# The effect of an experimental infection of the nematode *Heterakis gallinarum* on hand-reared grey partridges *Perdix perdix*

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SUMMARY

We compared 26 hand-reared grey partridges given an experimental infection of the caecal nematode *Heterakis gallinarum* with 26 uninfected ones. Under laboratory conditions after 91 days, there were no measurable clinical effects of the infection. We found no effect of treatment on the amount of food eaten or on caecal dropping production. However, treated birds, in particular females, developed slightly lower body mass (around 2%) compared to the controls. At *post-mortem* examination, we found a positive relationship between breast muscle mass and the number of worms collected from the caeca of treated birds. Treated birds with no worms when examined had smaller breast muscle mass (4·6%) compared to the uninfected control birds. These results are largely different to those found in a similar study that documented significant negative impacts on most of these factors in 8 infected birds compared to 6 controls. Its findings were used in a published model to support a hypothesis that *H. gallinarum* maintained in the environment by common pheasants, the primary host for this worm, could negatively affect wild grey partridge productivity and survival. In the same model our data would not support this hypothesis. Possible explanations for the different results from the 2 experiments are discussed. Together they suggest that only in certain, as yet unidentified circumstances, could experimental *H. gallinarum* infections have deleterious effects on hand-reared grey partridges.

Key words: *Heterakis gallinarum*, caecal nematode, *Perdix perdix*, grey partridge.

## INTRODUCTION

Of several galliforms in which the caecal nematode *Heterakis gallinarum* (Schrank) is found, the common pheasant *Phasianus colchicus* (hereafter, pheasant) is its most productive host (Lund & Chute, 1972) and the worm is widespread and abundant in this species in the UK (Draycott *et al.* 2000). In comparison the grey partridge *Perdix perdix* is not a productive host and the worm has been found to be less common and abundant in this bird (Clapham, 1935, 1961; Lund & Chute, 1972, 1974).

In a laboratory experiment by Tompkins, Greenman & Hudson (2001) *H. gallinarum* affected these 2 gamebird species differently. By 50 or so days following infection with around 100 embryonated worm eggs, 8 grey partridges had suffered a decrease in caecal activity of around 30% compared to 6 uninfected control birds, a decrease in food consumption of 20% and a decrease in body mass of over 10%. Mean breast muscle mass was also thought to have been reduced by the infection. A similar number of pheasants showed only a decrease in caecal activity compared to control birds, with no effect on food consumption or body mass.

These findings have been used to support a model

that suggests pheasants carrying *H. gallinarum* could compete with the grey partridge via the parasite alone (Tompkins *et al.* 2000). Pheasants infected with *H. gallinarum* are required in the model because the partridge itself does not sustain viable *H. gallinarum* populations. The model predicts the eventual exclusion of the grey partridge because the pheasant is comparatively unaffected and continues to produce the infection regardless of the density of grey partridges.

Until this recent work, *H. gallinarum* had been found to be relatively benign to its main hosts in most circumstances in which they are found (Clapham, 1935, 1961; Draycott *et al.* 2002; Robertson & Hillgarth, 1994; Woodburn, 1999). The protozoan disease *Histomonas meleagridis* (e.g. Lund & Burtner, 1957) is associated with the parasite, but has not been important to either pheasants or grey partridges for many years (Pennycott, 1997). High numbers of *H. gallinarum* adults (around 100 or more) have been found in free-living pheasants in poor condition but it is not clear whether these infections cause this poor condition or are a response to it (Robertson & Hillgarth, 1994; Draycott *et al.* 2002). Similarly, Tompkins, Dickson & Hudson (1999) found correlations between poor condition and the presence of *H. gallinarum* in grey partridges kept in pens placed near to pheasant release pens.

The study reported by Tompkins *et al.* (2001) is

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the only one that provides experimental evidence of a significant effect of *H. gallinarum* on grey partridges. As they stated, 'verification that the impact of *H. gallinarum* is indeed as high on the grey partridge and as low on the ring-necked pheasant as the correlational work suggests is essential to this demonstration of apparent competition'. We therefore considered it necessary to repeat the experimental work on grey partridges. Our aims were, first to demonstrate the repeatability of the results in Tompkins *et al.* (2001) concerning caecal activity, food consumption and body mass of the grey partridge, second to confirm the hypothesis that breast muscle mass is reduced in grey partridges infected with *H. gallinarum* and third, through *post-mortem* examination, to investigate any pathology of the infection.

## MATERIALS AND METHODS

We purchased 140 one-day-old grey partridge chicks from a commercial game farm and reared them in a single unit on a concrete base designed to prevent natural infection with endo-parasites. When the birds were 14 weeks old they were given a course of the anthelmintic flubendezole for 1 week prior to caging, to ensure that they remained free of any parasitic infections.

When the birds were 15 weeks old, 54 were randomly selected, weighed, sexed and placed into individually numbered cages supported in racks inside a brooder house facility. Twenty-six birds, 13 of each sex, were randomly selected and assigned to the treatment group and a further 26 assigned to the control group. The sample size was calculated from a power analysis based on data provided by Tompkins *et al.* (2001) to achieve, with 80% probability, a statistically significant effect on breast muscle mass of an infection, given the difference between their mean values for the two groups.

A period of 2 weeks was allowed to elapse following the end of treatment with flubendezole before experimental treatments began. This period enabled the birds to acclimatize to their new environment and to ensure that there was no residual effect of the anthelmintic. On 23 November 2000, when the birds were 17 weeks of age, experimental treatments were applied. The 2 unassigned caged birds, together with 3 randomly selected birds from the original rearing unit, were culled and examined, to confirm absence of infection by *Heterakis* and other disease. Each of the 26 birds in the treatment group were then given approximately 100 embryonated worm eggs in 1 ml of saline solution administered orally. The 26 control birds were given a similar dose of saline without nematode eggs. Embryonation prior to treatment was achieved by maintaining worms at 21 °C for 21 days in 0·5% formalin solution. Eggs were then separated from worms and counted in a saline solution, adjusted to give 100 eggs/ml.

Treated and control birds were observed several times each day for 91 days before culling. Daily minimum and maximum air temperatures were recorded inside the facility. Pelleted food, water and grit were supplied in hoppers *ad libitum*. The mass of pellets required to refill hoppers and the mass of spilt pellets were recorded to calculate food consumption per bird per week. Similarly at 1-week intervals caecal droppings collected in trays beneath cages were weighed fresh and again after drying for 24 h at 50 °C. Caecal droppings look very different to intestinal droppings so their collection, while time consuming, was accurate. Each bird was weighed to the nearest 5 g each week. To assess the concentration of worm eggs in the caecal droppings a 2·5 g subsample of droppings from each bird each week was suspended in 10 ml of saturated salt solution and 0·1 ml of this was then placed into a faecal egg-counting chamber and examined under a microscope. This was repeated 5 times for each sample. At the end of the study, all birds were culled. Birds were given an *ante-mortem* examination and *post-mortem* examinations and measurements were undertaken by a team of 8 staff on fresh carcasses over 2 consecutive days.

The weekly mean food consumption, caecal dropping mass and body mass were compared between treatment and control birds using repeated measures analysis of variance (sex * treatment * time) using the computer program SYSTAT Version 9 (Anon, 1999). To account for small differences in these measures between treatment and control birds at the beginning of the experiment, analyses were conducted on the ratio of the actual weekly value to the initial (week 0) value. Residuals were normally distributed so no data transformations were necessary. SYSTAT 9 uses several tests for these within-subjects analyses over time. The classical univariate test assumes that compound symmetry holds, i.e. that the covariance between all pairs of repeated measures is equal (Anon, 1999). It was ambiguous as to whether this assumption actually held for our data for, in particular, body mass. The multivariate test does not rely on this assumption but as a consequence is less powerful. Consequently, for within-subjects repeated measures analyses of relative body mass, food intake and caecal dropping production in relation to treatment both univariate and multi-variate test statistics are presented. These two tests represent the extremes in terms of their underlying assumptions.

When examined *post-mortem*, the right-hand major and minor pectoral muscles were dissected from the carcass and immediately weighed. When deviations from the average body size are accounted for, breast muscle mass is a good indicator of condition in game birds (Brittas & Marcstrom,
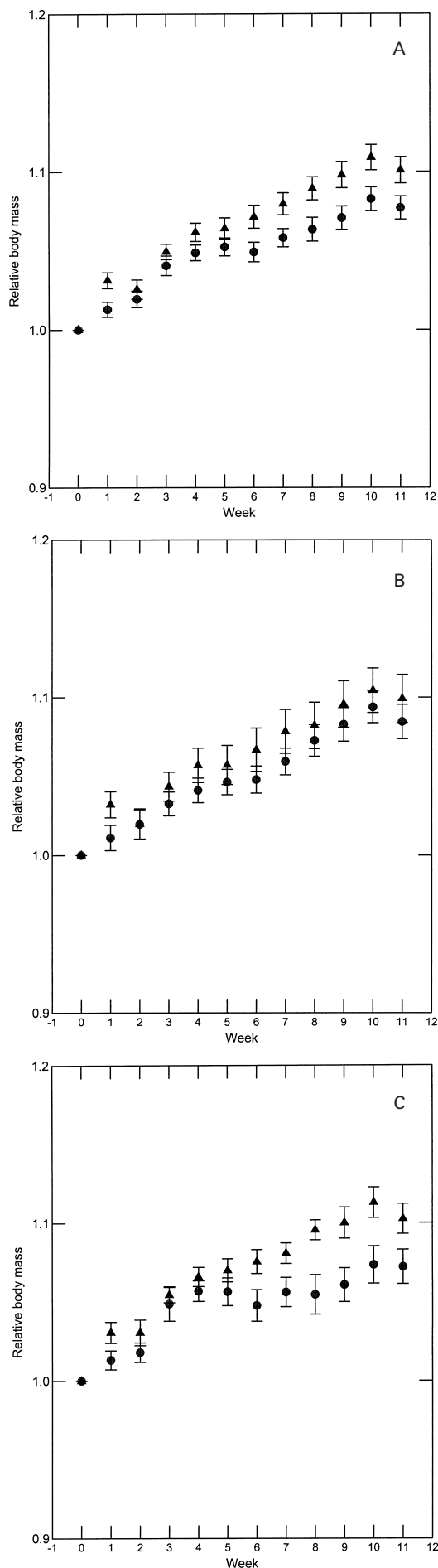
1982). To account for these deviations, tarsal length was included as an independent variable in an analysis of the effect of treatment on breast muscle mass (ANCOVA of sex * treatment with tarsal length). The relationships between worm burden at *post-mortem* examination and breast muscle mass were investigated using an otherwise similar ANCOVA (where worm number replaced treatment). Both breast muscle mass (3 dimensions) and tarsal length (1 dimension) were log-transformed to ensure linearity in the allometric relationship between the two variables.

A parallel exercise was undertaken, in order to demonstrate the infectivity of our embryonated *H. gallinarum* eggs, using pheasants. The reproductive potential of *H. gallinarum* eggs has been found to be over 200 or 300 times more in pheasants than in grey partridges (Tompkins *et al.* 2000; Lund & Chute, 1974). Although these estimates were very approximate, it was likely that little evidence of an infection would be revealed in our grey partridges, even if our solution was 100% infective. Pheasants would be much more sensitive indicators. Twelve game-farm adult pheasants, maintained in pens on concrete and previously treated with flubendezole, were used. Six were given an identical oral dose of the embryonated egg solution as that given to the grey partridges and 6 the saline solution only. All 12 were culled 25 days later and the caeca searched for *H. gallinarum*.

### RESULTS

The 5 untreated grey partridges culled at the beginning of the experiment were free of *H. gallinarum* infection. The 6 treated pheasants contained $33 \cdot 8 \pm 12 \cdot 1$ (1 standard error) worms when culled at 25 days post-infection. One of the 6 untreated control pheasants contained 1 adult worm. During the course of the experiment, no *H. gallinarum* eggs were detected in samples of caecal droppings from any of the experimental or control grey partridges. When examined *post-mortem*, it was confirmed that 1 female bird had been initially incorrectly sexed as a male. Consequently we had 13 treated and 14 untreated female, and 13 treated and 12 untreated male grey partridges in the trial.

The mean body mass of all 52 treatment and control birds increased during the course of the experiment from $336 \cdot 0 \pm 3 \cdot 1$; at week 0 to $366 \cdot 2 \pm$

Fig. 1. Comparison of body mass between treated (●) and control (▲) grey partridges. Means with standard errors. (A) All birds. Significant overall effect of treatment and possible significant effect of 'time * sex * treatment' interaction (univariate test, Table 1). (B) 13 treated male and 12 control male grey partridges. No effect of treatment (Table 1). (C) 13 treated female and 14 control female grey partridges. A possible effect of treatment (univariate test, Table 1).

Table 1. *Repeated measures ANOVA of relative body mass over time in relation to sex and treatment*

(See Materials and Methods for explanation of presentation of 2 tests for within-subjects analyses.)

| | Between-subjects | | Within-subjects interaction with time | | | |
| | | | Univariate | | Multivariate | |
|---|---|---|---|---|---|---|
| All birds | $F_{1,48}$ | $P$ | $F_{10,480}$ | $P$ | $F_{10,39}$ | $P$ |
| Sex | 0·078 | 0·782 | 2·627 | 0·004 | 1·541 | 0·162 |
| Treatment | 5·523 | 0·023 | 2·246 | 0·014 | 1·348 | 0·240 |
| Sex * treatment | 0·440 | 0·510 | 2·036 | 0·028 | 1·947 | 0·068 |
| Females | $F_{1,25}$ | $P$ | $F_{10,250}$ | $P$ | $F_{10,16}$ | $P$ |
| Treatment | 7·006 | 0·014 | 3·934 | < 0·001 | 1·790 | 0·144 |
| Males | $F_{1,23}$ | $P$ | $F_{10,230}$ | $P$ | $F_{10,14}$ | $P$ |
| Treatment | 1·011 | 0·325 | 0·659 | 0·761 | 2·570 | 0·052 |
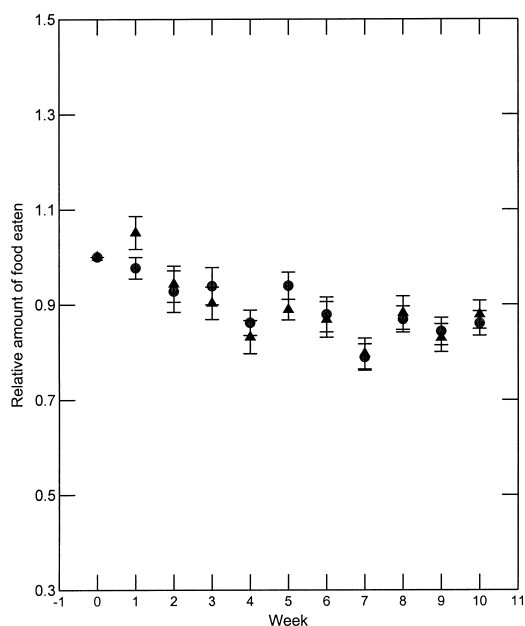


Fig. 2. Comparison of food intake between 26 treated (●) grey partridge and 26 control (▲) grey partridges. Means and standard errors. No overall difference between treatments and no different trend between treatments over time (Table 2).

3·9 g (within-subjects univariate test on relative body mass, $F_{10,480} = 94·296$, $P < 0·001$, Fig. 1A). This increase was due largely to the recovery of weight lost during the 2-week acclimatization period prior to experimental treatments being applied, plus perhaps, the last stages of growth to adult size.

Overall, treated birds had lower relative body mass than the controls (Fig. 1A). This reduction due to treatment peaked at 2·3 % of body mass in week 11 and remained at or just over 2 % for the second half of the trial. In the analysis of body mass over time, the 'sex * treatment * time' interaction was significant according to the within-subjects univariate test but not according to the multivariate test (see Table 1). This suggested a different response between

sexes. Considered separately, there was no effect of treatment on relative body mass overall or over time in males (Fig. 1B) but there was on growth in females (Fig. 1C), according to the between-subjects test and the within-subjects univariate test (but not the multivariate test, Table 1).

Relative food intake decreased during the course of the experiment (Fig. 2, univariate within-subjects test: $F_{9,369} = 11·619$, $P < 0·001$). This trend was not different between treatments (Table 2). The relative dry mass of caecal droppings produced varied over time as indicated in Fig. 3 (univariate within-subjects test: $F_{9,432} = 3·845$, $P < 0·001$) but was also not different between treatments (Table 2). The between-subjects test in Table 2 indicates that the relative mass of caecal droppings was lower in females than males, i.e. all females tended to produce fewer droppings following treatment while all males produced more.

At the *ante-mortem* examination, several individuals showed varying degrees of bill over-growth and 1 treated female bird was clinically lame. With the possible exception of the lame bird, which had an infection in its joint, there was no evidence at *post-mortem* examination of any pathology that could impact on any of the measurements recorded in this study.

The treated female grey partridges contained $0·62 \pm 0·24$ *H. gallinarum* adults or juvenile larvae in the caeca at *post-mortem* examination and the males $1·39 \pm 0·38$ (difference not significant, Mann–Whitney $U = 52·0$, $P = 0·074$). In all, 20 juveniles, 4 adult males and 2 adult female worms were found. All the control birds had no worms.

In the analysis of breast muscle mass at *post-mortem* examination, log tarsal length was not significant ($t_{4,46} = 0·721$, $P = 0·474$) and the treatment * sex interaction was ($t_{4,46} = 2·200$, $P = 0·033$). Considering sexes separately, while treated females tended to have lower breast muscle mass than control females, and treated males tended to have higher

Table 2. Repeated measures ANOVA of relative food intake and production of caecal droppings in relation to sex and treatment

(See Materials and Methods for explanation of presentation of 2 tests for within-subjects analyses.)

| | Between-subjects | | Within-subjects interaction with time | | | |
| | | | Univariate | | Multivariate | |
| Food | $F_{1,41}$ | $P$ | $F_{9,369}$ | $P$ | $F_{9,33}$ | $P$ |
|---|---|---|---|---|---|---|
| Sex | 0·089 | 0·766 | 1·165 | 0·317 | 1·134 | 0·368 |
| Treatment | 0·356 | 0·554 | 0·926 | 0·499 | 0·778 | 0·638 |
| Treatment $*$ Sex | 0·009 | 0·924 | 0·917 | 0·510 | 1·125 | 0·374 |
| Droppings | $F_{1,48}$ | $P$ | $F_{9,432}$ | $P$ | $F_{9,33}$ | $P$ |
| Sex | 7·704 | 0·008 | 0·873 | 0·549 | 1·321 | 0·257 |
| Treatment | 0·209 | 0·650 | 0·152 | 0·178 | 1·553 | 0·163 |
| Treatment $*$ Sex | 0·018 | 0·895 | 1·320 | 0·224 | 1·057 | 0·414 |
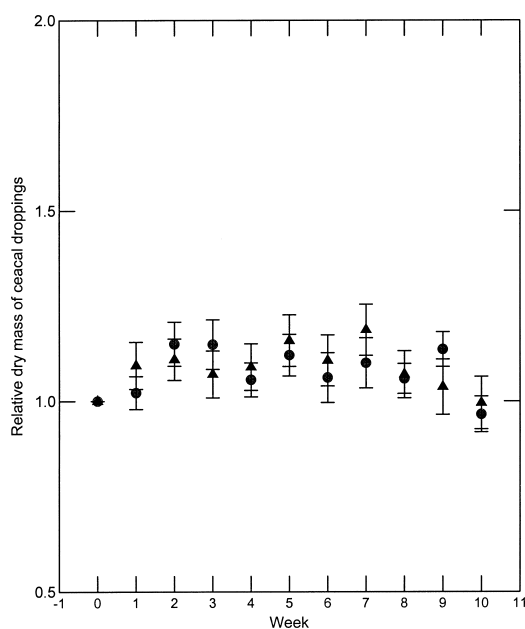


Fig. 3. Comparison of dry caecal dropping mass between 13 treated (●) female grey partridge and 14 control (▲) female grey partridges. Means and standard errors. No overall difference and no different trend between treatments over time (Table 2).

breast muscle mass than control males, neither result was significant ($t_{2,21} = 1.552$, $P = 0.136$ and $t_{2,21} = -1.492$, $P = 0.149$ respectively).

When this analysis was restricted to only those birds that contained no worms (26 control and 10 treated birds), there was no significant interaction ($t_{4,30} = 1.724$, $P = 0.095$) but a significant effect of treatment ($t_{4,30} = 2.394$, $P = 0.023$). Treated partridges with no worms had smaller breast muscle mass (one side, $38.7 \pm 2.4$ g) than the control birds ($40.6 \pm 2.9$ g).

In the analysis of the treated birds only in relation to worm counts, log tarsal length and the sex $*$ worms interaction were not significant (($F_{1,21} < 0.001$, $P = 0.983$, $F_{1,21} = 2.041$, $P = 0.168$ respectively).

Breast muscle mass at *post-mortem* examination was positively related to the number of worms found ($F_{1,21} = 8.289$, $P = 0.009$), after accounting for sex ($F_{1,21} = 13.664$, $P = 0.001$) (Fig. 4).

The weekly mean daily minimum temperature inside the experimental facility varied between 5·7 and 9·0 °C for the first 5 weeks following treatment. In week 6, this mean temperature dropped to 2·6 °C and remained under 3·6 °C for the following 4 weeks.

DISCUSSION

The experimental infection of 100 *H. gallinarum* eggs in 6 pheasants gave rise to levels of infection ($33.8 \pm 12.1$ worms after 25 days) that were lower but not significantly different ($t_{10} = 1.32$, $P > 0.1$) to those found by Tompkins *et al*. (2001) ($59.0 \pm 14.8$ after 40 days). Tompkins *et al*. (1999), amongst others, have found only slight changes in the number of worms in pheasants between 25 and 40 days post-infection with 100 eggs indicating that these data are comparable. The likelihood therefore is that there was no difference in infectivity between the two studies although the possibility cannot be ruled out. The level of infection in the grey partridges in the main experiment after 91 days was $1.0 \pm 0.3$ worms/bird. Tompkins *et al*. (2000) found $6.5 \pm 3.6$ worms 40 days after a similar infection of grey partridges. No comparison can be made in these data as *H. gallinarum* have been found to have a life-span in pheasants of between around 50 and 100 days (Anon, 1998; Tompkins *et al*. 2000).

In terms of effects on body mass our results showed similar trends but of different magnitudes to those of Tompkins *et al*. (2001). All birds gained weight during the trial. The mean body mass of the female grey partridges given worms was lowered very slightly by the treatment and this difference was not recovered by the end of the trial. This response to treatment did not become apparent until week 6 of the experiment. This coincided with the first cold

Fig. 4. Relationship between breast muscle mass and the number of worms found in the caeca at *post-mortem* examination for treated grey partridges, with significant effect of sex (●, female; ○, male; see text).

spell of the winter. The difference in mean body mass between groups was less than one fifth of that recorded by Tompkins *et al.* (2001) where it peaked at around 15 % of initial body weight and was maintained at over 10 % for most of the trial.

We found no reduction in food intake or the production of caecal droppings in treated birds. We did find a relationship between breast muscle mass and worm burden at *post-mortem* examination for the 26 treated birds. However, this relationship was positive (i.e. birds with worms at *post-mortem* examination had larger breast muscles) while Tompkins *et al.* (2001) found a negative relationship. Furthermore, the group of 10 treated birds with no worms had a breast muscle mass that was 4·7 % lower than the 26 controls. In a slightly different comparison, Tompkins *et al.* (2001) indicated that the 6 treated birds that had no worm eggs had much higher breast muscle mass (around 92 g) than 2 treated birds in which worm eggs were found (around 52 g). In the model of Tompkins *et al.* (2000), the exclusion of grey partridges caused by the presence of pheasants infected by *H. gallinarum* stemmed from the loss in condition and implicit increased mortality associated with reduced breast muscle mass in the partridge. They used the negative relationships between worm counts when examined *post-mortem* and breast muscle mass from correlational data (e.g. Tompkins *et al.* 1999) for 2 important model parameters: the increase in grey partridge mortality per worm per year; and the

decrease in fecundity per worm per year. Using the same model, our data indicate opposite outcomes, decreased mortality per worm per year and increased fecundity.

The minor effect on body mass and lack of any clinical implications of the experimental infections in this study suggests that we may have been measuring the result of metabolic activity, and hence use of resources, associated with the grey partridges immune system response. This activity was not particularly costly. Our results relating to worm counts, where treated birds with no worms had smaller muscle mass than treated birds with worms, is also consistent with this idea. An individual that succeeds in completely removing an infection may use more resources (Klasing, Johnstone & Benson, 1991). No studies of the immune system in challenged grey partridges have, however, been undertaken. Immune defence has a genetic base (Wakelin & Apanius, 1998), and it is conceivable that the selection pressure that would normally suppress an over-costly anti-parasite response in an animal (Behnke, Barnard & Wakelin, 1992) has not properly occurred for the grey partridge/*H. gallinarum* interaction. On the other hand, native grey partridges in the UK have been 'exposed' to introduced poultry and pheasants bearing *H. gallinarum* over a period of at least several hundred years.

Why were the results of our experiment and that of Tompkins *et al.* (2001) different in several important respects? We can identify several methodological differences that could be relevant. As indicated above, it is possible that the infectivity of our solution of *H. gallinarum* eggs was slightly different to that used in the study of Tompkins *et al.* (2001). There can be no doubt, however, that it was infective. It is perhaps more likely that the age of the study birds could have been relevant. The ability of young gallinaceous birds to express an effective immunological response to parasites is thought to develop throughout at least the first 6–12 weeks following hatching (e.g. Rose, 1967; Suresh, Sharma & Belzer, 1993; Wakelin & Apanius, 1998). In the infectivity test, our pheasants were 1 year old while Tompkins *et al.* (2001) used juveniles. Similarly, in the main experiment, the age of our grey partridges when infected was 17 weeks, 5 weeks older than those in the study of Tompkins *et al.* (2001). We also used different UK sources for both our infection and host animals. There are examples of categorical variations in host susceptibility to parasites including nematodes between individuals or particular strains (Wakelin & Apanius, 1998; Wassom *et al.* 1986). As examples, both Tompkins *et al.* (2001) and Lund & Chute (1972) found that while most experimentally infected partridges did not contain egg-laying females, a small minority did. The different facilities and conditions in which the experiments were undertaken may also be relevant. For example, stress

of one form or another has the potential to have a compounding effect on the effectiveness or cost of an immune response (Coop & Holmes, 1996; Klasing *et al.* 1991). In our experiment only 1 bird out of 52 developed any sort of clinical condition (lameness). In the study of Tompkins *et al.* (2001) 4 birds (3 controls and 1 treatment bird) out of 18 were killed due to husbandry problems.

Whatever the reasons, our findings in conjunction with those of Tompkins *et al.* (2001) indicate that only in certain circumstances is it possible that hand-reared grey partridge will show deleterious effects of exposure to an experimental *H. gallinarum* infection. In the first instance, further laboratory studies could investigate, for example, the possible interaction with host age or stress. An immunological investigation should also be undertaken. Even then, however, very little would be known about the relationships between these laboratory-based studies and the effects of exposure on grey partridges in the wild. In the study reported by Tompkins *et al.* (2000) the model as parameterized predicts decreased wild partridge population size with increased exposure to pheasants bearing *H. gallinarum*. If the data presented here relating to breast muscle mass and worm counts were used to parameterize the same model, increased exposure to these pheasants would not lead to reduced partridge population size.

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