Helminth parasites of the wild rabbit *Oryctolagus cuniculus* near Malham Tarn, Yorkshire, UK

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Abstract

Between 1992 and 1996, 95 rabbits from the immediate locality of Malham Tarn, North Yorkshire, UK were examined for the presence of helminth parasites. All the examinations took place in late September or October. Three species of nematodes, *Graphidium strigosum, Passalurus ambiguus* and *Trichostrongylus retortaeformis* and two species of cestodes, *Taenia pisiformis* and *Cittotaenia pectinata* were identified. There were no associations between helminth species richness and year of sampling, host weight or sex. A logistic model was fitted to the prevalence data from these helminths as was an over-dispersed Poisson model to the worm burden data. *Graphidium strigosum* was the most frequently identified species with an average prevalence of 78%. The mean prevalence and intensity of *Graphidium* infection were significantly effected by sampling year. The lower than normal rainfall recorded at the Tarn during the years 1995 and 1996 may have be one reason for this pattern. The worm burden of *G. strigosum* was significantly positively associated with rabbit body weight. The intensity of infection with *P. ambiguus* was significantly higher in female rabbits. There was a significant non-linear relationship between *P. ambiguus* worm burden and rabbit weight ($P = 0.002$) with worm burdens being highest in the 1000 g to 1499 g weight cohort. *Trichostrongylus retortaeformis* was only identified in 1994 and male rabbits harboured significantly higher worm burdens than females (48 vs. 7, $P = 0.022$). Over the five years, the average *Taenia pisiformis* prevalence was 31% and there was a significant positive association between worm burden and rabbit weight ($P = 0.001$). *Cittotaenia pectinata* had a prevalence of 37% over the whole study period with no interactions between prevalence or intensity and body weight, year of sampling or rabbit sex. All five helminths showed an overdispersed distribution with k values less than 1.

Introduction

There has been relatively little published data on the helminth parasites of rabbits (*Oryctolagus cuniculus*) in the British Isles in the last thirty years, the period after the arrival of myxomatosis which decimated rabbit populations in the 1950s. Some work has been carried out in north east England (Boag, 1972), Scotland (Boag, 1985; Boag & Iason, 1986) and a national survey of anoplocephaline cestodes has been made (Mead-Briggs & Page, 1975). More recently, a small survey of rabbit parasites was also made in south-west Ireland (Butler, 1994).

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These studies have indicated that *Cittotaenia pectinata* is the most abundant of the anoplocephaline cestodes in rabbits in the British Isles, infecting 25% of those examined by Mead-Briggs & Page (1975). *Graphidium strigosum*, the stomach worm, appears to be one of the most common nematode parasites in rabbits in the UK. It has also been shown, however, that the helminth parasites of rabbits, such as *Passalurus ambiguus* and *C. pectinata,* have a discontinuous distribution, being completely absent from some areas whilst being present in others (Boag, 1972). Some species, such as *G. strigosum*, are suggested to be affected by desiccation of their sensitive L3 stage, thus affecting their regional distribution. In contrast, *P. ambiguus* is a pinworm of the large intestine with potential for direct transmission.

The current study was carried out in North Yorkshire in north west England over a five year period. No previous studies had been undertaken in this area, which has a large rabbit population.

Materials and methods

Rabbits were obtained from a lightly wooded and limestone moorland area adjacent to Malham Tarn (02°10′W, 54°07′N, 380 m) as part of an undergraduate field course on animal parasitology held annually in late September or early October from 1992 to 1996. All the rabbits had been killed with an air rifle.

Rabbits were weighed with a spring balance and sexed. The abdominal cavity was opened and the contents removed. The mesenteries, liver, pelvic area and body cavity were checked for the presence of parasites, particularly *Taenia pisiformis*. The stomach contents were examined dry (without saline) in a large glass petri dish. The small and large intestines were initially examined directly in sections and their contents washed with normal saline into large petri dishes. Total worm or cyst counts were made for each rabbit. Helminths were identified using previously published descriptions (Soulsby, 1983).

Rainfall data was collected from the Malham Tarn Field Centre's weather station. Total rainfall in mm was calculated for each month. Such records were available from 1949 up to and including the period of the study.

Data summary and analysis was carried out using SAS 6.12 (SAS Institute, Cary, North Carolina, USA). Prevalence and mean worm burden for each species were calculated for each year and sex. The mean number of species per rabbit was also calculated for each year and sex.

Prevalence of each species was analysed using a logistic model with year, sex, year × sex interaction and weight as independent variables. *Trichostrongylus retortaeformis* was only found in 1994, and so only sex and weight was fitted to this data. For *G. strigosum*, the full model could not be fitted and so the year × sex interaction term was dropped.

Worm burdens were analysed using a generalized linear model with an over-dispersed Poisson distribution. Additionally, a quadratic weight term was fitted to test for non-linearity of the relationship between worm burden and weight, but not included in the final model if it was not statistically significant. *Trichostrongylus retortaeformis* was only found in 1994, and so only sex and weight was fitted to this data.

The parameter k, a measure of parasite distribution based on a negative binomial distribution, was calculated for each species using the mean worm burden, μ, and the over-dispersion parameter, ϕ, of a Poisson distribution. This relationship is: $k = \mu/(\phi\text{-}1)$.

Number of helminth species per rabbit was analysed using a generalized linear model with a Poisson distribution with year, sex, year × sex interaction and weight as independent variables.

Results

Over the five year study period, a total of 95 rabbits were examined (66 females and 29 males) with body weights ranging from 700 to 2020 g. Two cestode species and three nematode species were recovered and 95% (90/95) of the rabbits harboured at least one species of helminth parasite. Unadjusted data for these species are summarized in tables 1 and 2.

Analysis of species richness indicated that over the whole study period five rabbits were uninfected, 33 rabbits harboured one species, 38 harboured two species, 17 harboured three species and two harboured four species (table 3). There were no effects on parasite species richness of sampling year, host sex, weight or year × sex interactions.

Table 1. Nematode species: crude unadjusted data for prevalence, mean worm burden, variance and range for each year and host sex.

	Number of rabbits	*Passalurus ambiguus*				*Graphidium strigosum*				*Trichostrongylus retortaeformis*			
		Prevalence (%)	Mean worm burden	Variance	Range	Prevalence (%)	Mean worm burden	Variance	Range	Prevalence (%)	Mean worm burden	Variance	Range
1992	14	43	257	179403	0–1000	93	108	33559	0–500	0	–	–	0
1993	21	29	114	216111	0–2136	95	95	20369	0–660	0	–	–	0
1994	28	14	33	28903	0–900	82	46	28903	0–1000	25	17	3610	0–300
1995	11	73	408	279052	0–1500	55	27	279052	0–263	0	–	–	0
1996	21	19	97	72846	0–1085	48	17	72846	0–130	0	–	–	0
Female	66	36	182	177151	0–2136	76	62	26457	0–1000	19*	7*	682*	0–120
Male	29	14	49	41698	0–1085	76	47	9504	0–500	43*	47*	12526*	0–300
Total	95	30	141	138744	0–2136	76	57	21175	0–1000	7	5	1026	0–300*

* Based on 1994 data only.

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Table 2. Cestode species: crude unadjusted data for prevalence, mean worm burden, variance and range for each year and host sex.

	Number of rabbits	*Taenia pisiformis*				*Cittotaenia pectinata*			
		Prevalence (%)	Mean worm burden	Variance	Range	Prevalence (%)	Mean worm burden	Variance	Range
1992	14	36	1.1	3.5	0–5	50	4.9	105	0–33
1993	21	14	0.3	0.6	0–3	19	0.2	0.2	0–1
1994	28	29	0.7	3.7	0–10	32	1.1	5.8	0–3
1995	11	46	1.0	1.8	0–4	36	2.2	13	0–10
1996	21	29	0.6	1.5	0–5	48	1.4	4.8	0–7
Female	66	26	0.6	2.5	0–10	35	1.8	27	0–33
Male	29	36	0.8	1.9	0–5	38	1.3	6.5	0–11
Total	95	28	0.7	2.3	0–10	36	1.6	20	0–33

Table 3. Frequency distribution of rabbits according to the number of helminth species harboured.

	Number of species				
	0	1	2	3	4
Year	n	n	n	n	n
1992	0	4	4	5	1
1993	0	12	6	3	0
1994	0	1	13	5	0
1995	0	3	5	2	1
1996	5	4	10	2	0
Total	5	33	38	17	2

n = Number of rabbits examined.

Subsequent data reported here use the results of the logistic model for prevalence and the over-dispersed Poisson model for analysis of worm burden. These data are shown in table 4 with the respective *P* values in tables 5 and 6.

Passalurus ambiguus

Twenty eight percent of rabbits were found to be infected with this pinworm which occurs in the colon and caecum. The mean intensity of infection was higher in females (128) compared with males (28) ($P = 0.018$). Worm burden was also significantly affected by the year of study ($P = 0.031$) but there was no interaction between year and

Table 4. Estimated prevalence and mean worm burden for each helminth species using a logistic model for prevalence and an over-dispersed Poisson model for worm burden.

	Number of rabbits	*Passalurus ambiguus*		*Graphidium strigosum*		*Trichostrongylus retortaeformis*		*Taenia pisiformis*		*Cittotaenia pectinata*	
		Prevalence (%)	Mean worm burden	Prevalence (%)	Mean worm burden	Prevalence (%)	Mean worm burden	Prevalence (%)	Mean worm burden	Prevalence (%)	Mean worm burden
1992	14	27	86	93	93	0	0	47	1.3	44	3.7
1993	21	30	79	94	18	0	0	11	0.2	19	0.2
1994	28	12	16	87	24	29	27	31	0.6	40	1.6
1995	11	72	237	57	16	0	0	47	0.9	36	2.1
1996	21	19	76	47	5	0	0	28	0.5	48	1.4
Female	66	40	128	77	32	17*	7*	26	0.6	37	2.0
Male	29	12	28	79	35	41*	48*	37	0.7	38	1.4
Total	95	28	83	78	33	29*	27*	31	0.6	37	1.8

* Based on 1994 data only.

Table 5. *P*-values from a logistic model for prevalence.

	P-values			
	Effect			
Helminth species	Year	Sex	Year × host sex	Host weight
Passalurus ambiguus	0.084	0.084	0.266	0.179
Graphidium strigosum	0.001	0.768	–	0.016
Trichostrongylus retortaeformis	–	0.204	–	0.496
Taenia pisiformis	0.087	0.223	0.291	0.117
Cittotaenia pectinata	0.365	0.954	0.319	0.772

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Table 6. *P*-values, overall mean and parameter estimates from an over-dispersed Poisson model for worm burden.

Helminth species	*P*-values: Effect: Year	Host sex	Year × host sex	Host weight: Linear	Host weight: Quadratic	Overall adjusted mean	Parameter estimates: Over-dispersion (ϕ)	estimated k-value
Passalurus ambiguus	0.031	0.018	0.074	0.002	0.002	83.3	400.0	0.21
Graphidium strigosum	0.001	0.290	0.036	0.001	–	33.0	85.0	0.39
Trichostrongylus retortaeformis	–	0.022	–	0.932	–	27.3	70.6	0.39
Taenia pisiformis	0.014	0.536	0.054	0.002	–	0.6	1.74	0.81
Cittotaenia pectinata	0.078	0.835	0.056	0.638	–	1.8	4.38	0.53

sex. There was a significant non-linear relationship between worm burden and weight (table 6).

The unadjusted mean number of worms per 500 g weight cohort was highest in the 1000 to 1499 g weight cohort (sub-adult to young adult). Rabbits above or below this weight cohort were found to harbour fewer *Passalurus* worms (fig. 1).

Graphidium strigosum

Seventy eight percent of rabbits harboured *G. strigosum*, almost always exclusively in the stomach. The mean prevalence of this parasite declined over the study period. Additionally the mean intensity of *G. strigosum* was significantly affected by the year of sampling being highest in first year, 1992 (93) and lowest in the final year, 1996 (5). This represented a significant effect of year ($P = 0.001$). Worm burden also showed a positively linear association with rabbit body weight ($P = 0.001$). There were no effects of host sex on either helminth prevalence or intensity although there was a significant interaction between year and host sex ($P = 0.036$).

Trichostrongylus retortaeformis

This species was only recovered from the upper small intestine of rabbits sampled in 1994, in that year 29% of the rabbits examined were infected. The mean worm burden was significantly higher in male rabbits (48) compared with female rabbits (7) ($P = 0.022$).

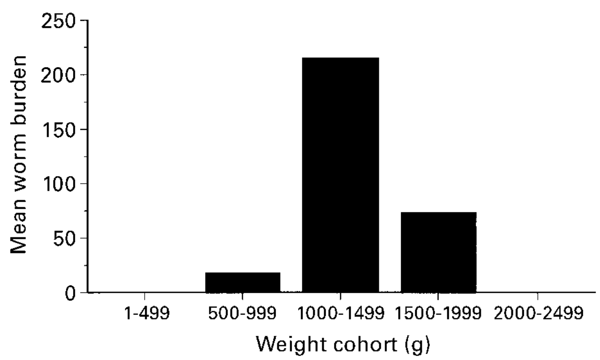

Fig. 1. Mean worm burden of *Passalurus ambiguus* by weight cohort. There were no animals below 500 g in weight, ten animals in the 500 g to 999 g weight cohort, 50 animals in the 1000 g to 1499 g weight cohort, 34 in the 1500 g to 1999 g weight cohort and one, uninfected, animal above 2000 g.

Taenia pisiformis

Thirty one percent of rabbits were infected with this species. Metacestodes were located either attached to the liver, the mesenteries of the alimentary canal, the abdominal wall or within the pelvic cavity. The intensity of infection was positively associated with rabbit body weight ($P = 0.002$). The worm burden also varied significantly with year ($P = 0.014$) but there was no significant association between helminth prevalence or intensity and host sex.

Cittotaenia pectinata

Thirty seven percent of rabbits were infected with this species which occurs in the small intestine. There were no differences between frequency or intensity of infection between males and females nor were there any interactions between worm burden and host weight or year of study.

Helminth distribution

The distribution of the worm burdens of all five rabbit helminth species were found to be overdispersed. All five species had an estimated k value of less than 1 (table 6).

Discussion

Rabbits are relatively large, wild, herbivorous mammals and, in the UK, act as hosts for a number of helminth parasites transmitted either directly or through predator–prey relationships. Not unexpectedly, trichostrongylid nematode and anoplocephalid cestode species such as *Graphidium* and *Cittotaenia* respectively occur in the alimentary canal, while the larval taeniid cestode *T. pisiformis* may occur in tissue or organs. Only a few studies have sought to investigate helminth parasites of *O. cuniculus* in the British Isles and none have studied the large population of rabbits at Malham Tarn in North Yorkshire. The woods and limestone moorland surrounding the Tarn (the only upland marl in England) are subject to cold and wet weather with a mean annual temperature of 8°C and a mean annual rainfall of 1455 mm

With *T. pisiformis*, whilst some previous studies recorded prevalences of up to 77.7% (Evans, 1940b),

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more recent studies have either not found the species or recorded prevalences below 5% (Boag, 1972, 1985; Butler, 1994). The current study estimated 31% of rabbits to be infected. The presence of *T. pisiformis* in any particular area probably depends on the numbers of red foxes (*Vulpes vulpes*) and perhaps dogs preying on rabbits. There was a positive association between rabbit body weight and worm burden, a pattern of age prevalence and intensity of infection common for larval taeniid cestodes (Gemmell *et al*., 1987). Exposure to oncospheres of taeniid cestodes is known to elicit a protective immune response in the intermediate host which acts as a density dependent regulatory mechanism on the parasite populations (Gemmell *et al*., 1987). Determination of host age intensity and age prevalence data for this parasite would allow its population status (i.e. endemic, hyperendemic) to be determined (Gemmell *et al*., 1987).

Boag (1985) suggested that the stomach worm *G. strigosum* may be more common in the south and west of the British Isles due to the wetter environment improving the survival of the L3 stage. Data from the north east of England and south east of Scotland indicated that *Graphidium* was either absent or present at less than 40% prevalence (Boag, 1972, 1985). The present data (78% average prevalence) together with data from Wales (Evans, 1940a) and more recent data from Ireland (Butler, 1994), where the prevalence was 60% or higher, tend to support the notion of greater prevalence in wetter regions. In the current study, there were significant interactions between year of sampling and worm burden and between sampling year and prevalence. Fluctuations in the prevalence and intensity of *G. strigosum* over the five year study period may also be indicative of this rainfall effect. The mean annual rainfall for Malham Tarn was 1508 mm in 1992 and 1474 mm in 1993 with lower rainfall values being recorded in 1995 and 1996, at 1154 and 1215 mm, respectively. The worm burden of *G. strigosum* was also positively correlated with host weight suggesting that if weight can be used as a corollary of age, older animals harbour significantly higher burdens of *G. strigosum*.

Other studies have indicated *T. retortaeformis* to be the most prevalent species of nematode in British rabbits often reaching a value above 80% (Boag, 1972, 1985; Boag & Iason, 1986). In the current study, however, *T. retortaeformis* was only present in one (1994) of the five sampling years with a comparatively low prevalence of 29%. Immune mechanisms are known to regulate the populations of this nematode in rabbits (Michel, 1952, 1953) and it appears to be more abundant in rabbits affected by myxomatosis (Boag, 1985, 1988) perhaps due to the impact of this disease on the immune status of the host. In the current study the condition of the rabbits with respect to myxomatosis was not recorded, but this may be useful in future.

The pinworm *P. ambiguus* was found to infect female rabbits at higher intensities than in male rabbits ($P = 0.031$). This host sex difference had not been recorded in previous studies. The distribution of *P. ambiguus* by host weight, where worm burdens first rose and then fell, was similar to previous findings (Evans, 1940a). More recent studies have shown that *Passalurus* numbers increase with host age (Boag, 1985) but the weight cohorts used in that study, where the upper cohort started at 1250 g, may have masked the distribution shown here where animals with weights above 1500 g actually showed some decline in worm burdens. A decline in pinworm burdens in heavier rabbits may be associated with age effects or with acquired resistance, a pattern seen with other oxyurid species (Soulsby, 1983).

The only species of intestinal tapeworm recovered in the current study was *C. pectinata*. A national survey of intestinal tapeworms of rabbits in the UK has indicated that this was the most common species and the average prevalence in the current study (37%) was similar to those reported previously (Mead-Briggs & Page, 1975). *Cittotaenia* is transmitted by oribatid mites that are present in high densities in the topsoil and herbage. However, the drier years of 1995 and 1996 appear not to have affected transmission between the arthropod and rabbit hosts.

Worm burdens often follow a negative binomial distribution. While such a distribution can be fitted to data from a single population, it can be difficult to fit models directly to examine explanatory factors (Shaw *et al*., 1998). However, the negative binomial distribution can be considered as an over-dispersed Poisson distribution (McCullagh & Nelder, 1989), and models using this distribution can be fitted with standard software for generalized linear models. The parameter, k, for a negative binomial is related to the mean, μ, and the over-dispersion parameter, ϕ, of a Poisson distribution. This relationship is: $k = \mu/(\phi\text{-}1)$. It is known, however, that estimating k from this relationship, which is equivalent to estimating k from the mean and variance, may not be efficient (Bliss & Fisher, 1953).

A recent survey of data from 49 published wildlife host–macroparasite systems indicated that 43 of the data sets had an estimated k value (k is an inverse measure of aggregation) of less than 1 (Shaw *et al*., 1998). The data from the current study was consistent with this with all five rabbit helminths having an estimated k value of less than 1.

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