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### Parasites of European hedgehogs (*Erinaceus europaeus*) in Britain: epidemiological study and coprological test evaluation

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Abstract Seventy-four European hedgehogs (Erinaceus europaeus) that had died in wildlife rehabilitation centres were dissected and their parasite burdens documented. Overall parasite prevalence was 91%, and a total of six helminth species were isolated: five nematodes (Crenosoma striatum, Eucoleus aerophilus, Capillaria erinacei, Capillaria ovoreticulata and Capillaria spp.), one trematode (Brachvlaemus erinacei) and one acanthocephalan (Oliganthorhynchus erinacei). The tick Ixodes hexagonus and flea Archeopsylla erinacei were also collected. The effect of parasite infection on body condition was assessed by correlation of burdens with the residuals of weight-skeletal length regression. Tick presence was positively related to body condition; for other parasites, no significant relationship was found. Faecal egg or larval count was closely correlated with adult parasite burden for C. striatum and Capillaria/Eucoleus spp., but not for other species. Coprological analysis should therefore be useful for in vivo studies of nematode parasite infection in hedgehogs. The epidemiology of parasites in hedgehogs and their possible role in recent population declines are discussed.

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S. Allen Gower Bird Hospital, Sandy Lane, Parkmill, Swansea SA3 2EW, UK **Keywords** Hedgehog · Helminth · Ectoparasite · Epidemiology · Coprological diagnosis

#### Introduction

Hedgehogs (Erinaceus europaeus) are one of the most distinctive and familiar groups of mammals and are widely distributed throughout the Old World (Reeve 1994). In Great Britain, the hedgehog is found almost everywhere, but tends to be scarce or absent from wet areas and extensive pine forests (Morris 1987). In recent years, the number of hedgehogs in Great Britain appears to have markedly declined (The Mammal Society 1999). The cause of this decline is not known. Reeve and Huijser (1999) reported that just over 40% of hedgehog mortalities were thought to be caused by road traffic accidents, garden and pet injuries and predation, with 59% of deaths resulting from 'natural cases' such as disease. Hedgehogs are the most common species of mammal admitted to wildlife hospitals in the UK (Bullen 2002; Molony 2006). As nonterritorial nocturnal species with a flexible diet consisting of a wide range of invertebrates, as well as small vertebrates and carrion (Reeve 1994), rehabilitated hedgehogs are sometimes released at novel locations under the assumption that they are readily capable of adapting to their new environs. For this reason, Molony et al. (2006) utilised hedgehogs as a focal species for translocation studies. Their results suggest that direct translocation had a damaging effect on survival, but a short period in captivity prior to release greatly improved the chance of survival. These beneficial effects of a period of retention in captivity were also evident for those individuals that had been rehabilitated following injury or illness.

Given the recent and possibly ongoing population declines, rehabilitation of sick or injured hedgehogs could

have conservation as well as welfare value. Anecdotal evidence from rehabilitation centres suggests that parasite infection, especially lungworm, is an important cause of illness in hedgehogs. Parasites are also known to have significant effects on the population dynamics of their hosts in other systems (Irvine 2006), and increased levels of infection could contribute to population declines. Unfortunately, clinical and ecological studies on parasites of hedgehogs are held back by a lack of validated tests for infection in the living animal.

The present study aims to record the parasite species present in British hedgehogs, along with typical levels of infection in animals admitted to rehabilitation centres, and to assess impacts on individual fitness through decreased body condition. Coprological test results are compared with burdens of adult parasites in order to assess their value as indicators of infection status.

#### Materials and methods

#### Study design and sampling method

Between November 2008 and July 2009, 74 hedgehogs (42 males and 32 females) were examined for parasites. The animals had died of various causes between December 2006 and September 2008 in two wildlife rehabilitation centres in Great Britain, one in south Wales and the other in the east of England. Hedgehogs that had spent more than 1 week in the rehabilitation centre before death were excluded in order to reduce bias arising from weight loss during prolonged captivity. Carcasses were frozen for storage and transport. After defrosting overnight and before dissection, weight and overall body length were noted, as well as the distance between the carpus and the end of the distal phalanx of the second digit of the left forelimb (=manus length).

#### Adult parasite counts

The stomach and intestines of hedgehogs were removed, and parasites were extracted using the total worm count technique (Euzeby 1981). Briefly, the intestines were separated from the mesentery and expressed before being incised along their length, and the mucosa was rinsed in water with firm digital pressure. The stomach was similarly, but separately, processed. The heart and lungs were removed, the aorta clamped using forceps and the lungs flushed using a technique modified from foxes (Morgan et al. 2008): Tap water was forced into each lobe of the lung using a 10-ml syringe with a 24-gauge needle to disrupt the pulmonary tissue and dislodge any nematodes. The fluid was collected as it flowed out through the trachea. Blood clots and mucus that blocked the trachea were removed using a pipette and deposited with the rest of the lung flush to be examined for nematodes. Extracts from guts and lungs were inspected under the dissection microscope and parasites counted, removed and placed in lactophenol on a standard microscope slide for identification. The integument and the bag in which hedgehogs were frozen were inspected for ectoparasites, which were removed for counting and identification. Parasites were identified to species level according to Yamaguti (1958, 1963), Skrjabin et al. (1970), Hillyard (1996) and Whitaker (2007).

#### Faecal egg/larva counts

Two grammes of faeces was taken from the rectum during dissections. Faecal egg and larval density were estimated using a modified McMaster technique (MAFF 1986), with a sensitivity of 50 eggs/larvae per gramme, using a salt and sucrose flotation solution (specific gravity, 1.280). Where <2 g of faeces was available, dilution factors were modified accordingly.

#### Statistical analysis

Descriptive statistics, including the prevalence (P), the mean intensity  $(I_m)$  and the mean abundance  $(A_m)$ , were calculated for each parasite species or genus recovered, as proposed by Bush et al. (1997). Abundance was compared between sexes and seasons (summer/winter) using Student's t test. The relationships between different parasite species, between parasite burden and body condition (as estimated by the residual of mass/manus length regression) and between adult parasite burden and faecal egg or larval count were compared using Pearson correlation, with Bonferroni correction for multiple testing. In each case, overdispersion in parasite burden was dealt with by log(x+1) transformation. After checking for normal distribution of residuals, linear regression was used to predict the number of adult parasites present from the density of eggs or larvae in the faeces and body mass from manus length. The sensitivity of coprological tests was estimated as the number of infected animals identified coprologically as a proportion of those found to carry parasites on postmortem examination.

#### Results

#### Adult parasites

Helminth parasites were present in 67 out of 74 hedgehogs examined (91%). A total of four species of nematode (*Crenosoma striatum*, *Eucoleus aerophilus* syn. *Capillaria aerophila*, *Capillaria erinacei*, *Capillaria ovoreticulata*,

	Lungs		Stomach	Intestine		Intestine and	Skin	
	Crenosoma striatum	Eucoleus aerophilus	Capillaria spp.	Capillaria spp.	Brachylaemus erinacei	gut serosa Oliganthorhynchus erinacei	Ixodes hexagonus	Archeopsylla erinacei
Р	71	32	66	61	55	18	59	8
Im (±SD)	71 (±153)	9 (±15)	110 (±135)	92 (±126)	85 (±179)	3.5 (±2.8)	24 (±57)	11 (±13)
$A_{\rm m}$ (±SD)	50 (±132)	3 (±9)	73 (±122)	56 (±108)	47 (±140)	0.6 (±1.7)	14 (±45)	0.9 (±1.7)
R	1–961	1–56	3-591	2-605	1–991	1–11	1-283	2-33

Capillaria spp. consisted of C. erinacei and C. ovoreticulata as well as females identified to genus only

P percent prevalence,  $I_m$  mean intensity,  $A_m$  mean abundance, SD standard deviation, R range

plus *Capillaria* sp. females), one trematode (*Brachylaemus* erinacei) and one acanthocephalan (*Oliganthorhynchus* erinacei syn. Echinorhynchus erinacei) were detected. The ectoparasites *Ixodes hexagonus* and Archeopsylla erinacei were also found. A summary of levels of infection is presented in Table 1. The most common parasite was *C. striatum* in the lungs, and the most abundant was *Capillaria* spp. in the stomach and small intestine. Gut flukes (*Brachylaemus*), *Eucoleus* in the lungs and ticks (*I. hexagonus*) were also common, with some high individual burdens, whilst acanthocephalans and fleas were less common.

#### Correlations between parasite species

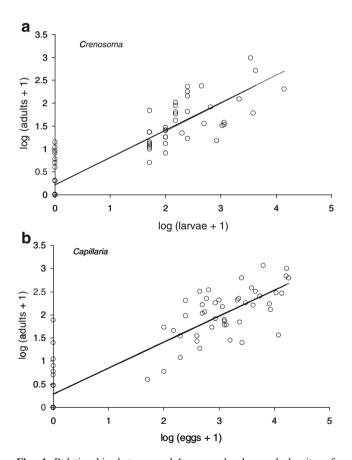
Using serial bivariate correlation, positive relationships were found between burdens of *C. striatum* in the lung and *Capillaria* spp. in the stomach (r=0.497, p<0.001) and intestine (r=0.646, p<0.001), *Capillaria* spp. in the stomach and intestine (r=0.809, p<0.001) and *B. erinacei* and *O. erinacei* (r=0.473, p<0.001).

#### Coprological test results

Parasite eggs or larvae were found in 51 of 74 (69%) hedgehogs. *Capillaria* sp. eggs (including *E. aerophilus*, which are morphologically similar) were found in 46 (62%), *Crenosoma* larvae in 38 (51%) and trematode eggs in 10 (13%). Test sensitivity, as defined above, was 78% for *Crenosoma*, 89% for *Capillaria* and 24% for *Brachylaemus*, with no false positives detected for any species. The relationship between adult worm burden and faecal egg/ larval count is shown for *Crenosoma* and *Capillaria* in Fig. 1. The density of trematode and acanthocephalan eggs correlated poorly with corresponding adult burdens.

#### Variation with sex, season and body condition

There were no significant differences in the abundance of any parasite species between sexes or seasons. The regression of body weight and manus length used to estimate body condition is shown in Fig. 2. Tick abundance was positively related to body condition (r=0.417, p<0.001).



**Fig. 1** Relationship between adult worm burden and density of corresponding immature parasite stages in the faeces for **a** *C*. *striatum* larvae: regression equation log (adults+1) =  $0.231+0.578 \times \log(\text{larvae}+1)$ ). Pearson correlation coefficient 0.895, analysis of variance (ANOVA),  $F_{1, 72} = 289.18$ , p < 0.001. **b** *Capillaria* spp. eggs, including *E*. *aerophilus*: regression equation log(adults+1) =  $0.293+0.560 \times \log(\text{eggs}+1)$ ). Pearson correlation coefficient 0.897, ANOVA,  $F_{1, 72} = 295.61$ , p < 0.001. Egg and larval density are treated as predictors of adult burden

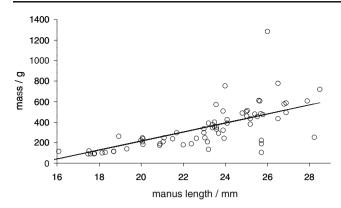


Fig. 2 Regression of body mass (g) against manus length (mm). Residuals were used as an index of body condition. Pearson correlation coefficient = 0.704; regression equation: Mass =  $-662+43.9 \times \text{manus length}$ . ANOVA,  $F_{1, 71} = 69.65$ , p < 0.001

#### Discussion

In Europe, epidemiological studies on the parasite fauna of hedgehogs have been conducted in Germany (Laux 1987; Wieland 2007), Norway (Keymer et al. 1991) and Italy (Poglayen et al. 2003). Studies on British hedgehog parasites focused particularly on parasitic lung disease (Majeed and Cooper 1984; Majeed et al. 1989; Robinson and Routh 1999; Cousquer 2004; Molenaar 2008, unpublished data), and few epidemiological studies of hedgehog parasites have been undertaken in Great Britain. Boag and Fowler (1988) found prevalence of 77% for C. striatum, 85% for C. erinacei, 8% for Capillaria spp. and 8% for Vampirolepis erinacei. Bunnell (2001) found I. hexagonus (14%), C. striatum (11%) and sarcoptic mange (6%). Reeve (1994) and Bexton and Robinson (2003) wrote about clinical signs during lung and intestinal Capillaria, trematode, acanthocephalan and ectoparasite infections. Severe infections of intestinal parasites can cause lethargy, weight loss and diarrhoea, and lung parasites can cause lethargy and dyspnoea (Bexton and Robinson 2003), whilst ectoparasites, especially ticks, can induce anaemia (Pfäffle et al. 2009). Sainsbury et al. (1996) considered parasitism to be involved in mortality of rehabilitated hedgehogs after release. Our results confirm that hedgehogs are commonly parasitised and can carry high burdens particularly of lungworms (especially C. striatum), gastrointestinal Capillaria spp., gut flukes (B. erinacei) and ticks (I. hexagonus). Flea burdens are likely to be underestimated in this study since fleas leaving the host after death and before storage of the carcase were not counted.

The high levels of parasitism found in hedgehogs, together with apparent increases in the abundance of some parasites in other wildlife species but relevant to hedgehogs, such as ticks (Scharlemannn et al. 2008) and slug-transmitted lungworms (Morgan et al. 2008, 2009), make

increased parasitism a plausible factor in observed population declines. However, the effect of parasites on hedgehogs at individual and population levels is difficult to ascertain. The results reported here are from animals admitted to rehabilitation centres and might not be representative of the general population, but could nevertheless be more sensitive indicators of negative effects of parasitism on individual fitness. The present study, however, failed to find negative correlations between body condition, as estimated by residuals of skeletal length/mass regression, and the abundance of any parasite species or total parasite burden. At face value, this might suggest that parasites at these levels of infection do not have negative effects on hedgehog body condition or fitness. However, such crosssectional correlations are notoriously insensitive indicators of effects on host fitness, which could be subtle, and restricted to certain times of year or population subclasses (Irvine 2006). Moreover, antagonistic relationships between parasitism and resource acquisition could obscure observed effects. For example, many parasite species are acquired from food, so well-fed individuals might accumulate higher parasite burdens. This is compounded by food choice, e.g. between gastropod molluscs (intermediate hosts of Crenosoma and Brachylaemus) and insects/earthworms, which itself is likely to be influenced by age (Dickman 1988) as well as by nutritional state and habitat use (Jones et al. 2005). Gastropods form a small percentage of hedgehog diets, and marginalised animals with poor prey choice might be more likely to ingest these, and to acquire infection, as well as being more likely to present at rehabilitation centres. Similarly, the positive correlation observed in the present study between body condition and tick burden could reflect greater range size or habitat quality in fitter animals. Habitat type and vegetation cover are well known to influence tick abundance (Medlock et al. 2008), and ticks have been used as markers of habitat use in hedgehogs (Thamm et al. 2010). It is also possible that feeding whilst in rehabilitation enabled parasitised animals to gain weight, obscuring negative relationships between burden and body condition. Positive correlations between burdens of different parasite species, as found here amongst helminths, could indicate co-transmission through diet or habitat use or synergistic interaction in the host, for example via immunosuppression.

The use of mass-length regression as an indicator of body condition in hedgehogs could be a useful tool to detect effects of parasites on fitness, especially in living animals. However, this approach is not without problems. Although residuals were normally distributed, smaller hedgehogs tended to be in poorer condition (Fig. 2), and there is likely to be confounding of this statistic with age. Since parasite accumulation is age dependent, it is possible that older individuals are generally not only in generally better body condition but also more heavily infected with parasites. If this is the case, even strong negative effects of parasitism on body condition could be obscured. This would further be compounded by the tendency to underestimate parasite burdens in older age classes of free-living animals (Pacala and Dobson 1988). Unfortunately, few helpful, validated measures of age are available in hedgehogs. Dentition (Delecourt and Herve 1981) and skeletal maturity (Morris 1971) can be useful early in life, but not to reliably distinguish the ages of mature hedgehogs, whilst eye lens weight (Augusteyn 2007; Gacic et al. 2007; Janova et al. 2007) has yet to be validated for this species. Better methods for age determination in hedgehogs are needed if the effects of parasites on individual fitness and population viability are to be determined.

Observation of parasite eggs or larvae in the faeces was found to be a useful indication of infection and, for C. striatum and combined Capillaria and Eucoleus species, a good reflection of adult parasite burden. This suggests that there is little density dependence in egg output, e.g. mediated by acquired immunity. Moreover, the strong correlation between egg or larval density and adult burden validates the use of coproscopy as a measure of parasitism in living animals. This opens the way for more detailed studies of parasite dynamics, especially longitudinal studies in free-living animals, as well as the effects of these parasites and their removal on the clinical performance of animals in rehabilitation. Such studies are needed if the value of antiparasitic treatment in captivity is to be evaluated and will form an important part of ongoing investigations into the possible role of parasites and other diseases in population decline.

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