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## Changes in parasite transmission stage excretion after pheasant release

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### Abstract

The production of parasite transmission stages was investigated in the faeces of 77 farm-bred ring-necked pheasants (*Phasianus colchicus*). Coccidian oocysts (*Eimeria* sp.), and nematode eggs (*Heterakis* sp., and *Capillaria*-like eggs) were recovered before and after release but all birds were treated prior to release. Treatment with fenbendazole significantly reduced the abundance of transmission-stage excretion for all parasites, and reduced the prevalence in the case of *Eimeria* sp. and *Heterakis* sp. Nonetheless, a significant increase in the excretion abundance for all parasites and in the prevalence of *Eimeria* sp. and *Heterakis* sp. was found after release. Eggs of *Ascaridia* sp. were found only after releasing, suggesting infection occurred in the wild. A negative relationship was found between the pheasant body condition and *Heterakis* excretion abundance and a higher abundance of *Capillaria* sp. eggs in female birds. No significant relationship was found between parasite excretion abundance and pheasant survival. Despite this, results suggest that an increase in the excretion of parasite transmission stages follows the release of captive pheasants into the wild. This can in part explain restocking failures, but also means that autochthonous free-living birds may become exposed to new and potentially harmful pathogens. To avoid these risks it is proposed that improved prophylactic measures should be taken.

### Introduction

Survival of farm-reared gamebirds after their release into the wild is known to be greatly reduced (Robertson, 1988; Sodeikat *et al.*, 1995; Gortázar *et al.*, 2000; Millán *et al.*, 2002, 2003). Dowell (1992) reviewed the causes of mortality and these included behavioural problems (Csermely *et al.*, 1983), diseases (Temple, 1987; Hoodless *et al.*, 2003) and the vulnerability of game birds when highly aggregated (Robertson, 1988; Gortázar *et al.*, 2000). Any of these conditions make game birds highly vulnerable to predation and favour predation by the red

fox *Vulpes vulpes* (Papeschi & Petrini, 1993; Gortázar *et al.*, 2000).

The effect of parasites on the survival of gamebirds in general (Hudson *et al.*, 1992) and released gamebirds in particular (Millán *et al.*, 2002) have received some attention recently. Millán *et al.* (2002) observed that farmed ring-necked pheasants (*Phasianus colchicus*) excreting *Eucoleus contortus* eggs showed reduced survival and were more frequently taken by foxes than uninfected pheasants. Also, the release of farmed game animals and the potential parasites or pathogens they may be harbouring can pose a risk for autochthonous populations, as observed by Fernández de Mera *et al.* (2003) in the case of the wild boar *Sus scrofa* and by Millán *et al.* (2004) for the red-legged partridge *Alectoris rufa*. The annual release of farmed pheasants in the United

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Kingdom is believed to be the cause of the maintenance or even the increase of *Heterakis gallinarum* burdens in wild pheasant populations (Draycott *et al.*, 2000), which in turn could be one of several factors involved in the decline and lack of recovery of the grey partridge *Perdix perdix* (Tompkins *et al.*, 2001).

Pheasants are usually subject to routine antiparasitic treatment when reared in captivity. Upon release, supplementation with antiparasitic drugs ceases and stress increases from handling and exposure to an unknown and potentially hostile environment. As parasite burdens of birds have been shown to increase in relation to stressful situations (Moreno *et al.*, 1999; Moller *et al.*, 2003), we postulated that stress coupled with the lack of antiparasitic treatment after release would lead to increased parasite intensity in released birds. This, in turn, would affect the survival of farmed birds and, in addition, increase the production of parasite transmission stages, thus favouring the transmission of the parasites to autochthonous wild species.

The aim of the present study was to test the hypothesis that parasite transmission stages, an indirect measure of parasite burden, would increase after the release of farmed pheasants into the wild.

## Materials and methods

### Study site

Fieldwork was undertaken in the El Portal hunting area (Asturias, northern Spain 5°34'W 43°48'N) between February and November 2003. The climate is humid with warm summers and the average temperature in 2003 was 15.1°C, the average rainfall ranges from 150 to 200 mm, and the total annual rainfall is between 1200 and 1400 mm (<http://www.ine.es>). The study area is covered by a mixed vegetation of eucalyptus (*Eucalyptus globulus*) woodlands, bramble (*Rubus* sp.) and gorse (*Ulex* sp.) scrubs, grasslands, small mixed crops and apple orchard plots.

The red fox is the most abundant predator, but feral cats (*Felis catus*), European wildcats (*F. sylvestris*), stone martens (*Martes foina*), goshawks (*Accipiter gentilis*), and buzzards (*Buteo buteo*) are also present.

### Maintenance and treatment of pheasants

Seventy seven juvenile pheasants (38 males, 39 females) were maintained in large open-air aviaries with natural vegetation and reared in a facility located 25 km from the release area. Each pheasant was measured (tarsus length and pectoral angle) and weighed, ringed and fitted with a necklace radio-transmitter (Biotrack, Dorset, UK) 10 days prior to release.

Prior to release, a faecal sample was obtained from each pheasant and the bird was then treated with 20 mg kg<sup>-1</sup> fenbendazole (Panacur®) orally (Newborn & Foster, 2002; Woodburn *et al.*, 2002). Specific treatment against coccidia was not applied, but from the time of treatment until release, pheasants were maintained in large wire cages (2 × 2 × 1.5 m) off the ground in order to interrupt the coccidial life cycle and minimize exposure to these and other parasites. Pheasant body condition was estimated

using three different measurements: an arbitrary score of the degree of fat deposition; the pectoral angle (a measurement of the width of the pectoral muscles, see Millán *et al.*, 2003); and the residuals from the regression of the body mass on the cube of tarsus length (Andersson, 1992). A second faecal sample was obtained from each bird just prior to its release, 10 days after application of the anthelmintic treatment.

Pheasants were released on 25 February (*n* = 19), 8 April (*n* = 19), 20 April (*n* = 16), 10 October (*n* = 12) and 12 November (*n* = 11) of the year 2003, and released over a wide area to avoid multiple predation by foxes (Robertson & Hill, 1986; Gortázar *et al.*, 2000). Over the subsequent two months, each pheasant was located twice daily using a hand-held antenna and a receiver (Wagener Telemetrieanlagen, Köln, Germany). When a radio-tagged pheasant was found dead, and the cause was thought to be predation, an attempt was made to identify the predator species involved by the presence of faeces, feathers or footprints and by inspecting carcass remains and toothmarks on transmitters or any other relevant signs. It was also noted whether the carcass was buried (Millán *et al.*, 2002). Whenever possible, pheasant carcasses were necropsied and a faecal sample obtained (table 1).

All faecal samples (prior to treatment; 10 days later, prior to release; and when found dead) were analysed for the relative abundance of transmission stages through flotation in a saturated ZnSO<sub>4</sub> solution, counts in MacMaster chambers, and identification of parasite transmission stages according to Melhorn *et al.* (1992).

### Statistical analysis

The relationship between various factors (time of coprological analysis, sex, and body condition) and parasite excretion parameters was evaluated using general linear mixed models (GLIMMIX). Prevalence and abundance variation was tested in two different models for each parasite. A binomial error distribution and a logit link function, in the case of the prevalence model, and Poisson error distribution and a logarithmic link function were included in the abundance model (Wilson & Grenfell, 1997) using a backward stepwise procedure (Crawley, 1993). In both models we controlled for the bird as a random factor. After this analysis, treatment and release effect were analysed independently in other GLIMMIX, using the same random factor, to evaluate the effect of each factor.

Differences in pheasant survival in relation to excretion of parasite transmission stages were tested by Kaplan-Meier logrank tests (Church, 1993). This excretion was categorized into two groups: (i) moderate excretion; and (ii) low excretion or absence of excretion. Moderate excretion was defined as more than 200 oocysts per g of faeces for *Eimeria* sp.; 100 eggs per g of faeces for *Heterakis* sp. (Tompkins *et al.*, 2000); and 26 eggs per g of faeces for *Capillaria*-like eggs (Millán *et al.*, 2002).

A Spearman's rank correlation was used to test for relationships between individual excretion of transmission stages before and after release and for relationships between different parasites after release.

Table 1. Excretion abundance and prevalence general linear mixed models (GLIMMIX) for three parasite species (*Eimeria*, *Heterakis* and *Capillaria*) in pheasants before treatment, before release and after release.

	Excretion abundance			Excretion prevalence		
	P-value	Estimate	% Explained deviance	P-value	Estimate	% Explained deviance
<i>Eimeria</i> sp. oocysts						
Time of coprological analysis						
Before treatment	0.9443	0.0198	79.31	0.5127	-0.5802	61.18
Before release	<.0001	-3.4170		0.0103	-2.3110	
Post-release		0			0	
<i>Heterakis</i> sp. eggs						
Time of coprological analysis						
Before treatment	0.0095	-1.4328		0.8596	0.1310	
Before release	<.0001	-2.7258	53.78	0.0004	-2.6458	31.22
Post-release		0			0	
Body condition	0.0464	-2.19E-6				
<i>Capillaria</i> sp. eggs						
Time of coprological analysis						
Before treatment	<.0001	0.4541		0.65	0.1621	
Before release		0		0.71	-0.3534	11.16
Post-release	0.9999	-28.8895	74.01		0	
Sex						
Female	0.0018	0.5246				
Male		0				

## Results

Before treatment, pheasants were found to excrete coccidian oocysts (*Eimeria* sp.), and nematode eggs (*Heterakis* sp. and *Capillaria*-like eggs).

The abundance models showed that the time of coprological analysis (before treatment, post-treatment and post-release) was significantly associated with the excretion of transmission stages in all parasites (table 1). Two other factors were included in the final abundance models, namely the body condition in the case of *Heterakis* sp. and host sex in the *Capillaria* model (table 1). Pheasants with lower body condition showed the highest *Heterakis* sp. excretion abundances, with the excretion abundance of *Capillaria* sp. being higher in female pheasants. Treatment with fenbendazole and housing in elevated wire cages reduced excretion abundance in oocysts of *Eimeria* sp. ( $F = 125.5$ ,  $P < 0.0001$ ), and eggs of *Heterakis* sp. ( $F = 2103.2$ ,  $P < 0.0001$ ) and *Capillaria* sp. ( $F = 2277.6$ ,  $P < 0.0001$ ) (fig. 1). The excretion increased significantly with release in the case of *Heterakis* sp. ( $F = 19.6$ ,  $P < 0.0005$ ) and *Eimeria* sp. ( $F = 120.7$ ,  $P < 0.0001$ ). In the case of eggs of *Capillaria* sp., a similar trend was found (fig. 1), but this increase did not reach the 95% level ( $F = 0.36$ ,  $P > 0.05$ ).

The prevalence models showed that the time of coprological analysis was significantly associated with excretion of *Eimeria* sp. oocysts and eggs of *Heterakis* sp., but not with the excretion of eggs of *Capillaria* sp. (table 1). Treatment with fenbendazole and housing in elevated wire cages significantly reduced the prevalence of excretion of eggs of *Heterakis* sp. ( $F = 13.1$ ,  $P < 0.005$ ) and oocysts of *Eimeria* sp. ( $F = 17.1$ ,  $P < 0.005$ ) (fig. 1). Excretion of eggs of *Capillaria* sp. followed similar trend in prevalence, but this did not reach the significant level ( $F = 0.36$ ,  $P > 0.05$ ) (fig. 1). Nonetheless, the release

produced an increase in the excretion prevalence of all parasites (fig. 1), which was only statistically significant for *Heterakis* sp. ( $F = 3.9$ ,  $P < 0.05$ ). Values for oocysts of *Eimeria* sp. were  $F = 3.1$ ,  $P = 0.08$ , and for eggs of *Capillaria* sp.  $F = 0.01$ ,  $P > 0.05$ .

There was no correlation between excretion of oocysts of *Eimeria* sp. and eggs of *Heterakis* sp. after release ( $n = 16$ ,  $P > 0.05$ ). Individual pheasants with high excretion levels of oocysts of *Eimeria* sp. or eggs of *Heterakis* sp. before treatment were not the same as those that showed high excretion after release ( $n = 11$  and  $P > 0.05$  in both cases).

Pheasant survival was apparently not related to coccidia oocyst excretion intensity (WW = -45; test statistic = -0.29;  $P > 0.05$ ), nor to *Heterakis* sp. (WW = 34; Test statistic = 0.71;  $P > 0.05$ ) or *Capillaria* sp. egg excretion (WW = 47; Test statistic = 1.38;  $P > 0.05$ ).

When the prevalence of transmission stage excretion in birds killed by foxes was compared with that of pheasants that died due to other causes, neither *Eimeria* sp. coccidia oocyst excretion ( $\text{Chi}^2 = 0.04$ ; 1 d.f.;  $P > 0.05$ ), nor *Heterakis* ( $\text{Chi}^2 = 0.05$ ; 1 d.f.;  $P > 0.05$ ) or *Capillaria* egg excretion ( $\text{Chi}^2 = 0.05$ ; 1 d.f.;  $P > 0.05$ ) appeared to be related to the causes of death.

In addition, eggs of *Ascaridia* sp. were found in the faeces of one of the dead pheasants (0.06%; 179 eggs per g), although this type of transmission stage had not been found in any of the previous analyses, suggesting infection in the wild.

## Discussion

Fenbendazole has proved to be effective in the treatment of nematode infections in captive pheasants

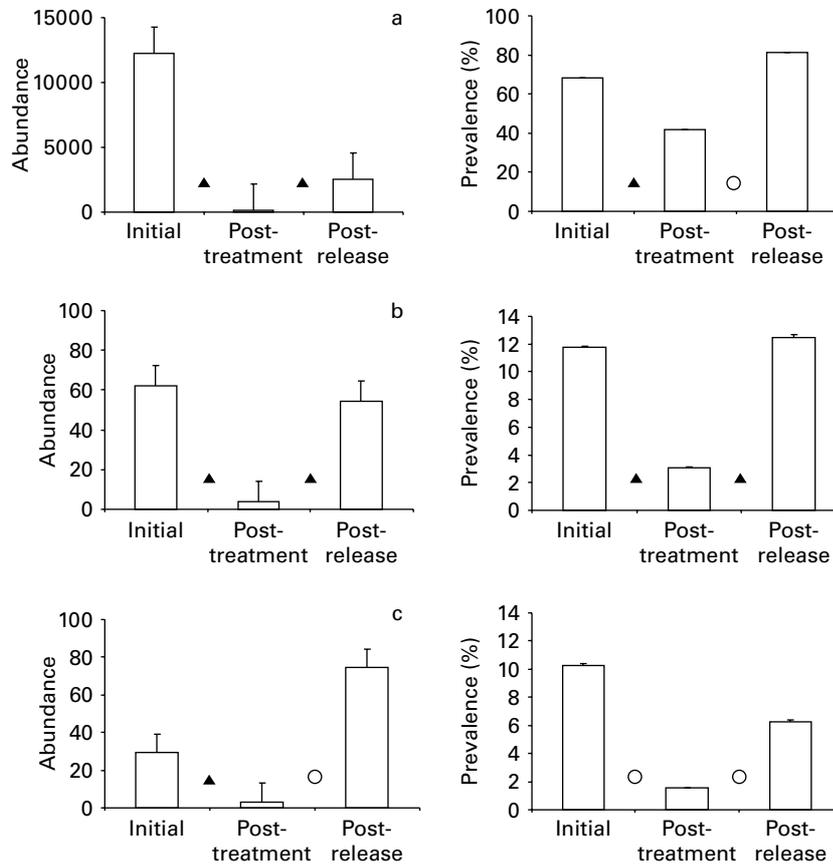


Fig. 1. Changes in the abundance (number of oocysts or eggs per gram) and prevalence (%) of transmission stages excreted in pheasants, with treatment and housing in elevated wire-cages and after release (significance level; ▲,  $P < 0.05$  and ○,  $P > 0.05$ ). (a) Oocysts of *Eimeria* sp.; (b) eggs of *Heterakis* sp.; (c) *Capillaria*-like eggs.

(Kirsch, 1983). However, treatment was applied for four consecutive days and did not prevent re-infection. A compound of the same family, flubendazole, demonstrated its effectiveness in the reduction of *Heterakis gallinarum* burdens in ring-necked pheasants (Tompkins & Hudson, 1999, Woodburn *et al.*, 2002). In the present study, treatment of pheasants with fenbendazole and housing them in elevated cages apparently reduced the abundance of the three parasite species found and also reduced the prevalence of *Heterakis* sp. and *Eimeria* sp.

The fact that both the prevalence and intensity of excretion of oocysts of *Eimeria* sp. decreased after treatment with the anthelmintic is somewhat surprising. Although there is no report of the effectiveness of fenbendazole against coccidia, other benzimidazole antiparasitic drugs have demonstrated effectiveness against other intestinal protozoa (Blanshard *et al.*, 1992; Dieterich *et al.*, 1994; Qing *et al.*, 1996). However, a decrease in the shedding of oocysts may well also be related to the interruption of the coccidian life cycle by housing pheasants in elevated wire cages until release.

The relationship between pheasant body condition and the abundance of *Heterakis* has been reported previously

(Tompkins *et al.*, 2001). High numbers of *H. gallinarum* adults have been found in pheasants in poor condition but, as in the present study, it is not clear whether these infections cause this poor condition or are a response to it (Robertson & Hillgarth, 1994). Tompkins *et al.* (2001) found no effect of *Heterakis gallinarum* on pheasant condition, whereas this nematode did affect body condition in grey partridges. The higher abundance of *Capillaria* in female pheasants is difficult to explain.

In the present study, no apparent relationship between parasite burdens and survival or cause of death of the pheasants was found. This is in contrast to the findings of Millán *et al.* (2002), who found significant differences in pheasant survival depending on the presence of *Eucoleus* eggs. This difference may be explained by the lack of anthelmintic treatment in the study by Millán *et al.* (2002), with far higher excretion values being recorded than in the present case.

An increase in the prevalence and abundance of *Heterakis* and *Eimeria* after release may be the consequence of two main factors, namely the cessation of treatment in the wild on one hand and the release-derived stress on the other. Various authors have shown in

different avian species that immune suppression can be related to an increase of parasite burden (Blanco *et al.*, 2001; Moller & Erritzoe, 2000; Moller *et al.*, 2003). In this respect, moult (Moller *et al.*, 2003) or reproductive effort (Moreno *et al.*, 1999) have been shown to be stress factors to birds that reduce their immune response and promote the increase of parasite burdens. In the present study the new environmental circumstances that the pheasants find after release, such as the absence of feed, cold and presence of predators, could act as stress factors affecting the birds' ability to mount an adequate immune response, benefiting the parasites. The finding of *Ascaridia* eggs in one released pheasant also suggests the potential adverse effects of any new parasite being acquired in the wild (e.g. Höfle *et al.*, 2004b).

In conclusion, these results show that an increase in the excretion of parasite transmission stages, and probably in parasite burdens, follows the release of captive pheasants into the wild. Parasites may be one of many reasons that explain restocking failures. Also, an increase in the excretion of transmission stages by released pheasants can expose autochthonous free-living birds to new and potentially harmful pathogens, as parasite species are generally not shared between free-living and captive game birds (Tompkins *et al.*, 2000; Millán *et al.*, 2004). A single dose of fenbendazole prior to release has proved to be insufficient, and continuous prophylactic measures should be taken during the rearing of pheasants, and should be based as little as possible on medication. However if treatment is applied, a re-treatment after release will be necessary. Some studies focus on nematode treatment in the field. Newborn & Foster (2002) for example, using fenbendazole administered with the grit in a wild grouse population, obtained promising results, with an improved breeding success and lower parasite burdens (see also Hudson, 1986 and Woodburn *et al.*, 2002). Nevertheless, some authors think that treatment of free-living birds is not practical (Cole, 1999) and the advantages and disadvantages of drug administration to wild birds have to be considered carefully, taking ethical and public health concerns into account (Höfle *et al.*, 2004a). Drugs that should be used must be safe and stable, with a broad spectrum, and effective at a low concentration (Hudson *et al.*, 1992). The use of such drugs must be evaluated with care in each particular case.

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