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

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ABSTRACT

Coprological analysis is the most widely used diagnostic tool for helminth infection in both domestic and wild mammals. Evaluation of the efficacy of this technique is rare, due to the lack of availability of adult worm burden. Where information is available the majority of studies are in small ruminants and seldom in a wild host. This study of 289 wild badgers is the first to report the relationship between faecal egg/larval counts and adult worm burden in badgers whilst also evaluating the reliability of coprological analysis as a diagnostic tool for hookworm (*Uncinaria criniformis*) and lungworm (*Aelurostrongylus falciformis*) infection. The prevalence of hookworm and lungworm infection, as assessed through adult worm burden was 59.2% and 20.8% respectively. For both species of helminth, infection was consistently under-reported by coprological analysis compared to adult worm burden with a reported 41% sensitivity for hookworm and 10% for lungworm. A significant positive relationship was found between faecal counts and adult worm burden for both species of helminths. Additionally the density –dependent relationship often reported in helminth infection appears to be weak or non-existent in this study, up to the observed worm intensity of 500.

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 or female 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 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; Fogarty unpublished results). 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. 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 lung tissue 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. Materials and methods

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 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 board (code:2016–28).

2.2. Collection of samples

Hearts, lungs and gastrointestinal tracts were collected and frozen without preservative, at − 20 °C until examination. Faecal samples were collected from the rectum of gastrointestinal tracts and fixed in 10% formalin (Garcia and Bruckner, 2001).

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 °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 parasites. Whenever they were found, helminth parasites were removed, counted and preserved in 70% ethanol. Species identification was confirmed by parasitologist, Eileen Harris at the Natural History Museum, London.

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.

2.5. Statistical 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).

2.5.1. 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;

$$CI = 100 \left(p \ \pm 1.96 \ \sqrt{\frac{p(1-p)}{n}} \right)$$

Where:

n = sample size
p = mean prevalence (Agresti, 1996).

2.5.2. Abundance and intensity
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 (V:$\bar{}$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.

2.5.3. Sensitivity and specificity

To evaluate egg and larval 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.

3. Results

Within the GI tract, the prevalence of hookworm *U. 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 1. In heart and lung samples, the prevalence of lungworm *A. falciformis* was 20.8% (CI 95% 16.2%–25.9%) and within the faeces, the prevalence of lungworm larvae was 18.3% (CI 95% 14.0%–23.3%). As shown in Tables 1 and 2, the mean abundance and intensity was higher for hookworm, for both adult worm burden and faecal egg count.

Both hookworm and lungworm had an aggregated distribution, as shown in Table 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 Fig. 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).

Using abundance data, the relationship between adult worm burden and e.p.g./l.p.g was explored for each helminth species (Figs. 2 and 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) (see Fig. 2) or larvae (n = 54) were detected in the faeces.

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% (CI 95% 34.05%–49.29%) and a specificity of 100% (CI 95% 96.92%–100%)(see Table 3). There were no individuals positive for eggs but negative for worms.

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 4). There were only 6

instances where 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.

For each helminth species (*U. 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.

4. Discussion

Within our sample of 289 badgers, the prevalence of both hookworm (*U. criniformis*) and lungworm (*A. 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 lower 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 that reported 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 sample of badgers 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 (ESCCAP, 2018; Lloyd-Smith et al., 2005). Interestingly, we found that adult hookworm demonstrated greater aggregation than adult lungworm, however in contrast, based upon faecal counts, lungworm larvae demonstrated higher aggregation than hookworm eggs. Results such as these underline the importance of diagnostic accuracy in future control programmes that hope to utilise targeted treatment strategies and highlight the need for a deeper understanding of the relationship between egg/larval output in to the faeces and adult worm burden present in hosts.

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 (Walker et al., 2013). 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

Table 1
Prevalence and abundance for adult hookworm (*U. criniformis*) and adult lungworm (*A. 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**).

| Helminth species	Prevalence		Abundance			
	Adult worm	Eggs/larvae	Adult worm		Egg/larvae	
	% (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 ± 20.0	0–4050
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

Table 2

Intensity and aggregation of adult hookworm (*U. criniformis*) and adult lungworm (*A. falciformis*) worm burden and corresponding faecal egg/larval counts (e.p.g/l.p.g)(n = 289).

Helminth species	Intensity				Aggregation (v:̄ 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	266.9 ± 143.7	10–4050	127.8	20.9
A. falciformis (L)	11.1 ± 1.6	1–53	154.2 ± 48.5	10–2120	23.6	921.8

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) additionally, due to the unpleasant nature of worm collection, compliance can be low within the sample population (Phiri, 2001). 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-dependent suppression of egg or larva production, as described by Anderson and 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 a reported worm intensity up to 8000 worms, in red grouse (Seivwright et al., 2004). Interestingly, the relationship between worm burden and their reproductive products in
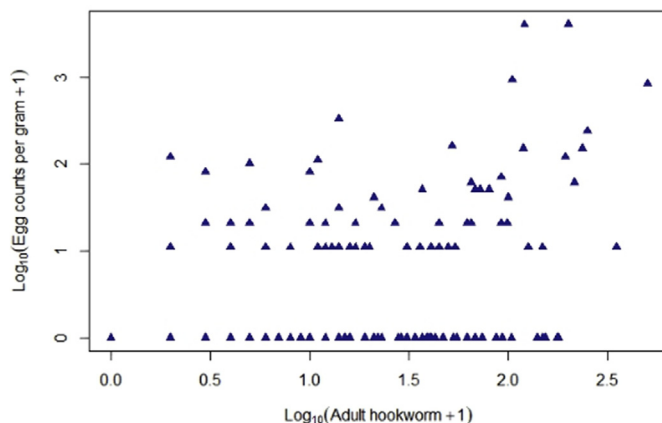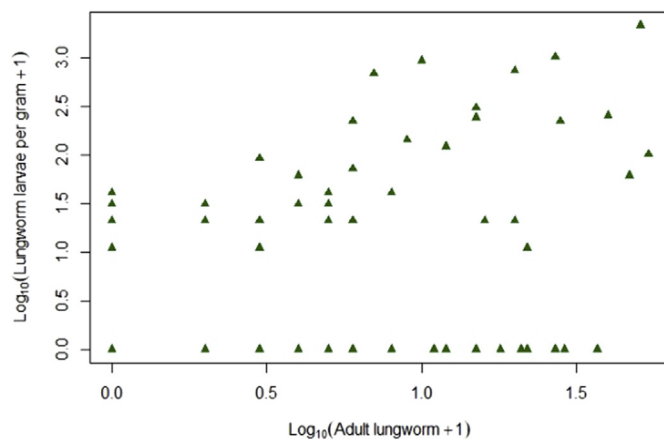

Fig. 2. The relationship between adult hookworm (*U. criniformis)* burden and faecal egg counts (e.p.g) in Irish badgers (n = 289).

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-dependent 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
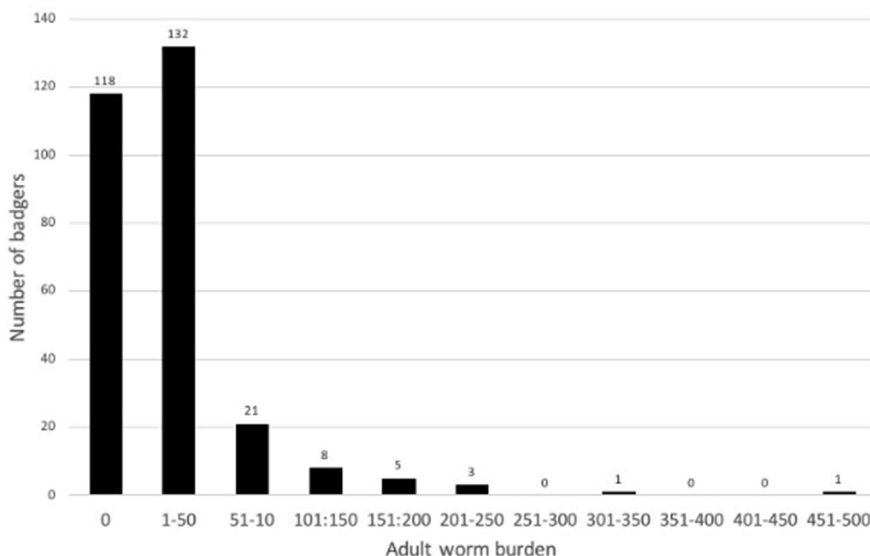
Fig. 1. The frequency distribution of adult hookworm (*U. criniformis*) in Irish badgers (n = 289).

Fig. 3. The relationship between adult lungworm (*A. falciformis*) burden and number of larvae per g of faeces in Irish badgers (n = 289).

Table 3
A comparison of faecal egg count and adult hookworm (*U. criniformis*) 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		Positive	Negative
Faecal egg count	Positive	**TP** 71	**FP** 0
	Negative	**FN** 100	**TN** 118
	Total	171	118

Table 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		Positive	Negative
Larval count	Positive	**TP** 6	**FP** 47
	Negative	**FN** 54	**TN** 182
	Total	60	229

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 are 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.

5. Conclusion

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 or 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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2018.11.003.

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