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Endoparasites of the endemic Irish hare *Lepus timidus hibernicus*

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The Irish hare *Lepus timidus hibernicus* is an endemic subspecies of Mountain hare and Ireland's only native lagomorph. The endoparasite community composition of the Irish hare was examined from 22 carcasses opportunistically sourced from wildlife strike events (with aircraft and vehicles) from three counties in the Republic of Ireland. Three parasite taxa were identified from the stomach and small intestines: *Trichostrongylus retortaeformis*, *Graphidium strigosum* and a tapeworm belonging to the genus *Mosgovoyia*. Overall, 50% of hares examined were host to at least one endoparasite taxon and 18% were host to more than one taxon. There was no significant correlation between parasite burden and host weight. This is the first known study of endoparasites in the Irish hare.

Keywords: ecology, gastrointestinal helminths, Ireland, mountain hare

The Irish hare *Lepus timidus hibernicus* is a distinct endemic subspecies of the mountain hare *Lepus timidus* which is an ecologically and economically important game species across its range. While mountain hares are generally arctic–boreal in range, the Irish hare is an exception, inhabiting a temperate environment (Hamill et al. 2006) and numerous habitat types within Ireland (Lysaght and Marnell 2016). Irish hare populations have undergone rapid population declines (Dingerkus and Montgomery 2002, Reid et al. 2010a) and are now considered a priority species for conservation action in Ireland (Caravaggi et al. 2015). Agricultural intensification is thought to be the primary cause of decline, resulting in the loss of herbaceous biodiversity required for both food and shelter (Dingerkus and Montgomery 2002, McGowan et al. 2019). The current population is estimated at 223 000 hares (McGowan et al. 2019). While there is an open hunting season, they are rarely taken as a game species in the Republic of Ireland. However, hare coursing, whilst banned in most countries, is a regulated and widespread practice (Reid et al. 2007, 2010b, Kelly 2020). Despite their cultural significance, little research has been conducted on the endoparasites of lagomorphs in Ireland. This is with the exception of Butler (1994), who recorded the arthropod and helminth parasites of rabbits *Oryctolagus cuniculus*.

Endoparasite infestations in lagomorphs reduce host survival by impacting on body condition and increasing predation (Murray et al. 1997, Lello et al. 2005). Mountain hares are known to host heavy helminth infestations and are particularly susceptible to high burdens of the two gastrointestinal species *Graphidium strigosum* and *Trichostrongylus retortaeformis* (Newey et al. 2005). High levels of infestation can negatively impact on female fecundity and are suspected to be a contributor to the unstable population dynamics observed in mountain hares (Newey et al. 2005). We hypothesise that endoparasite communities in the Irish subspecies will be similar to those recorded in other mountain hare populations and the European rabbit. Here we describe the endoparasite community and associated prevalence of three helminth taxa from Irish hare carcasses opportunistically sourced from wildlife-strike incidents from the Republic of Ireland.

Methods

Carcass collection

A total of 22 Irish hare carcasses were examined, the majority of which (n = 19) were obtained from Dublin Airport (DUB, 53°42′64″N, 6°24′99″W), following fatal collisions with aircraft (2010–2015). The grasslands at DUB are maintained using a 'long grass' management policy consisting of a blend of tall fescue *Festuca arundinacea* and Italian ryegrass *Festuca perennis*. Carcasses were collected by DUB personnel from runways and the conditions surrounding the strike

Table 1. Hare *Lepus timidus hibernicus* specimens opportunistically sourced from strike events with aircraft (n = 19) and vehicle (n = 3) collisions in the Republic of Ireland. Due to the nature of the specimen's deaths, not all carcasses were intact for analysis. Only the weights of intact specimens are presented. Unk = Unknown.

ID	Condition	Age	Sex	Weight (g)	Season	Year	Location
1*	Intact	Adult	Unk	1680	Winter	2013	DUB
2*	Intact	Adult	Male	1720	Winter	2013	DUB
3*	Intact	Adult	Male	2020	Winter	2014	Clonmel, Co. Tipperary
4*	Intact	Adult	Unk	2480	Winter	2014	Nad, Co. Cork
5*	Intact	Adult	Male	2340	Winter	2014	Lyre, Co. Cork
6*	Intact	Adult	Male	2140	Autumn	2014	DUB
7*	Intact	Adult	Unk	2060	Winter	2014	DUB
8*	Intact	Adult	Unkn	1995	Autumn	2014	DUB
9*	Intact	Adult	Ukn	1980	Winter	2014	DUB
10*	Intact	Adult	Male	2190	Winter	2015	DUB
11*	Intact	Adult	Ukn	1825	Unk	Unk	DUB
12*	Intact	Adult	Female	2965	Unk	Unk	DUB
13*	Intact	Juvenile	Male	1320	Unk	Unk	DUB
14	Fragmented	Adult	Ukn	NA	Summer	2010	DUB
15	Fragmented	Adult	Ukn	NA	Spring	2014	DUB
16	Fragmented	Adult	Ukn	NA	Winter	2014	DUB
17	Fragmented	Adult	Ukn	NA	Winter	2014	DUB
18	Fragmented	Adult	Ukn	NA	Winter	2015	DUB
19	Fragmented	Adult	Ukn	NA	Winter	2015	DUB
20*	Fragmented	Adult	Male	NA	Unk	Unk	DUB
21	Fragmented	Juvenile	Male	NA	Unk	Unk	DUB
22*	Fragmented	Adult	Ukn	NA	Unk	Unk	DUB

* Indicates entire digestive tract.

incident recorded. Carcasses were recorded to have been collected in the spring (n = 1), summer (n = 1), autumn (n = 2) and winter (n = 9). Six carcasses from the airfield had no date associated with them. Post collection, all carcasses were immediately frozen. The carcasses were then made available from the Dublin Airport Authority, in line with their policy of making scientific use of wildlife-strike data (Kelly et al. 2017). A further three specimens were opportunistically collected from road side accidents in the counties of Cork (n = 2) (52°08′12″N, 8°86′32″W/52°06′70″N, 8°83′16″W) and Tipperary (n = 1) (52°36′27″N, 7°58′80″W) in the winter of 2014–2015.

Carcass processing

There was an apparent preponderance of males (14 males, 1 female, 7 unknown) and adults (20 adults, 2 juveniles). Due to the nature of the specimens' deaths, some carcasses were incomplete, resulting in a total of 13 whole hares and a total of 15 complete digestive tracts (Table 1). All carcasses were thawed for 24 h at room temperature prior to processing. Where possible, specimens were identified to sex by visual inspection and aged based on the presence or absence of epiphyseal distal cartilage (Fernández et al. 2010).

The digestive tract was sectioned into the 1) stomach, 2) large intestine and 3) small intestine. The 4) heart, 5) lungs, 6) liver and 7) kidney were also examined and sectioned. Digestive tract sections were separately opened and examined for macroparasites which were removed and counted (Kornaś et al. 2014). Each section and digestive contents were washed through successive sieves (250, 150 and 90 μm) (Kornaś et al. 2014). Sieve contents were washed with 70% ethanol into sample containers and parasites were counted and identified under binocular microscope (Chroust et al. 2012). Cestodes were isolated from the small intestine, without the presence of an intact scolex and were therefore cleared and identified using other morphological features against reference material. To count the number of cestodes present in a host, the immature, smaller strobila at the anterior end of the cestode were located and their presence considered to be a single cestode.

Statistical analysis

The full set of host specimens (n = 22) were used to determine the overall prevalence (%) of each parasite taxon. Only intact specimens (n = 13) were used to determine parasite burden and organ burden. Intact specimens (n = 13) were used to examine the effect of parasite burden for each taxon on host weight (g) and data were tested for normality using the Shapiro–Wilk test. Kendall correlations were fitted for non-parametric data. All statistical analysis was carried out in programme R ver. 3.6.1 (<www.r-project.org>). We obtained Fisher's index of aggregation ($F = \sigma^2/\mu$), where $F > 1$ indicates an aggregated pattern, followed by a χ^2-test. To determine if parasite burdens were aggregated in the host population, the 'epiphy' package (Gigot 2018) was used. We did not investigate the effects of sex, age, season or location on parasite burden due to the small sample size.

Results

In total, three endoparasite taxa were recorded in the Irish hare with 50% being host to at least one taxon (Table 2). Overall, 18% (n = 4) of hares were host to two parasite taxa. Across all specimens (n = 22), *Trichostrongylus retortaeformis* was the most prevalent helminth (43%, n = 9) followed by *Graphidium strigosum* (20%, n = 3). A tapeworm belonging to the *Mosgovoyia* genus was also recorded (14%,

Table 2. Prevalence (%), mean intensity ($\mu \pm SE$) and range of infected hares *Lepus timidus hibernicus* for each endoparasite taxon identified from 1) all hosts (n = 22) where not every target organ was available and 2) from intact specimens (n = 13) with all target organs available.

Parasite taxa	Target organ and number available	Prevalence (%)	Mean intensity (± SE)	Range (n–n)
All specimens (n = 22 in total)				
T. retortaeformis	Small intestine (n = 21)	43 (n = 9)	868.7 (± 301.2)	112–2560
G. strigosum	Stomach (n = 15)	20 (n = 3)	12.3 (± 0.8)	11–14
Mosgovoyia sp.	Small intestine (n = 21)	14 (n = 3)	4.7 (± 1.2)	3–7
Intact specimens (n = 13)				
T. retortaeformis	Small intestine (n = 13)	38 (n = 5)	850 (± 428)	112–2000
G. strigosum	Stomach (n = 13)	23 (n = 3)	12.5 (± 1.5)	11–14
Mosgovoyia sp.	Small intestine (n = 13)	23 (n = 3)	5.0 (± 2.0)	3–7

n = 3). *Trichostrongylus retortaeformis* was the most numerous helminth amounting to 99% of individual endoparasites recorded.

Only hares sourced from DUB (n = 19) were positive for helminths, with a mean burden of 292.7 (SE ± 163.64) for infested intact specimens (n = 13). Although several organs were examined, all parasites were confined to either the stomach (*G. strigosum*) or the small intestine (*T. retortaeformis*, *Mosgovoyia* sp.). The small intestine had a higher mean parasite burden of 262.3 (SE ± 163.9) than the stomach (1.9 SE ± 1.3) in intact specimens (n = 13). The mean weight of hosts examined was 2055 g (range 1320–2480 g), with little difference in the mean observed between seasons (autumn = 2068 g, winter = 2059 g). There was a weak correlation between host parasite burden and body weight. This correlation was not significant for either *T. retortaeformis* ($r = -0.09$, n = 13), *G. strigosum* ($r = -0.09$, n = 13) or the *Mosgovoyia* tapeworm species ($r = -0.17$, n = 13). Parasite burden was highly aggregated for all three parasites: *T. retortaeformis* ($F = 769.4$; $\chi^2_{(12,\ 13)} = 9232$, $p < 0.001$); *G. strigosum* ($F = 10.4$; $\chi^2_{(12,\ 13)} = 124.97$, $p < 0.01$); and the *Mosgovoyia* tapeworm species ($F = 4.5$; $\chi^2_{(12,\ 13)} = 54.71$, $p < 0.001$).

Discussion

Here we identify three helminth endoparasite taxa hosted by the endemic Irish hare. This is somewhat lower than studies in continental Europe, where members of the genus *Lepus* have been recorded to host between five and 10 endoparasite taxa (Soveri and Valtonern 1983, Bordes et al. 2007, Chroust et al. 2012, Lukešová et al. 2012, Diakou et al. 2014, Kornaś et al. 2014). It is possible that the Irish hares geographic and genetic isolation from other mountain hare populations (Hamill et al. 2006) may be a contributing factor to this low helminth diversity, as may the small sample size from a predominantly single location (DUB). We do not believe in this instance that season played a major role in parasite diversity but it is known to impact on parasite burden and aggregation (Newey et al. 2005). Six helminth species have been identified in rabbits in Ireland (Butler 1994), three of which were present in hares included in this study, confirming low host specificity.

Overall, 50% (n = 11) of hare specimens examined were host to at least one parasite taxon, the most prevalent of which was *Trichostrongylus retortaeformis* (43%, n = 9). Higher prevalence rates of 99% (n = 589) have been found in mountain hares in Scotland (Newey et al. 2005) and 76% (n = 109) in Finland (Soveri and Valtonern 1983). The *Mosgovoyia* sp. is likely to be *Mosgovoyia pectinata* which has been identified previously in rabbits in Ireland (Butler 1994) and mountain hares in Scotland (Hulbert and Boag 2001). However, due to the absence of the scolex, it was not possible to identify this cestode with certainty. We found a *Mosgovoyia* species to be infecting 14% of Irish hares, similar to levels recorded in Spain (17% (n = 487); Segovia et al. 2014). Contrastingly, we found *Graphidium strigosum* prevalence (20%) to be higher than in other parts of Europe, where prevalence has been recorded between 3% (n = 225) and 4% (n = 137) (Chroust et al. 2012). However, differences in prevalence rates recorded between these studies and that of the Irish hare may be as a result of the small sample size used (n = 22). All three parasite taxa recorded in the Irish hare were aggregated ($\sigma^2 \geq \mu$), which can influence individual fitness, parasite transmission and population regulation (Poulin 2013). For example, aggregation in hares is highest during the winter months due to reduced transmission rates and is thought to negatively impact on body condition (Newey et al. 2005).

We found that *T. retortaeformis* burden was not correlated with host weight. Whilst we found no correlation to *G. strigosum* burden and host weight, Allan et al. (1999) found a positive correlation between increasing host weight and *G. strigosum* burden, as a consequence of this parasite generally being associated with older individuals (Dudzinski and Mykytowycz 1963). Interestingly, in this study a juvenile yielded the highest *G. strigosum* burden.

Habitat type and quality have been shown to impact on gastrointestinal helminth prevalence in mammals (Hulbert and Boag 2001, Froeschke and Matthee 2014, Santicchia et al. 2015). Therefore, it is not certain if the prevalence rates recorded in this study are reflective of the overall Irish hare population or a reflection of the habitat type (i.e. airfield semi-natural grassland). The three parasite taxa recorded in hares in this study are transmitted through the consumption of vegetation contaminated with either free-living larvae (*T. retortaeformis* and *G. strigosum*) or orbatid mites (*Mosgovoyia* sp.). Lagomorphs can share and potentially transmit parasite species with commercially important ruminants (Audebert et al. 2002), with evidence that they may transmit anthelminthic resistant parasite strains between livestock grazing areas (Stott et al. 2009). Data on the helminth fauna of the Irish hare is, therefore, important from an agricultural and habitat management perspective.

Coursing is a standard practice in the Republic of Ireland and is affiliated with the administration of anthelmintic treatments (e.g. Ivermectin) to wild populations of hares. It is notable that in this study hares, albeit in small numbers, sourced in parts of the country distant from the airport had no helminths. Two of these hares were not tagged and so unlikely to have received anthelmintic treatments. An ear tag confirmed that the remaining hare from Tipperary had been coursed and therefore treated, but in a previous year. Comparing the endoparasite community composition and parasite burden of Irish hares in the Republic to those in Northern Ireland where coursing is not a regulated practice, would give valuable insights into any population level effects as a result of anthelminthic treatments. As the endoparasite community of the Irish hare was unrecorded until now, this study can help inform ongoing management practices as to the appropriateness of these current anthelminthic treatments being administered. We suggest that the regular treatment of hares countrywide, over a prolonged period, has the potential to impact on parasite–host dynamics.

Conclusion

Gaining an understanding of a species parasitofauna composition is important from both a resource management and conservation perspective. In the case of the endemic subspecies of Irish hare, we identified parasite species known to impact on population dynamics (e.g. *T. retortaeformis* and *G. strigosum*). Further research is needed to quantify the prevalence and range of the parasite community of Irish hares across the island of Ireland. Such data is crucial to investigate and understand the impact of current treatment and management practices on hare population dynamics in order to ensure continued conservation of this iconic Irish mammal.

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Conflict of interest – This work was conducted as part of a research project in 2015. As of 2018 the lead author works with the Dublin Airport Authority as a project partner to conduct PhD research.
Ethics – This article does not contain any studies with human participants or live animals. All specimens were opportunistically sourced from wildlife collisions.

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