2009	United Kingdom	28						Egg/larval count
Gerrikagoitia *et al.*, 2010	Spain	50	Nematoda	*Angiostrongylus daskalovi*	Pulmonary	24	N/A	Worm burden
Ribas *et al.*, 2011	Catalonia	44	Nematoda	*Troglotrema acutum*	Nasal sinuses	2.27	8	Worm burden
Moskwa *et al.*, 2012	Poland	7	Nematoda	*Trichinella britovi*	Intestine	28.5	N/A	Worm burden
Stuart *et al.*, 2013	Republic	50	Nematoda	*Eucoleus aerophilus*	Pulmonary	6	N/A	Egg/larval count
	of Ireland			*Uncinaria criniformis*	Intestine	40	N/A	Egg/larval count
Demiaszkiewicz *et al.*, 2017	Poland	9	Nematoda	*Aelurostrongylus falciformis*	Pulmonary	22.2	N/A	Worm burden
Byrne *et al.*, 2018	Republic	289	Nematoda	*Aelurostrongylus falciformis*	Pulmonary	20.7	11.05	Worm burden
(present study)	of Ireland			*Crenosoma melesi.*	Pulmonary	1.03	2	Worm burden
				Eucoleus aerophilus	Pulmonary	8.3	23.3	Egg/larval count
				Species A	NA	27.6	58.6	Egg/larval count

	Strongyloides spp.	Intestine	28.08	50.1	Egg/larval count
	Uncinaria criniformis	Intestine	59.2	38.2	Worm burden
	Unknown 1	Intestine	1.03	2	Worm burden
	Unknown 2	Intestine	2.07	2.1	Worm burden

[a]N/A= Not available

One important caveat is that for 3 of the 8 species reported here, population parameters were calculated using faecal egg and larval counts. This method of detection is known to have low sensitivity for diagnosing helminth infection compared to adult worm burden in wild hosts (Gassó *et al.,* 2015; Byrne *et al.,* 2018). However, in a recent analysis, utilising both adult worm burden data and egg/larval counts (Byrne *et.al.,* 2018), we were able to demonstrate that although coprological analysis was not a sensitive test for diagnosing helminth infection, where eggs or larvae were detected, the abundance of these eggs or larvae in the faeces was a suitable indicator of intensity of infection.

In this study, the helminth parasite community of European badgers (*Meles meles*) from the Republic of Ireland (ROI) is reported for the first time. In total, 8 distinct helminth types were described (1 genus, 4 species and 3 unknown), all of which were nematodes. This is the first report of *Crenosoma melesi* in the ROI. Helminth infection was found to be endemic throughout the Irish community irrespective of host sex, region of origin or season of sampling. The present study, is to our knowledge, the most comprehensive study of badger helminths conducted within their geographical range. Observations from this study can serve as a baseline for badger health surveillance as well as an important comparison with studies of helminth parasitism in badgers from other countries across the badgers' range.

Chapter 4

General Discussion

We have provided a detailed description of the helminth community of 289 European badgers (*Meles meles*) from the Republic of Ireland. The helminth community consisted of 8 nematodes and included 4 identified to species, 1 identified to genus and 3 unknown. *Aelurostrongylus falciformis; Crenosoma melesi.; Eucoleus aerophilus; Uncinaria criniformis; Strongyloides spp; '*Species A' and two unidentifiable but morphologically distinct nematodes *(*nematode 1 and 2*)*. *A. falciformis* and *Crenosoma spp.* adult worms were recovered from the lungs and *U. criniformis*, nematode 1 and nematode 2 adults were recovered from the gastrointestinal tract. *E. aerophilus* (eggs), *Strongyloides spp.* (larvae) and *Species A* (larvae) were diagnosed by coprological analysis.

For diagnosis of helminth infection, coprological analysis is a common tool utilised in both animal and human health (Jourdan *et al.,* 2018). In Chapter 2, we evaluated coprological analysis against the reference standard of gross organ dissection for two helminth species, hookworm (*U. criniformis*) derived from the gastrointestinal tract and lungworm (*A. falciformis*) derived from the lungs. This is the first time such an evaluation has taken place in a badger host, due in part to the logistical and ethical challenges associated with post-mortem examination. We reported that individuals positive for hookworm infection were more likely to be diagnosed incorrectly than correctly due to the low sensitivity (41.5%) of faecal egg counts compared to adult worm burden. Notably, for lungworm infection, similar prevalence values were reported for both coprological analysis (18.3%) and gross organ dissection (20.8%). At first glance it would appear that coprological analysis for diagnosing lungworm is

closer to the reference standard than that for hookworm. However, when investigating the diagnostic parameters of the two tests (See Table 2.3 and 2.4), it became apparent that neither faecal larval counts (FLC) or gross organ dissection were sensitive for diagnosing lungworm infection and despite the similar reported prevalence for the two methods, individuals were rarely positive for both the reference test (adult worm burden) and the index test (FLC) (n=6). In light of this finding, we recommended that a composite reference standard be used for lungworm diagnosis. Therefore, if we assume that those labelled 'false positives' (n=47) in Table 2.4 are indeed true positives, this would result in a lungworm prevalence of 37%, rather than 20.8% reported when using adult worm burden alone.

It is not just the ability to compare coprological analysis as a diagnostic test that is hindered by the lack of available data on faecal egg/larval counts and adult worm burden, but also the opportunity to investigate the relationship between egg/larval output and adult worm burden. Additionally in Chapter 2, the relationship between the two was evaluated for hookworm and lungworm infections and we found there was a statistically significant positive relationship between egg/larval output and the number of worms recovered. Because of the widespread use of faecal analysis for helminth diagnosis, these data will be of interest not just to badger parasite ecologists but also to the wider diagnostic parasitological community. Additionally, the positive relationship between worm burden and egg/larval output in badger hosts differs from reports of the density-dependent relationship between eggs and worms in human helminth infections (Anderson and Schad, 1985). Further investigation is required to establish the reasons behind the observed differences in the relationship between worm burden and egg or larval production in this wildlife host and whether this is linked to an adaptive immunological response.

In Chapter 3, we reported that the helminth parasite community of 289 badgers was entirely dominated by nematode species. This is the only study across the badgers' distribution to describe a helminth community dominated by only one Taxon (Table 4.1). Other studies from the UK, Spain, Italy and Portugal described badgers infected with trematode and cestode helminths. Additionally of the known species in this study, the majority have a direct life cycle (Table 3.2). Parasites that exhibit direct life

cycles are reliant on only one definitive host (Olsen, 1986), whereas those with indirect life cycles such as *A. falciformis* rely on intermediate or paratenic hosts such as snails and slugs. It is interesting that the two most prevalent species in this study were *U. criniformis* and *Strongyloides spp.* both of which are direct life cycle helminths. Anderson (1980) whilst working on human helminth infections put forward the theory that helminths with direct life cycles are more successfully transmitted as they are not reliant on intermediate hosts, and thus are amongst the most prevalent in the community, to which our results offer support. Additionally, figures reported by the World Health Organization (WHO) state that the four most prevalent helminths infecting humans (*Ascaris lumbricoides, Trichuris trichiura, Necator americanus and Ancylostoma duodenale*) are all transmitted directly (World Health Organization, 2017).

Also in Chapter 3, a comparison was made between the present study and studies from a range of countries (Table 3.4) which explored the helminth species recorded in European badgers. What emerged from this comparative analysis was that few studies provided quantitative measures of helminth parasite community structure, in particular worm abundance, despite the recent call for parasite ecologists to improve the standardisation of reporting of helminth parasite communities (Poulin, 2018b). Since 1980 only 5 reports from the UK (Jones *et al.,* 1980), Spain (Millan *et al.,* 2004; Torres *et al.,* 2001), Italy (Magi *et al.,* 1999) and Portugal (Rosalino *et al.,* 2006) have described the helminth community of badgers, four of which merely reported the prevalence of helminth infection.

In addition to Table 3.4, here, we present a comparison of helminth species richness between two Mustelid species – the European badger and the European otter (*Lutra lutra*) –and the Red fox (*Vulpes vulpes*) (Table 4.1). Only one other helminth parasite study recorded mean species richness among red foxes sampled from Portugal (Eira *et al.,* 2006), so the total number of helminth species recorded was used as the unit for comparison (Table 4.1). The total number of helminth species reported from badgers sampled from the ROI is 8 and that is consistent with reports from the UK (Jones *et al.,* 1980) (see Table 4.1). This is higher than the number of helminth species recorded for both Italy (n=6) and Portugal (n=5). However, reports from Spain provide

comparatively high total numbers of species (Table 3.4) in two distinct regions of the country: Mainland Spain (Torres *et al.,* 2001) and the Basque Country (Millan *et al.,* 2004). With a small number of studies, some of which only reported prevalence data, it is difficult to hypothesise any reasons for the variability in the total number of helminth species reported.

Table 4.1 The helminths of the European badger (*Meles meles*), the European Otter (*Lutra lutra*) and the red fox (*Vulpes vulpes*) by total species number, taxon, species name and nematode proportion within the community.

Name of study	**Location**	**Host**	**Mean species richness**	**Total number of species**	**Helminth taxon**	**Names of species**	**Nematode proportion**
Jones *et al*. 1980	UK	Badger	N/A	8	Nematoda	*Capillaria sp.*	62.5
						Uncinaria stenocephala	
						Molineus patens	
						Strongyloides spp.	
						Aelurostrongylus falciformis	
					Cestoda	*Mesocestoides lineatus*	
						Dilepis undula	
					Trematoda	*Ityogonimus lorum*	
Magi *et al.* 1999	Italy	Badger	N/A	6	Nematoda	*Aelurostrongylus falciformis*	83
						Capillaria sp.	
						Crenosoma melesi	
					Cestoda	*Mesocestoides*	
						Molineus patens	
						Uncinaria criniformis	
Torres *et al.* 2001	Spain	Badger	N/A	17	Nematoda	*Aelurostrongylus pridhami*	70.5
						Aelurostrongylus vasorum	
						Aonchotheca putorii	
						Crenosoma melesi	
						Mastophorus muris	
						Molineus patens	
						Pearsonema plica	
						Physaloptera sibrica	
						Strongyloides sp.	
						Trichinella sp.	
						Uncinaria criniformis	
						Vigisospirura potekhina	
					Cestoda	*Atriotaenia incisa*	
						Mesocestoides sp.	
					Trematoda	*Brachylaima sp.*	

						Euparyphium melis *Euryhelmis squamula*	
Millian *et al*. 2004	Spain	Badger	N/A	12	Nematoda Cestoda	*Aonchotheca putorii* *Physaloptera sp.* *Molineus patens* *Uncinaria criniformis* *Strongyloides sp.* *Angiostrongylus sp.* *Crenosoma sp.* *Atriotaenia incisa* *Mesocestoides sp.*	58.3

Table 4.1 continued

Name of study	Location	Host	Mean species richness	Total number of species	Helminth taxon	Names of species	Nematode proportion
Millian *et al*. 2004 cont.					Cestoda Trematoda	*Taenia sp.* *Euryhelmis squamula* *Brachylaima sp.*	
Rosalino *et al.* 2006	Portugal	Badger	N/A	5	Nematoda Cestoda	*Mastophorus muris* *Molineus patens* *Uncinaria criniformis* *Strongyloides sp.* *Atriotaenia incisa*	80
Byrne *et al.* 2018 Present study	ROI	Badger	1.43	8	Nematoda	*Aeluerostrongylus falciformis* *Crenosoma spp.* *Eucoleus aerophilus* Species A *Strongyloides spp.* *Uncinaria criniformis* *Unknown 1* *Unknown 2*	100
Torres *et al.* 2004	France, Portugal and Spain	Otter	N/A	7	Nematode Trematoda Acanthocephala	*Aonchotheca putorii* *Eucoleus schvalovoj* *Strongyloides lutrae* *Anisakis (L3)* *Dirofilaria immitis* *Phagicola sp.* *Gigantorhynchus sp.*	71.4
Ross and Fairley, 1969	ROI	Fox	N/A	6	Nematoda Cestoda	*Toxocara canis & leonina* *Uncinaria stenocephala* *Taenia pisiformis* *Taenia serialis* *Taenia taeniaformis*	33

						Taenia hydatigena	
Wolfe *et al.*, 2001	ROI	Fox	N/A	8[a]	Nematoda	*Ascarid spp.*	71.4
						Spirurida spp	
						Toxocara canis	
						Uncinaria stenocephala	
					Cestoda	*Taenia spp.*	
					Trematoda	*Alaria alata*	
					Trematoda	*Brachylaima spp*	
						Echniostome spp	

Table 4.1 continued

Name of study	**Location**	**Host**	**Mean species richness**	**Total number of species**	**Helminth taxon**	**Names of species**	**Nematode proportion**
Stuart *et al.*, 2013	ROI	Fox	N/A	4[b]	Nematoda	*Eucoleus aerophilus*	100
						Trichuris vulpis	
						Toxocara canis	
						Uncinaria stenocephala	
Eira *et al.* 2006	Portugal	Fox	2.7	19	Nematoda	*Angiostrongylus vasorum*	60
						Crenosoma vulpis	
						Dirofilaria immitis	
						Molineus patens	
						Pearsonema plica	
						Pterygodermatites affinis	
						Ricturlaria proni	
						Spirocerca lupi	
						Toxocara canis	
						Trichuris vulpis	
						Uncinaria stenocephala	
					Cestoda	*Mesocestoides spp*	
						Taenia pisiformis	
						Taenia taeniaeformis	
					Trematoda	*Alaria alata*	
						Ascoctyle longa	
						Pseudamphistomum truncatum	
					Acanthocephala	*Centrorhynchus sp.*	
						Macracanthorhynchus catulinus	

[a]diagnosed by coprological analysis

It is, however, interesting to relate our finding to the theory presented by Kennedy *et al.* (1986), that mammals harbour a greater number of helminth species than fish or birds. If we compare our data to that of Kennedy *et al.*, (1986) we find that the mean species richness (1.43) recorded for badgers in the present study is actually similar to that of a range of studies on freshwater fish (1.5). However, Kennedy *et. al*., acknowledged the lack of data on mammals in their data set, represented by a single species, the rice rat.

The ROI is home to a range of Mustelid species (McDonald, 2002), the Family to which the badger belongs. Typically, when discussing the helminth community of one member of a Family, where possible, a comparison should be drawn with other native species present, for example, the European Otter (*Lutra lutra*). However, to our knowledge there have been no published studies on the helminth communities of the native Mustelids of the ROI. Looking more broadly across the overlapping range of badgers and otters, the otter's helminth community was described from the South-West of continental Europe, spanning France, Spain and Portugal (Torres *et. al*., 2004) (Table 4.5). A total of 109 cadavers were examined for helminths, with a further 89 individuals analysed using faecal samples only. Seven helminths in total were described; 5 nematodes, 1 trematode and 1 acanthocephalan. This is similar to the findings in the present study, with badgers exhibiting an even lower total amount of species and a domination of nematodes. The caveat should be added that despite belonging to the same Family, the badger and otter differ in their habitat, behaviour and feeding preferences, and as such comparison of the characterisation of parasite communities might be better suited to other wild carnivores native to the Republic of Ireland.

The Red fox (*Vulpes vulpes)* shares a similar distribution to that of badgers in ROI, and is common throughout rural and urban areas (Wolfe *et al.,* 2001). It has even been reported that foxes and badgers can share setts in high density regions (Roper, 2010). Additionally, although foxes are often viewed as predators, they are better described as opportunistic carnivores as they mostly scavenge for carrion, arthropods and various fruits and berries (Padial *et al.,* 2003) and thus often feed on or around soil,

like the fossorial feeding badger. There are numerous reports of the helminths of Red foxes in the ROI (McCarthy *et al.,* 2018; Ross and Fairley, 1969; Stuart *et al.,* 2013; Wolfe *et al.,* 2001). However, only Ross and Fairley (1969) attempted to characterise the entire helminth community with the remainder either focusing on the gastrointestinal helminth community (Wolfe *et al.*, 2001; Stuart *et al.,* 2013) or reporting the incidence of important zoonotic species, such as *Angiostrongylus vasorum* (McCarthy *et al.,* 2018). Ross and Fairley (1969) found the helminth community of foxes to be dominated by various *Taenia* species (Table 4.1). Interestingly, in the two subsequent studies of the GI helminth communities of foxes, *Taenia* infection was either reported at a low prevalence (Wolfe *et al.,* 2001) or not at all (Stuart *et al.,* 2013). However, all three studies describe the occurrence of *Toxocara canis* and *Uncinaria stenocephala* infection.

Toxocara canis is the one of the most widely distributed ascarid nematode parasite known to infect mammals and is of particular research interest because of its zoonotic potential (Holland, 2017). It utilises paratenic hosts including earthworms and beetles (Strube *et al.,* 2013). Earthworms are an occasional component of nearly every badger's diet in Ireland (Cleary *et al.,* 2009) but the main component for some regions of Europe (Roper, 2010). It is therefore surprising that *Toxocara spp.* has not been reported in European badgers (see Table 3.4) yet is reported to infect Japanese badgers (*Meles anakuma*) (Kamiya and Suzuki, 1975). *Uncinaria stenocephala* is similar to *U. criniformis,* a nematode specific to Mustelids, notably the badger (see Table 4.1).However, unlike *U. criniformis, U. stenocephala* is a hookworm of veterinary importance due to its ability to infect both dogs and cats (Stuart *et al.,* 2013). From the studies in the ROI only one helminth was found to be present in both badger and fox populations: *Eucoleus aerophilus* (syn. *Cappilaria aerophilus*). It is primarily known to parasitize foxes, but can also be found to incidentally infect dogs and other carnivores (Stuart *et al.,* 2013).

Looking more broadly across the fox's range, it is notable that the helminth parasite community follows the same trend observed in the badger (see Table 3.4), in that hosts in continental Europe appear to have a richer and more diverse helminth community than that in the ROI and the UK (Table 4.5). However, the caveat should be

added that this observation is limited only to a small number of countries across the badger's and fox's range.

Knowledge of the helminth community of badgers in the ROI is vital, not only because it contributes to a greater understanding of macroparasitic infections but also in the context of the potential ability of helminths to interfere and interact with the host's immune system and its response to microparasites (Maizels and Yazdanbakhsh, 2003). The majority of co-infection studies have been conducted in laboratory mice or humans and much less is known about the effect of micro- and macroparasitic co-infection in wild hosts (Risco *et al.,* 2014). Ezenwa *et al.* (2010), utilised a disease dynamic model parameterised with empirical data to show that nematode-induced immune suppression facilitated the invasion of bovine tuberculosis (bTB) in African buffalo (*Syncerus caffer)* hosts. Additionally, Risco *et al.* (2014) investigated the relationship between bTB infection and numerous pathogens known to infect Wild boar (*Sus scrofa*) including *Metastrongylus spp.* Wild boar are a recognised wildlife reservoir of bTB in the Mediterranean ecosystem and, like badgers in ROI, are culled as part of a bTB control strategy (Mentaberre *et al.,* 2014). Risco *et al.* (2014) reported a positive relationship between individuals infected with *Metastrongylus spp.* and a sensitivity to bTB infection. Both studies recommend the need for further investigation into the relationship between bTB infection and severity of helminth infections, notably those that infect the gastrointestinal tract.

Helminths not only have the potential to interact with microparasitic infections but have also been known to reduce the efficacy of vaccines (Hotez *et al.*, 2008; Sabin *et al.,* 1996). Again, data from wild hosts are rare, with the vast majority of studies focusing on vaccines and diseases of human public health importance (Cooper *et al.*, 1998; Sabin *et al.,* 1996). However, for both human (*Mycobacterium tuberculosis*) and bovine tuberculosis (*M. bovis*) infections the same vaccination is utilised: the BCG vaccine. Human studies have focused on the effect of helminth infection on the host's response to the BCG vaccination (Elias *et al.,* 2008; Wammes *et al.,* 2010). Both these studies reported a reduction in immunogenicity, the ability of a substance to invoke an immune response in individuals with chronic helminth infections. As the ROI is now moving towards a vaccination-led strategy for the control of bTB, it is imperative that

work is continued to investigate the effect of underlying helminth infection on both bTB pathology and vaccination efficacy, in light of the helminth endemicity reported here and the well documented immunological effects of helminth infection.

Acknowledgements

Over the past two years I have been privileged to be supervised by two of the most empowering and inspirational professors- Celia Holland and Nicola Marples. Although this project is now complete, I know I have two amazing mentors at Trinity. There isn't enough room in this thesis to list all of the things you have done for me, both personally and professionally over the last two years, so I will keep this short. Thank you.

This work would not have been possible without Dr Ursula Fogerty at the Irish Equine Centre. Ursula, you are the loveliest most experienced woman with a knife I've ever met. Your love for your job is a joy to be around and I'm still in ore of your ability to remove organs from a badger in less than 5 minutes! Additionally, Noel and Sarah were invaluable to our cause and helped out with every crazy request (and searching bins when I accidently lost my ring during dissections).

Alison Boyce is a woman that cannot and should not be stopped. She is always around for technical (and emotional support). This project could not have happened without her and I hope she knows how invaluable her time and efforts have been to me, and every other person in the department – plus the liquid lunches helped too!

Thank you to the Department of Agriculture, Food and the Marine for the two years of funding to facilitate this project.

An important thank you goes to the inhabitants of Zoology at Trinity College Dublin. Notably, Aoibheann for infecting me with her love of badgers from our very first meeting and dragging me out to the sticks to drink wine and catch some live ones! Fiona for the laughs, tears and dramas that accompany a postgraduate project, for always talking me down off a ledge and going above and beyond to help. And of course to my partners in crime over the past two years- Andrew and Paula. Two of the most loving, genuine and caring people I've ever met. From bouncing ideas around about science and statistics to climbing mountains and running races you are the greatest people anyone could ever meet.

Thank you to my family for your endless support during my time in Dublin- Especially mum and dad and for all grocery shopping every month because Dublin is the most expensive place on Earth!

My wonderful partner John, whose put up with me talking about badger poo over dinner and calling him screaming because I actually found worms! You are a once in a life time person. To my fluff balls Thumper and Moon who welcomed me home after lonely hours in the lab, you deserve to be co-authors.

---------- and of, course, thank you to the parasites.

References

Abate, E., Belayneh, M., Idh, J., Diro, E., Elias, D., Britton, S., Aseffa, A., Stendahl, O., Schön, T., 2015. Asymptomatic Helminth Infection in Active Tuberculosis Is Associated with Increased Regulatory and Th-2 Responses and a Lower Sputum Smear Positivity. PLoS Negl. Trop. Dis. 9, e0003994. https://doi.org/10.1371/journal.pntd.0003994

Akdesir, E., Origgi, F.C., Wimmershoff, J., Frey, J., Frey, C.F., Ryser-Degiorgis, M.-P., 2018. Causes of mortality and morbidity in free-ranging mustelids in Switzerland: necropsy data from over 50 years of general health surveillance. BMC Vet. Res. 14, 195. https://doi.org/10.1186/s12917-018-1494-0

Allen, A.V.H., Ridley, D.S., 1970. Further observations on the formol-ether concentration technique for faecal parasites. J. Clin. Pathol. 23, 545–546.

Altman, D.G., Bland, J.M., 1994. Diagnostic tests. 1: Sensitivity and specificity. Br. Medicial J. (Clinical Res. ed.) 308, 1552. https://doi.org/10.1136/BMJ.308.6943.1552

Anderson, R., Truscott, J., Hollingsworth, T.D., 2014. The coverage and frequency of mass drug administration required to eliminate persistent transmission of soil-transmitted helminths. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 369, 20130435. https://doi.org/10.1098/rstb.2013.0435

Anderson, R.M., 1980. The Dynamics and Control of Direct Life Cycle Helminth Parasites. Springer, Berlin, Heidelberg, pp. 278–322. https://doi.org/10.1007/978-3-642-93161-1_16

Anderson, R.M., Schad, G.A., 1985. Hookworm burdens and faecal egg counts: an analysis of the biological basis of variation. Trans. R. Soc. Trop. Med. Hyg. 79, 812–825. https://doi.org/10.1016/0035-9203(85)90128-2

Arneberg, P., Skorping, A., Grenfell, B., Read, A.F., 1998. Host densities as determinants of abundance in parasite communities. Proc. R. Soc. B Biol. Sci. 265, 1283–1289. https://doi.org/10.1098/rspb.1998.0431

Aznar, I., McGrath, G., Murphy, D., Corner, L.A.L., Gormley, E., Frankena, K., More, S.J., Martin, W., O'Keeffe, J., De Jong, M.C.M., 2011. Trial design to estimate the effect of vaccination on tuberculosis incidence in badgers. Vet. Microbiol. 151, 104–111.

https://doi.org/10.1016/j.vetmic.2011.02.032

Bengis, R.G., Kock, R.A., Fischer, J., 2002. Infectious animal diseases: the wildlife/livestock interface. Rev. Sci. Tech. 21, 53–65.

Bethony, J., Brooker, S., Albonico, M., Geiger, S.M., Loukas, A., Diemert, D., Hotez, P.J., 2006. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. Lancet 367, 1521–1532. https://doi.org/10.1016/S0140-6736(06)68653-4

Bielby, J., Vial, F., Woodroffe, R., Donnelly, C.A., 2016. Localised Badger Culling Increases Risk of Herd Breakdown on Nearby, Not Focal, Land. PLoS One 11, e0164618. https://doi.org/10.1371/journal.pone.0164618

Bloom, B.R., 1994. Tuberculosis : pathogenesis, protection, and control. ASM Press.

Bobat, S., Cunningham, A.F., 2014. Bacterial Infections and Vaccines. pp. 75–98. https://doi.org/10.1007/978-1-4939-1489-0_4

Bradley, D.J., May, R.M., 1978. Consequences of helminth aggregation for the dynamics of schistosomiasis. Trans. R. Soc. Trop. Med. Hyg. 72, 262–73.

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., Revisited, A., Busht, A.O., Laffertyt, K.D., Shostakll, A.W., 1997. Parasitology Meets Ecology on Its Own Terms. Source J. Parasitol. J. Parasitol 83, 575–583.

Byrne, A., 2013. Studies relating to the population dynamics of the European badger (Meles meles) in Ireland.

Byrne, A.W., Paddy Sleeman, D., O'Keeffe, J., Davenport, J., 2012. The ecology of the European badger (Meles meles) in Ireland: A review. Biol. Environ. 112, 105–132. https://doi.org/10.3318/BIOE.2012.02

Churcher, T.S., Ferguson, N.M., Basáñez, M.G., 2005. Density dependence and overdispersion in the transmission of helminth parasites. Parasitology 131, 121–32.

Cleary, G.P., Corner, L.A.L., O'Keeffe, J., Marples, N.M., 2009. The diet of the badger Meles meles in the Republic of Ireland. Mamm. Biol. - Zeitschrift für Säugetierkd. 74, 438–447. https://doi.org/10.1016/J.MAMBIO.2009.07.003

Cooper, P.J., Espinel, I., Paredes, W., Guderian, R.H., Nutman, T.B., 1998. Impaired tetanus-specific cellular and humoral responses following tetanus vaccination in human onchocerciasis: a possible role for interleukin-10. J. Infect. Dis. 178, 1133–8.

Corner, L.A.L., Murphy, D., Gormley, E., 2011. Mycobacterium bovis Infection in the Eurasian Badger (Meles meles): the Disease, Pathogenesis, Epidemiology and Control. J. Comp. Pathol. 144, 1–24. https://doi.org/10.1016/j.jcpa.2010.10.003

Crofton, H.D., 1971. A quantitative approach to parasitism. Parasitology 62, 179. https://doi.org/10.1017/S0031182000071420

Daszak, P., Cunningham, A.A., Hyatt, A.D., 2000. Emerging infectious diseases of wildlife--threats to biodiversity and human health. Science 287, 443–9.

Davidson, R.K., Handeland, K., Gjerde, B., 2006. The first report of Aelurostrongylus falciformis in Norwegian badgers (Meles meles). Acta Vet. Scand. 48, 6. https://doi.org/10.1186/1751-0147-48-6

Delahay, R.J., Cheeseman, C.L., Clifton-Hadley, R.S., 2001. Wildlife disease reservoirs: the epidemiology of Mycobacterium bovis infection in the European badger (Meles meles) and other British mammals. Tuberculosis 81, 43–49. https://doi.org/10.1054/tube.2000.0266

Eira, C., Vingada, J., Torres, J., Miquel. J., 2006. The Helminth Community of the red fox, Vulpes vulpes, in Dunas de Mira (Portugal) and its effect on host condition. Wildl. Biol. Pract.

Elias, D., Akuffo, H., Britton, S., 2006. Helminthes could influence the outcome of vaccines against TB in the tropics. Parasite Immunol. 28, 507–13. https://doi.org/10.1111/j.1365-3024.2006.00854.x

Elias, D., Britton, S., Aseffa, A., Engers, H., Akuffo, H., 2008. Poor immunogenicity of BCG in helminth infected population is associated with increased in vitro TGF-β production. Vaccine 26, 3897–3902. https://doi.org/10.1016/j.vaccine.2008.04.083

Elkins, D.B., Sithithaworn, P., Haswell-Elkins, M., Kaewkes, S., Awacharagan, P., Wongratanacheewin, S., 1991. Opisthorchis viverrini: relationships between egg counts, worms recovered and antibody levels within an endemic community in northeast Thailand. Parasitology 102 Pt 2, 283–8.

Ezenwa, V.O., Etienne, R.S., Luikart, G., Beja-Pereira, A., Jolles, A.E., 2010. Hidden Consequences of Living in a Wormy World: Nematode-Induced Immune Suppression Facilitates Tuberculosis Invasion in African Buffalo. Am. Nat. 176, 613–624. https://doi.org/10.1086/656496

Flynn, J.L., Chan, J., 2001. Tuberculosis: latency and reactivation. Infect. Immun. 69,

4195–201. https://doi.org/10.1128/IAI.69.7.4195-4201.2001

Forrellad, M.A., Klepp, L.I., Gioffré, A., Sabio y García, J., Morbidoni, H.R., Santangelo, M. de la P., Cataldi, A.A., Bigi, F., 2013. Virulence factors of the *Mycobacterium tuberculosis* complex. Virulence 4, 3–66. https://doi.org/10.4161/viru.22329

Galvani, A.P., 2003. Immunity, antigenic heterogeneity, and aggregation of helminth parasites. J. Parasitol. 89, 232–41. https://doi.org/10.1645/0022-3395(2003)089[0232:IAHAAO]2.0.CO;2

Gassó, D., Feliu, C., Ferrer, D., Mentaberre, G., Casas-Díaz, E., Velarde, R., Fernández-Aguilar, X., Colom-Cadena, A., Navarro-Gonzalez, N., López-Olvera, J.R., Lavín, S., Fenández-Llario, P., Segalés, J., Serrano, E., 2015. Uses and limitations of faecal egg count for assessing worm burden in wild boars. Vet. Parasitol. 209, 133–7. https://doi.org/10.1016/j.vetpar.2015.02.006

Gaughran, A., 2018. The impact of roadworks on the ranging behaviour of European badgers (Meles meles).

Gaughran, A., Kelly, D.J., MacWhite, T., Mullen, E., Maher, P., Good, M., Marples, N.M., 2018. Super-ranging. A new ranging strategy in European badgers. PLoS One 13, e0191818. https://doi.org/10.1371/journal.pone.0191818

Gerrikagoitia, X., Barral, M., Juste, R.A., 2010. Angiostrongylus species in wild carnivores in the Iberian Peninsula. Vet. Parasitol. 174, 175–180. https://doi.org/10.1016/j.vetpar.2010.07.015

Gonzalez-Juarrero, M., Turner, O.C., Turner, J., Marietta, P., Brooks, J. V, Orme, I.M., 2001. Temporal and Spatial Arrangement of Lymphocytes within Lung Granulomas Induced by Aerosol Infection with Mycobacterium tuberculosis. Infect. Immun. 69, 1722–1728. https://doi.org/10.1128/IAI.69.3.1722-1728.2001

Gormley, E., Ní Bhuachalla, D., O'Keeffe, J., Murphy, D., Aldwell, F.E., Fitzsimons, T., Stanley, P., Tratalos, J.A., McGrath, G., Fogarty, N., Kenny, K., More, S.J., Messam, L.L.M., Corner, L.A.L., 2017. Oral Vaccination of Free-Living Badgers (Meles meles) with Bacille Calmette Guérin (BCG) Vaccine Confers Protection against Tuberculosis. PLoS One 12, e0168851. https://doi.org/10.1371/journal.pone.0168851

Górski, P., Zalewski, A., Lakomy, M., 2006. Parasites of carnivorous mammals in Białowieza Primeval Forest. Wiad. Parazytol. 52, 49–53.

Gulland, F.M., Fox, M., 1992. Epidemiology of nematode infections of Soay sheep (Ovis

aries L.) on St Kilda. Parasitology 105 (Pt 3), 481–92.

Guyatt, H.L., Bundy, D.A., 1993. Estimation of intestinal nematode prevalence: influence of parasite mating patterns. Parasitology 107 (Pt 1), 99–105.

Hancox, M., 1980. Parasites and infectious diseases of the Eurasian badger (Meles meles L.): a review. Mamm. Rev. 10, 151–162. https://doi.org/10.1111/j.1365-2907.1980.tb00236.x

Havlir, D. V, Wallis, R.S., Boom, W.H., Daniel, T.M., Chervenak, K., Ellner, J.J., 1991. Human Immune Response to Mycobacterium tuberculosis Antigens, Infection and immunity.

Holland, C. V., 2017. Knowledge gaps in the epidemiology of Toxocara: the enigma remains. Parasitology 144, 81–94. https://doi.org/10.1017/S0031182015001407

Holland, C. V., Asaolu, S.O., Crompton, D.W.T., Stoddart, R.C., Macdonald, R., Torimiro, S.E.A., 1989. The epidemiology of Ascaris lumbricoides and other soil-transmitted helminths in primary school children from Ile-Ife, Nigeria. Parasitology 99, 275. https://doi.org/10.1017/S003118200005873X

Horsnell, W., 2014. How helminths alter immunity to infection.

Hotez, P.J., Brindley, P.J., Bethony, J.M., King, C.H., Pearce, E.J., Jacobson, J., 2008. Helminth infections: the great neglected tropical diseases. J. Clin. Invest. 118, 1311–21. https://doi.org/10.1172/JCI34261

Houpin, E., McCarthy, G., Ferrand, M., De Waal, T., O'Neill, E.J., Zintl, A., 2016. Comparison of three methods for the detection of Angiostrongylus vasorum in the final host. Vet. Parasitol. 220, 54–58. https://doi.org/10.1016/j.vetpar.2016.02.023

Hudson, P.J., 1986. The Effect of a Parasitic Nematode on the Breeding Production of Red Grouse. J. Anim. Ecol. 55, 85. https://doi.org/10.2307/4694

Irvine, R.J., Stien, A., Dallas, J.F., Halvorsen, Langvatn, R., Albon, S.D., 2001. Contrasting regulation of fecundity in two abomasal nematodes of Svalbard reindeer (Rangifer tarandus platyrhynchus). Parasitology 122, 673–81.

Jones, G.W., Neal, C., Harris, E.A., 1980. The helminth parasites of the badger (Meles meles) in Cornwall. Mamm. Rev. 10, 163–164. https://doi.org/10.1111/j.1365-2907.1980.tb00237.x

Jourdan, P.M., Lamberton, P.H.L., Fenwick, A., Addiss, D.G., 2018. Soil-transmitted helminth infections. Lancet (London, England) 391, 252–265.

https://doi.org/10.1016/S0140-6736(17)31930-X

Judge, J., Wilson, G.J., Macarthur, R., Delahay, R.J., McDonald, R.A., 2015. Density and abundance of badger social groups in England and Wales in 2011–2013. Sci. Rep. 4, 3809. https://doi.org/10.1038/srep03809

Kamiya, H., Suzuki, Y., 1975. Parasites of the Japanese badger, Meles meles Anakuma Temmick, especially on Isthmiophora melis (Schrank, 1788) LUHE, 1909. Japanese J. 23, 125–130.

Kataranovski, M., Mirkov, I., Belij, S., Popov, A., Petrović, Z., Gačić, Z., Kataranovski, D., 2011. Intestinal helminths infection of rats (*Ratus norvegicus*) in the Belgrade area (Serbia): the effect of sex, age and habitat. Parasite 18, 189–196. https://doi.org/10.1051/parasite/2011182189

Kennedy, C.R., Bush, A.O., Aho, J.M., 1986. Patterns in helminth communities: why are birds and fish different? Parasitology 93, 205. https://doi.org/10.1017/S0031182000049945

Keymer, A.E., Slater, A.F., 1987. Helminth fecundity: density dependence or statistical illusion? Parasitol. Today 3, 56–8.

Kim, J.-H., Choi, M.-H., Bae, Y.M., Oh, J.-K., Lim, M.K., Hong, S.-T., 2011. Correlation between Discharged Worms and Fecal Egg Counts in Human Clonorchiasis. PLoS Negl. Trop. Dis. 5, e1339. https://doi.org/10.1371/journal.pntd.0001339

Lass, S., Hudson, P.J., Thakar, J., Saric, J., Harvill, E., Albert, R., Perkins, S.E., 2013. Generating super-shedders: co-infection increases bacterial load and egg production of a gastrointestinal helminth. J. R. Soc. Interface 10, 20120588. https://doi.org/10.1098/rsif.2012.0588

Li, X.-X., Zhou, X.-N., 2013. Co-infection of tuberculosis and parasitic diseases in humans: a systematic review. Parasit. Vectors 6, 79. https://doi.org/10.1186/1756-3305-6-79

Lloyd-Smith, J.O., Schreiber, S.J., Kopp, P.E., Getz, W.M., 2005. Superspreading and the effect of individual variation on disease emergence. Nature 438, 355–9. https://doi.org/10.1038/nature04153

Loos-Frank, B., Zeyhle, E., 1982. The intestinal helminths of the red fox and some other carnivores in southwest Germany. Z. Parasitenkd. 67, 99–113.

Loxton, K.C., Lawton, C., Stafford, P., Holland, C. V., 2017. Parasite dynamics in an invaded ecosystem: Helminth communities of native wood mice are impacted by

the invasive bank vole. Parasitology 144, 1476–1489. https://doi.org/10.1017/S0031182017000981

Loxton, K.C., Lawton, C., Stafford, P., Holland, C. V., 2016. Reduced helminth parasitism in the introduced bank vole (Myodes glareolus): More parasites lost than gained. Int. J. Parasitol. Parasites Wildl. 5, 175–183. https://doi.org/10.1016/j.ijppaw.2016.05.005

Magi, M., Banchi, C., Barchetti, A., Guberti, V., 1999. The parasites of the badger (Meles meles) in the north of Mugello (Florence, Italy). Parassitologia 41, 533–6.

Magi, M., Guardone, L., Dell'omodarme, M., Prati, M.C., Mignone, W., Torracca, B., Monni, G., Macchioni, F., 2009. Angiostrongylus Vasorum in Red Foxes (Vulpes Vulpes) and Badgers (Meles Meles) From Central and Northern Italy. Hystrix 20, 121–126.

Maizels, R.M., Bundy, D.A.P., Selkirk, M.E., Smith, D.F., Anderson, R.M., 1993. Immunological modulation and evasion by helminth parasites in human populations. Nature 365, 797–805. https://doi.org/10.1038/365797a0

Maizels, R.M., Yazdanbakhsh, M., 2003. Immune Regulation by helminth parasites: cellular and molecular mechanisms. Nat. Rev. Immunol. 3, 733–744. https://doi.org/10.1038/nri1183

Mccarthy, G., Ferrand, M., De Waal, T., Zintl, A., Mcgrath, G., Byrne, W., O 'neill, E.J., 2018. Geographical distribution of Angiostrongylus vasorum in foxes (Vulpes vulpes) in the Republic of Ireland. https://doi.org/10.1017/S0031182016000032

McDonald, R.A., 2002. Resource partitioning among British and Irish mustelids. J. Anim. Ecol. 71, 185–200. https://doi.org/10.1046/j.1365-2656.2002.00588.x

Medley, G.F., Turner, H.C., Baggaley, R.F., Holland, C., Hollingsworth, T.D., 2016. The Role of More Sensitive Helminth Diagnostics in Mass Drug Administration Campaigns: Elimination and Health Impacts. Adv. Parasitol. 94, 343–392. https://doi.org/10.1016/bs.apar.2016.08.005

Mentaberre, G., Romero, B., de Juan, L., Navarro-González, N., Velarde, R., Mateos, A., Marco, I., Olivé-Boix, X., Domínguez, L., Lavín, S., Serrano, E., 2014. Long-Term Assessment of Wild Boar Harvesting and Cattle Removal for Bovine Tuberculosis Control in Free Ranging Populations. PLoS One 9, e88824. https://doi.org/10.1371/journal.pone.0088824

Millan, J., Sevilla, I., Gerrikagoitia, X., Garcia Perez, A.L., Barral, M., 2004. Helminth

parasites of the Eurasian badger (Meles meles L.) in the Basque Country (Spain). Eur. J. Wildl. Res. 50, 37–40.

Moliva, J.I., Turner, J., Torrelles, J.B., 2015. Prospects in Mycobacterium bovis Bacille Calmette et Guérin (BCG) vaccine diversity and delivery: Why does BCG fail to protect against tuberculosis? Vaccine 33, 5035–5041. https://doi.org/10.1016/J.VACCINE.2015.08.033

More, S., Collins, D., 2016. The Centre for Veterinary Epidemiology and Risk Analysis, The TB Diagnostics and Immunology Research Centre, The Badger Vaccine Project. Biennial Report, 2014-15.

Morgan, E.R., Tomlinson, A., Hunter, S., Nichols, T., Roberts, E., Fox, M.T., Taylor, M.A., 2008. Angiostrongylus vasorum and Eucoleus aerophilus in foxes (Vulpes vulpes) in Great Britain. Vet. Parasitol. 154, 48–57. https://doi.org/10.1016/J.VETPAR.2008.02.030

Moskwa, B., Goździk, K., Bień, J., Bogdaszewski, M., Cabaj, W., 2012. Molecular identification of Trichinella britovi in martens (Martes martes) and badgers (Meles meles); new host records in Poland. Acta Parasitol. 57, 402–405. https://doi.org/10.2478/s11686-012-0054-1

Mueller, J.F., 1965. Helminth life cycles.

Mullineaux, E., Keeble, E.J., British Small Animal Veterinary Association, 2017. BSAVA manual of wildlife casualties, in: The Veterinary Report. p. 480.

Naaktgeboren, C.A., Bertens, L.C.M., Smeden, M. v., Groot, J.A.H. d., Moons, K.G.M., Reitsma, J.B., 2013. Value of composite reference standards in diagnostic research. BMJ 347, f5605–f5605. https://doi.org/10.1136/bmj.f5605

Naranjo Lucena, A., Garza Cuartero, L., Mulcahy, G., Zintl, A., 2017. The immunoregulatory effects of co-infection with Fasciola hepatica: From bovine tuberculosis to Johne's disease. Vet. J. 222, 9–16. https://doi.org/10.1016/J.TVJL.2017.02.007

Neal, E., Cheeseman, C., 1996. Badgers.

Nielsen, M.K., Baptiste, K.E., Tolliver, S.C., Collins, S.S., Lyons, E.T., 2010. Analysis of multiyear studies in horses in Kentucky to ascertain whether counts of eggs and larvae per gram of feces are reliable indicators of numbers of strongyles and ascarids present. Vet. Parasitol. 174, 77–84. https://doi.org/10.1016/j.vetpar.2010.08.007

Nikolay, B., Brooker, S.J., Pullan, R.L., 2014. Sensitivity of diagnostic tests for human soil-transmitted helminth infections: a meta-analysis in the absence of a true gold standard. Int. J. Parasitol. 44, 765–74. https://doi.org/10.1016/j.ijpara.2014.05.009

Noonan, N.L., Sheane, W.D., Harper, L.R. and Ryan, P.., 1975. Wildlife as a possible reservoir of bovine tuberculosis. Irish Vet. Journal.

O'corry-Crowe, G., Hammond, R., Eves, J., Hayden, T.J., 1996. The Effect of Reduction in Badger Density on the Spatial Organisation and Activity of Badgers Meles meles L. in Relation to Farms in Central.

O'Hara, R.B., Kotze, D.J., 2010. Do not log-transform count data. Methods Ecol. Evol. 1, 118–122. https://doi.org/10.1111/j.2041-210X.2010.00021.x

O'Meara, B., Mulcahy, G., 2002. A survey of helminth control practices in equine establishments in Ireland. Vet. Parasitol. 109, 101–110. https://doi.org/10.1016/S0304-4017(02)00249-2

Olsen, O.W. (Oliver W., 1986. Animal parasites : their life cycles and ecology. Dover.

Padial, J. M, 2003. Feeding habits and overlap among red fox (Vulpes vulpes) and stone marten (Martes foina) in two Mediterranean mountain habitats. Mamm Biol 67, 137–146.

Popiołek, M., Jarnecki, H., Łuczyński, T., 2009. A record of Crenosoma vulpis (Rudolphi, 1819) (Nematoda, Crenosomatidae) from the Eurasian badger (Meles meles L.) from Poland. Wiad. Parazytol. 55, 437–439.

Poulin, R., 2018a. Interactions between species and the structure of helminth communities. Parasitology 122, S3-11.

Poulin, R., 2018b. Best practice guidelines for studies of parasite community ecology 10–13. https://doi.org/10.1017/S0022149X18000767

Priemer, J., Lux, E., 1994. Atriotaenia incisa (Cestoda), a parasite of the badger, *Meles meles* , and the raccoon, Procyon lotor , in Brandenburg, Germany. Can. J. Zool. 72, 1848–1853. https://doi.org/10.1139/z94-250

Ribas, A., Molina-Vacas, G., Boadella, M., Rodríguez-Teijeiro, J.D., Fernández-Cardo, R., Arrizabalaga, A., 2012. First report of Troglotrema acutum (Digenea, Troglotrematidae) in the Eurasian badger Meles meles in the Iberian Peninsula and presumptive lesions caused in the host. J. Helminthol. 86, 222–227. https://doi.org/10.1017/S0022149X11000289

Risco, D., Serrano, E., Fernández-Llario, P., Cuesta, J.M., Gonçalves, P., García-Jiménez, W.L., Martínez, R., Cerrato, R., Velarde, R., Gómez, L., Segalés, J., Hermoso de Mendoza, J., 2014. Severity of bovine tuberculosis is associated with co-infection with common pathogens in wild boar. PLoS One 9, e110123. https://doi.org/10.1371/journal.pone.0110123

Roper, T., 2010. Badger, Badger. Collins.

Rosalino, L.M., Torres, J., Santos-Reis, M., 2006. A survey of helminth infection in Eurasian badgers (Meles meles) in relation to their foraging behaviour in a Mediterranean environment in southwest Portugal. Eur. J. Wildl. Res. 52, 202–206. https://doi.org/10.1007/s10344-006-0033-7

Ross, J.G., Fairley, J.S., 1969. Studies of disease in the Red fox (Vulpes vulpes) in Northern Ireland. J. Zool. 157, 375–381. https://doi.org/10.1111/j.1469-7998.1969.tb01708.x

Sabin, E.A., Araujo, M.I., Carvalho, E.M., Pearce, E.J., 1996. Impairment of Tetanus Toxoid-Specific Thl-like Immune Responses in Humans Infected with Schistosoma mansoni. J. Infect. Dis. 173, 269–272. https://doi.org/10.1093/infdis/173.1.269

Salgame, P., Yap, G.S., Gause, W.C., 2013. Effect of helminth-induced immunity on infections with microbial pathogens. Nat. Immunol. 14, 1118–1126. https://doi.org/10.1038/ni.2736

Schad, G.A., Anderson, R.M., 1985. Predisposition to hookworm infection in humans. Science 228, 1537–40.

Schluger, N.W., Rom, W.N., Magi, M., Guardone, L., Dell'omodarme, M., Prati, M.C., Mignone, W., Torracca, B., Monni, G., Macchioni, F., Garnier, T., Eiglmeier, K., Camus, J.-C., Medina, N., Mansoor, H., Pryor, M., Duthoy, S., Grondin, S., Lacroix, C., Monsempe, C., Simon, S., Harris, B., Atkin, R., Doggett, J., Mayes, R., Keating, L., Wheeler, P.R., Parkhill, J., Barrell, B.G., Cole, S.T., Gordon, S. V, Hewinson, R.G., McCarthy, G., Ferrand, M., De Waal, T., Zintl, A., McGrath, G., Byrne, W., O'Neill, E.J., Pedersen, A.B., Fenton, A., Gallagher, J., Clifton-Hadley, R.S., Bordes, F., Morand, S., Poulin, R., Mohd Zain, S.N.S., Behnke, J.J.M., Lewis, J.W.J., Gallagher, J., Clifton-Hadley, R.S., Rosalino, L.M., Torres, J., Santos-Reis, M., Cooper, P.J., Espinel, I., Paredes, W., Guderian, R.H., Nutman, T.B., To, O., This, S., Maizels, R.M., Yazdanbakhsh, M., Mohd Zain, S.N.S., Behnke, J.J.M., Lewis, J.W.J., Morgan, E.R., Tomlinson, A., Hunter, S., Nichols, T., Roberts, E., Fox, M.T., Taylor, M.A.,

2012. Tuberculosis in badgers; a review of the disease and its significance for other animals. Parasitology 5, 203–217. https://doi.org/10.1053/rvsc.2000.0422

Seivwright, L.J., Redpath, S.M., Mougeot, F., Watt, L., Hudson, P.J., 2004. Faecal egg counts provide a reliable measure of Trichostrongylus tenuis intensities in free-living red grouse Lagopus lagopus scoticus. J. Helminthol. 78, 69–76.

Shaw, J.L., Moss, R., 1989. The role of parasite fecundity and longevity in the success of Trichostrongylus tenuis in low density red grouse populations. Parasitology 99 Pt 2, 253–8.

Shen, C., Kim, J., Lee, J.-K., Bae, Y.M., Choi, M.-H., Oh, J.-K., Lim, M.K., Shin, H.-R., Hong, S.-T., 2007. Collection of Clonorchis sinensis adult worms from infected humans after praziquantel treatment. Korean J. Parasitol. 45, 149. https://doi.org/10.3347/kjp.2007.45.2.149

Sithithaworn, P., Tesana, S., Pipitgool, V., Kaewkes, S., Pairojkul, C., Sripa, B., Paupairoj, A., Thaiklar, K., 1991. Relationship between faecal egg count and worm burden of Opisthorchis viverrini in human autopsy cases. Parasitology 102 Pt 2, 277–81.

Sleeman, Paddy; Kelly, T., 1997. Parasites and diseases of Irish badgers. Small Carniv. Conserv. 17.

Sleeman, D.P., Davenport, J., More, S.J., Clegg, T.A., Collins, J.D., Martin, S.W., Williams, D.H., Griffin, J.M., O'Boyle, I., 2009. How many Eurasian badgers Meles meles L. are there in the Republic of Ireland? Eur. J. Wildl. Res. 55, 333–344. https://doi.org/10.1007/s10344-008-0244-1

Smal, C., 1995. The badger and habitat survey of Ireland. Stationery Office., Stationery Office.

Smith, I., 2003. Mycobacterium tuberculosis Pathogenesis and Molecular Determinants of Virulence. Clin. Microbiol. Rev. 16, 463–496. https://doi.org/10.1128/CMR.16.3.463-496.2003

Stear, M.J., Fitton, L., Innocent, G.T., Murphy, L., Rennie, K., Matthews, L., 2007. The dynamic influence of genetic variation on the susceptibility of sheep to gastrointestinal nematode infection. J. R. Soc. Interface 4, 767–76. https://doi.org/10.1098/rsif.2007.1104

Stromberg, B.E., 1997. Environmental factors influencing transmission. Vet. Parasitol. 72, 247-56; discussion 257-64.

Strube, C., Heuer, L., Janecek, E., 2013. Toxocara spp. infections in paratenic hosts.

Vet. Parasitol. 193, 375–389. https://doi.org/10.1016/j.vetpar.2012.12.033

Stuart, P., Golden, O., Zintl, A., De Waal, T., Mulcahy, G., Mccarthy, E., Lawton, C., 2013. A coprological survey of parasites of wild carnivores in Ireland. Parasitol. Res. 112, 3587–3593. https://doi.org/10.1007/s00436-013-3544-7

Stubbe, M., 1965. Zur Biologie der Raubtiere eines abgeschlossenen Waldgebietes. Z. Jagdwiss. 11, 73–102. https://doi.org/10.1007/BF01956001

Tchuem Tchuenté, L.A., 2011. Control of soil-transmitted helminths in sub-Saharan Africa: Diagnosis, drug efficacy concerns and challenges. Acta Trop. 120, S4–S11.

Tompkins, D.M., Hudson, P.J., 1999. Regulation of nematode fecundity in the ring-necked pheasant (Phasianus colchicus): not just density dependence. Parasitology 118 (Pt 4), 417–23.

Torres, J., Miquel, J., Motjé, M., 2001. Helminth parasites of the eurasian badger (Meles meles L.) in Spain: a biogeographic approach. Parasitol. Res. 87, 259–63.

Venables, W.N. (William N.., Ripley, B.D., Venables, W.N. (William N.)., 2002. Modern applied statistics with S.

Walker, M., Hall, A., Basanez, M.., 2013. Ascaris lumbricoides: new epidemiological insights and and mathematical approaches., in: Holland, C. V. (Ed.), Ascaris the Neglected Parasite. Elsevier, pp. 155–201.

Wammes, L.J., Hamid, F., Wiria, A.E., de Gier, B., Sartono, E., Maizels, R.M., Luty, A.J.F., Fillié, Y., Brice, G.T., Supali, T., Smits, H.H., Yazdanbakhsh, M., 2010. Regulatory T cells in human geohelminth infection suppress immune responses to BCG and *Plasmodium falciparum*. Eur. J. Immunol. 40, 437–442. https://doi.org/10.1002/eji.200939699

Wilson, K., Bjørnstad, O., Dobson, A., Merler, S., Poglayen, G., Randolph, S., Read, A., Skorping, A., n.d. Chapter 2: Heterogeneities in macroparasite infections: patterns and processes 1.

Wilson, K., Grenfell, B.T., 1997. Generalized linear modelling for parasitologists. Parasitol. Today 13, 33–38. https://doi.org/10.1016/S0169-4758(96)40009-6

Wolfe, A., Hogan, S., Maguire, D., Fitzpatrick, C., Vaughan, L., Wall, D., Hayden, T.J., Mulcahy, G., 2001. Red foxes (Vulpes vulpes) in Ireland as hosts for parasites of potential zoonotic and veterinary significance, Veterinary Record.

World Health Organization, 2017. WHO | Soil-transmitted helminthiases. WHO.

Zinsstag, J., Schelling, E., Roth, F., Kazwala, R., 2006. Economics of Bovine Tuberculosis,

in: Mycobacterium Bovis Infection in Animals and Humans. Blackwell Publishing Ltd, Oxford, UK, pp. 68–83. https://doi.org/10.1002/9780470344538.ch9