Journal of Helminthology (2004) **78,** 69–76 DOI: 10.1079/JOH2003220

Faecal egg counts provide a reliable measure of *Trichostrongylus tenuis* intensities in free-living red grouse *Lagopus lagopus scoticus*

L.J. Seivwright[1,2]*, S.M. Redpath[1], F. Mougeot[1], L. Watt[3] and P.J. Hudson[4]

[1]CEH Banchory, Hill of Brathens, Glassel, Banchory, Aberdeenshire, AB31 4BW, UK: [2]Department of Biological and Molecular Sciences, University of Stirling, Stirling, FK9 4LA, UK: [3]BioSciences Building, University of Liverpool, Crown Street, Liverpool, L69 7ZB, UK: [4]Biology Department, Mueller Laboratory, Penn State University, University Park, PA 16802, USA

Abstract

The reliability of different egg counting methods for estimating the intensity of *Trichostrongylus tenuis* infections in red grouse, *Lagopus lagopus scoticus*, was investigated in the autumn, when grouse may harbour high parasite intensities. Possible limitations to the use of these methods were also examined. Faecal egg counts were found to accurately estimate *T. tenuis* worm intensities, at least up to an observed maximum of *c.* 8000 worms. Two egg counting methods (smear and McMaster) gave consistent results, although the exact relationship with worm intensity differed according to the method used. Faecal egg counts significantly decreased with increasing length of sample storage time, but egg counts were reliable for estimating worm intensity for three weeks. The concentration of eggs in the caecum was also found to reliably estimate worm intensity. However, egg counts from frozen gut samples cannot be used to estimate worm intensities. These results conclude that, despite some limitations, faecal and caecum egg counts provide useful and reliable ways of measuring *T. tenuis* intensities in red grouse.

Introduction

Host–parasite interactions have long been of interest to ecologists, especially when they are thought to play an important role in host population dynamics (Anderson, 1978; May & Anderson, 1978; Hudson *et al.*, 1985; Albon *et al.*, 2002). Red grouse (*Lagopus lagopus scoticus*) populations exhibit cyclic fluctuations, and *Trichostrongylus tenuis* parasites are suspected to play a major role in causing the population cycles (Hudson *et al.*, 1992, 1998).

A quantitative understanding of the population dynamics of parasites and of their impact on individual hosts and populations requires a good estimation of the intensity of infection in each host and how the parasites are distributed through the host population. In some systems, faecal egg counts are often the only measure available to estimate parasite intensity in free-living animals, and are routinely used in a range of species (Gordon & Whitlock, 1939; Shaw & Moss, 1989b; Gulland & Fox, 1992; Guyatt & Bundy, 1993; Irvine *et al.*, 2001). The validity of using faecal egg counts to estimate host worm intensity has nevertheless been widely questioned and needs to be addressed in any host–parasite system (Michael & Bundy, 1989; Sithithaworn *et al.*, 1991; Guyatt

*Fax: 01330 823303
E-mail: ljse@ceh.ac.uk

& Bundy, 1993). The primary concern is that egg counting might be an unreliable index of worm intensity if it is influenced by a number of factors such as density-dependent constraints on worm fecundity (Anderson & Schad, 1985; Smith *et al.*, 1987; Tompkins & Hudson, 1999) or seasonal variation in worm egg production rate (Hudson, 1986a; Shaw & Moss, 1989a).

In the *T. tenuis*–red grouse system, Moss *et al.* (1990) showed that egg counts are a reliable measure of worm burdens. However, that work was limited to spring and used only one egg counting method. Moreover, worm intensities in that study were relatively low (only two of the 40 birds were found to have a worm intensity greater than 3000 worms). Since grouse are routinely sampled for parasites during autumn and may carry worm intensities of up to 24,000 worms (Hudson, 1986b), it is important for the validity of ecological studies involving red grouse–*T. tenuis* interactions, to establish whether the relationship between *T. tenuis* egg counts and adult worm intensity holds for different seasons and for higher worms intensities.

In the present paper, the reliability of various egg counting methods for estimating worm intensities of red grouse and possible limitations to the use of these methods are assessed. We examine the relationship between egg counts and *T. tenuis* worm intensities in red grouse harbouring up to 7992 *T. tenuis* worms, during the autumn. Egg count reliability may depend on the method used to count eggs, as well as variability in the concentration of eggs in faecal material (Sinniah, 1982). Three different ways of counting parasite eggs (smear and McMaster egg counts carried out on faecal samples, and McMaster egg counts carried out on material from the caecum) are examined and the reliability of each for estimating worm intensity is assessed. Egg counts on gut material from the caecum have already been used previously for estimating *T. tenuis* intensities (Hudson & Dobson, 1997) and can provide a quicker and easier method of estimating parasite infection levels than a straight worm count. However, *T. tenuis* worms are not evenly distributed within the caecum (Shaw, 1988b), so we examine in more detail spatial patterns in egg concentrations, and the applicability of using egg counts from different sections of the gut to estimate worm intensities. Lastly, we examine potential limitations to the use of egg counts, such as conditions for the storage of samples and storage time.

Materials and methods

Life cycle of Trichostrongylus tenuis

Trichostrongylus tenuis inhabits the relatively large caeca of the red grouse, and like most other gastrointestinal nematodes, has a simple, direct life cycle. Eggs laid by adult worms are voided onto the moor once daily in caecal droppings and, when the temperature exceeds 5°C, embryos develop and the first two larval stages complete their development in the faeces. Under conditions of adequate temperature and moisture, during May to November, they develop into infective larvae, which ascend the heather *Calluna vulgaris* (the main plant food of red grouse) and are ingested (Hudson, 1986b; Shaw *et al.*, 1989). During the summer the ingested larvae soon develop into adults, usually within 2 weeks. However, in autumn, ingested larvae may become hypobiotic and delay development until spring in March and April when many may mature simultaneously and begin to produce eggs (Shaw, 1988a). In spring, the numbers of eggs can increase suddenly even though no larvae have been ingested for months.

Study areas, material collection and storage

Parasite counts were conducted on 36 cocks from three study sites located in northeast Scotland. Previous studies have found no evidence for sex-biased parasitism in grouse, as males have been shown to have approximately the same number of worms as females (Wilson, 1983; Hudson, 1986b). Birds were caught at night using lamping techniques (Hudson & Newborn, 1995) in September and October 2001. Each bird was held in an individual holding box overnight and caecal faeces collected the following morning. Birds were then euthanased and their caeca removed for parasite and parasite egg estimation. Post-mortem parasite estimations were also conducted (using only the caeca) on 12 grouse shot on grouse moors in Aberdeenshire and Berwickshire in October 2001. All samples collected in the field were brought immediately to the laboratory and stored in a cold room at a constant temperature of 5°C to inhibit the hatching of eggs. The second caecum from each of 10 shot birds was stored in a freezer at a temperature of −40°C for two weeks to study the effect of freezing on caecum egg counts. In addition, the original data from 44 cocks from Moss *et al.* (1990) were used.

Laboratory analysis of intensity of T. tenuis *worm infection and egg counts*

Estimating worm intensity

Direct worm counts were carried out on one caecum from each grouse. Each caecum was opened lengthways and all the gut material flushed with water over 150 μm gauze to collect the worms. The contents of the sieve were washed into 300 ml of water, mixed thoroughly and adult worms counted with the aid of a binocular dissecting microscope with 25 × magnification. Where possible, all worms in the sample were counted and this number doubled to obtain the total number of worms per bird, as worm numbers do not significantly differ between the two caeca (Wilson, 1979, 1983). Otherwise, worms were counted from a minimum of three 10 ml sub-samples. The average of these counts was multiplied by 30 to obtain an estimate of the total number of worms in a single caecum, and this number was doubled to give the total number of worms per bird.

Estimating egg intensity

The intensity of parasite eggs per gram of caecal faeces was estimated using two different counting methods. First, a measure of eggs per gram (EPG) was carried out using the modified McMaster egg counting technique (MAFF, 1986). For this method, approximately 0.2 g (range 0.19–0.21 g) of well-mixed faecal material was put into a

shaker tube with approximately 10 glass balls and 5 ml of saturated NaCl solution. The tube was shaken until the faecal matter was suspended. Using a Pasteur pipette, a sample of the faecal suspension was extracted and carefully run into one chamber of a McMaster counting slide. The tube was shaken again and another sample extracted and run into the second section of the chamber. The saline suspension was left to settle for 2–3 min, allowing the eggs to float to the top of each chamber. Eggs were then counted beneath a marked grid on each chamber using a compound microscope with 100 × magnification. The number of eggs per gram of faeces was calculated by multiplying the total number of eggs counted under both grids by the total volume of faecal suspension contained in both chambers and then dividing this by the quantity of faeces used in the suspension.

A second egg counting method (smear) was carried out on the same faecal sample, after Moss *et al.* (1990). These were carried out on the same day the McMaster egg count was conducted, to allow a direct comparison between the two methods. In the smear method, caecal faeces were thoroughly mixed, and a 5–10 mg sub-sample was weighed onto a cover slip, which was then pressed onto a glass microscope slide. All the eggs in the sub-sample were counted using a compound microscope with 100 × magnification. Successive sub-samples from the same sample of faeces were carried out until two counts varied by no more than 10%. The number of eggs, either per 10 mg (to allow comparison with original data from Moss *et al.* (1990)) or per gram of faeces, was calculated according to the average of the two counts and the average weight of the sub-samples.

Having carried out a direct worm count on one caecum, the second caecum was used to investigate spatial variability in *T. tenuis* egg concentration in different sections of the gut. The weight and length of each caecum was recorded, then the caecum was divided into three sections of approximately equal length. These were, namely, the proximal section, which is the end nearest the opening into the intestine, the mid section, and the distal section, that nearest the blind end of the gut. The gut contents from each section were squeezed out, weighed and an egg count carried out on each section. The remaining material was combined, thoroughly mixed and also sampled for worm eggs. For gut material, only the modified Mcmaster egg counting technique was used.

Effect of freezing caeca on the relationship between egg counts and worm intensity

The second caeca of ten birds which had been shot in September were frozen and used to compare egg counts from the frozen caecum with direct worm counts carried out on the first caecum prior to freezing. The frozen caeca were kept in the freezer for 14 days, then thawed at room temperature and a McMaster egg count was carried out on thoroughly mixed caecal material from the whole gut.

Effect of sample storage time on the relationship between egg counts and worm intensity

Faecal samples from 13 birds were stored in a cold room at a constant temperature of 5°C (to inhibit the hatching of eggs) for a period of 14 weeks. For each sample, a first egg count was conducted on the day of sample collection, and thereafter on the same day weekly for a period of 10 weeks, with a final egg count 3.5 months after collection. The McMaster faecal egg count technique was used throughout this section of the study.

Statistical analyses

Statistical analyses were conducted using Minitab (version 13) and SAS (version 8.01, SAS, 2001). Where necessary, data were transformed to approximate normal distribution. All relationships between egg counts and worm intensity were tested using regression analysis. ANCOVAs were used to compare the relationships between worm intensity and egg counts obtained in different seasons; spring (original data used from Moss *et al.* (1990)) vs. autumn (this study), or obtained using different techniques (smear and McMaster). As the original data from Moss *et al.* (1990) included low values of eggs and worm intensities outside the range of the autumn data from this study, in order to directly compare the two data sets, these low values were excluded from the spring data in the ANCOVA. An ANCOVA was also used to compare the relationships between worm intensity and EPG counts obtained from each gut section.

Generalized linear mixed models (GLMMs) were used to test for differences in caecal egg counts between gut sections, and to test for an effect of storage time on faecal egg counts. Models were fitted to the data using a Poisson error distribution and a log link function. Denominator degrees of freedom were estimated using Satterthwaite's formula (Littell *et al.*, 1996). The total number of eggs counted in both chambers of the McMaster slide was used as the response variable, with the exact amount of faeces or caecal material sampled as an offset in the model. GLMMs were implemented in SAS using the GLIMMIX macro (Littell *et al.*, 1996). For differences in egg counts between gut sections, the model included 'individual gut' as a random effect, in order to identify counts within a given gut. For the effect of storage time of egg counts, the model included 'individual sample' and the 'individual sample*time' interaction as random effects, in order to account for the repeat sampling of individual faecal samples at different time periods. The data were unbalanced, as some faecal samples ran out before the end of the experiment. Time period (week number; 0–14) was included as a class variable. Difference of least square means (DLSM) was used to compare egg counts from different time periods and to determine when they differed significantly from the initial count (week 0).

Results

Relationship between faecal egg and worm counts

The number of adult *T. tenuis* worms found in the caeca of individual birds was significantly correlated with corresponding counts of eggs in caecal droppings (fig. 1; $F_{1,29} = 294.24$, $P < 0.001$). *Trichostrongylus tenuis* worm intensities in individual grouse ranged from 103 worms to 7992, with at least seven birds carrying intensities of greater than 4000 worms. The relationship found between

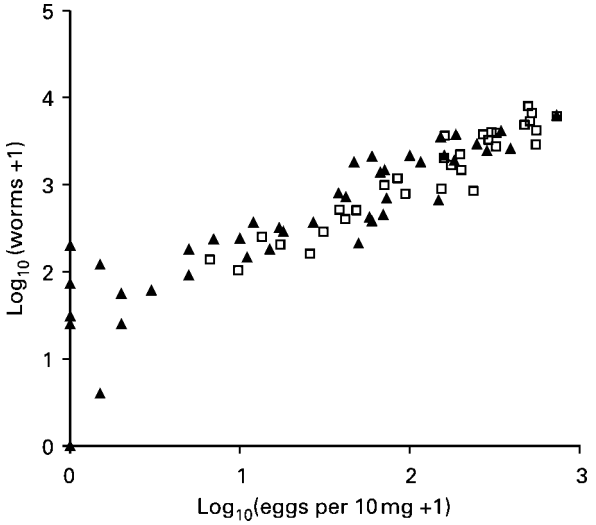

Fig. 1. Relationship between *Trichostrongylus tenuis* intensity (W, worms bird^{-1}) and egg concentration (E, eggs 10 mg^{-1}) using smear egg counting method in the caecal droppings of cock red grouse in relation to season. Symbols represent data for autumn from this study (□) and data for spring, from Moss *et al.* (1990) (▲).

numbers of adult worms (W worms bird^{-1}) and counts of eggs (E eggs 10 mg^{-1}) in faecal droppings in autumn, using the smear egg counting technique, was very similar to that found in spring, using data from Moss *et al.* (1990). Shown below are the regression equations for both studies. The regression equation from this study is also given with standard errors for the slope and intercept.

$$\text{Autumn:}\quad \log_{10}(W+1) = 0.917(\pm 0.053)\log_{10}(E+1) + 1.20(\pm 0.116)\ (r^2 = 0.91,\ n = 31, P < 0.001).$$

$$\text{Spring:}\quad \log_{10}(W+1) = 0.915\,(\pm 0.076)\log_{10}(E+1) + 1.28\,(\pm 0.121)\ (r^2 = 0.77, n = 44, P < 0.001).$$

There was no significant effect of season on the relationship between egg counts and worm intensity ($F_{1,63} = 1.95$, $P = 0.168$). 95% Confidence intervals for the slopes of both the autumn (0.917 ± 0.109) and spring (0.919 ± 0.153) regression equations include a slope of unity, further suggesting that there is no density-dependent reduction in worm fecundity.

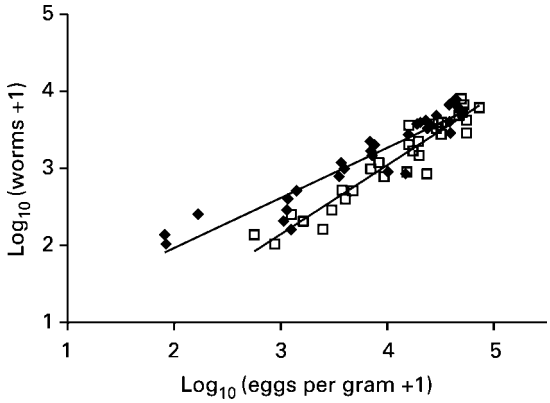

Fig. 2. Relationship between *Trichostrongylus tenuis* worm intensity (W, worms bird^{-1}) and egg concentration (E, eggs g^{-1}) in the caecal droppings according to egg counting method (□, Smear technique; ◆, McMaster technique).

Comparison of eggs counting methods

Regression analysis carried out on the two egg counting methods (smear and Mcmaster) showed a highly significant relationship between egg concentration and worm intensity for both methods (fig. 2, table 1; smear $r^2 = 0.90$, $F_{1,29} = 287.73$, $P < 0.001$; McMaster $r^2 = 0.88$, $F_{1,29} = 218.28$, $P < 0.001$ respectively). However, there were significant differences both in the slopes of the regression lines (smear slope = 0.895 (± 0.053), McMaster slope = 0.977 (± 0.143), $F_{1,58} = 12.20$, $P = 0.001$) and in the intercepts of the regression lines (smear intercept = −0.541 (± 0.218), McMaster intercept = −1.23 (± 0.689), $F_{1,58} = 18.15$, $P < 0.001$).

Spatial distribution of eggs in the caecum

EPG counts significantly differed between each of the three sections, with the proximal end carrying significantly more eggs (fig. 3; GLIMMIX; $F_{2,42} = 6.8$, $P = 0.003$). Results of a pairwise comparison showed a significant difference between the proximal and mid sections ($t = -2.511$, $P < 0.05$) and between the proximal and blind sections ($t = -3.595$, $P < 0.05$).

Relationship between caecum EPG counts and worm intensity

There were significant linear relationships between worm intensity and the EPG counts from each section of

Table 1. Relationships between *Trichostrongylus tenuis* worm intensity (W, worms bird^{-1}) and egg concentrations (eggs g^{-1}) obtained from various egg counting methods.

Egg counting method	Regression equation	r^2	n, *P*
Faecal sample McMaster technique (FM)	$\text{Log}_{10}(W+1) = 0.651(\pm 0.044)\ \text{log}_{10}\ (FM+1) + 0.664\ (\pm 0.17)$	0.88	$n = 31, P < 0.001$
Faecal sample Smear technique (S)	$\text{Log}_{10}\ (W+1) = 0.895\ (\pm 0.053)\ \text{log}_{10}(S+1) - 0.541\ (\pm 0.218)$	0.90	$n = 31, P < 0.001$
Caecum mixed contents McMaster technique (T)	$\text{Log}_{10}(W+1) = 0.646\ (\pm 0.047)\ \text{log}_{10}\ (T+1) + 0.547(\pm 0.194)$	0.86	$n = 32, P < 0.001$

Regression equations are given with standard errors for the slope and intercept.

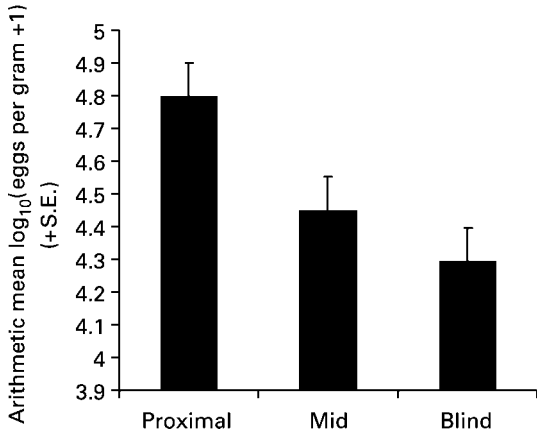

Fig. 3. Arithmetic mean (+ S.E.M.) of *Trichostrongylus tenuis* egg concentration ($\log_{10}$ eggs g^{-1}) in the proximal, mid and blind sections of the caecum (n = 15 guts), using the McMaster egg counting technique.

the gut (table 2; proximal $r^2 = 0.76$, n = 15, $P < 0.001$; mid $r^2 = 0.88$, n = 15, $P < 0.001$; and blind $r^2 = 0.87$, n = 15, $P < 0.001$, respectively). There was no significant difference between the slopes of the three regression lines ($F_{2,39} = 0.15$, $P = 0.858$) but the intercepts differed significantly ($F_{2,41} = 28.19$, $P < 0.0001$).

Effect of freezing on the relationship between caecum egg counts and worm intensity

Eggs per gram counts using the McMaster method from mixed caecum contents, were strongly correlated with worm intensity (table 1; $r^2 = 0.86$, $F_{1,30} = 188.72$, $P < 0.001$). However, it would appear that the freezing process resulted in the deterioration of eggs, since this relationship was no longer significant ($F_{1,8} = 0.05$, $P = 0.829$) when using egg counts carried out on frozen samples.

Effect of sample storage time on egg counts

Analysis of egg counts from 13 individual faecal samples, repeated over a period of 14 weeks, showed a significant decrease in egg counts with increased storage time ($F_{11,82} = 4.55$, $P < 0.001$). Pairwise comparisons (table 3) indicated that egg counts conducted at week 1–3 do not differ from the initial egg count (week 0), but from week 4 onwards, egg counts were significantly lower than those of the initial count.

Table 3. Results of an analysis testing for differences between initial week (week 0) and subsequent weekly egg counts (weeks 1 to 14) repeated on the same faecal samples.

Pair-wise comparisons	DF	t value	Pr > |t|
Week 0 – Week 1	58.4	−0.02	NS
Week 0 – Week 2	68.3	1.48	NS
Week 0 – Week 3	61.2	0.31	NS
Week 0 – Week 4	77.4	2.72	**
Week 0 – Week 5	79.1	3.05	**
Week 0 – Week 6	83.1	3.56	***
Week 0 – Week 7	83.6	3.5	***
Week 0 – Week 8	75.2	3.24	**
Week 0 – Week 9	68	2.81	**
Week 0 – Week 10	78.4	3.6	***
Week 0 – Week 14	77.1	3.89	***

Differences of least square means analyses were used for the pair-wise comparisons.
** $P < 0.01$; *** $P < 0.001$.

Discussion

Previous studies have shown that the reliability of using worm egg counts to indirectly estimate the worm intensity within a living host may be influenced by both seasonal variation in worm egg production (Shaw & Moss, 1989a; Moss *et al.*, 1993) and by density-dependent constraints in worm fecundity (Anderson & Schad, 1985; Smith *et al.*, 1987; Tompkins & Hudson, 1999). In the present study, however, we found that the strong relationship between faecal worm egg count and host worm intensity derived from samples collected in the autumn is similar to that derived from samples collected during spring (Moss *et al.*, 1990). Further, we found no evidence for a decline in egg count reliability with increasing worm intensity, up to an observed maximum worm intensity in this study, of *c.* 8000. Although worm intensities in grouse may occasionally reach numbers as high as 24,000 worms (Hudson, 1986b), the maximum observed value in this study represents a level of parasite intensity more routinely observed in the field.

These findings agree with a number of other studies of helminth infections, which have similarly demonstrated a significant relationship between parasite intensities and faecal egg counts. Faecal egg counts are strongly correlated with liver fluke (*Opisthorchis viverrini*) intensities in humans (Elkins *et al.*, 1991; Sithithaworn *et al.*, 1991). In sheep, there is a good correlation between egg counts and intensities of *Haemonchus contortus* (Le Jambre *et al.*, 1971; Roberts

Table 2. Relationships between *Trichostrongylus tenuis* intensity (W, worms $bird^{-1}$) and egg concentrations (E eggs g^{-1}) measured from three sections (proximal, mid and blind) of the gut.

Section	Regression equation	r^2	n, *P*
Proximal	$\text{Log}_{10}(W + 1) = 0.977 (\pm 0.143) \log_{10}(E + 1) - 1.23 (\pm 0.689)$	0.76	n = 15, $P < 0.001$
Mid	$\text{Log}_{10}(W + 1) = 1.07 (\pm 0.104) \log_{10}(E + 1) - 1.28 (\pm 0.464)$	0.88	n = 15, $P < 0.001$
Blind	$\text{Log}_{10}(W + 1) = 1.01 (\pm 0.104) \log_{10}(E + 1) - 0.85 (\pm 0.446)$	0.87	n = 15, $P < 0.001$

Regression equations are given with standard errors for the slope and intercept.

& Swan, 1981; Coadwell & Ward, 1982) and intensities of *Trichostrongylus colubriformis* (Gibson & Parfitt, 1973; Chiejina & Sewell, 1974). However, in contrast, there are also many studies of nematode infections where there is little or no correlation between the number of eggs that a host produces and worm intensity. This is particularly evident for *Ostertagia* species, important parasites of cattle and sheep (Michel, 1963, 1969; Callinan & Arundel, 1982; Coop *et al.*, 1985).

Such variation between parasitic helminth populations may be due to variations in the rate of worm egg production, a regulatory mechanism considered to be important in the regulation of many gastrointestinal parasite populations (Barger, 1987). In the red grouse–*T. tenuis* host–parasite system, our findings suggest that density-dependent suppression of egg production is weak or non-existent, at least up to the observed worm intensity of *c*. 8000 worms. A number of other factors may influence egg production, but there are two main mechanisms thought to dominate. The first of these mechanisms is the host's immunological response to infection, which may cause density-dependent suppression of egg production (Hudson & Dobson, 1997). However, evidence suggests that there is little or no effective acquired immunity in red grouse to infections of *T. tenuis* (Shaw & Moss, 1989b; Wilson, 1979). Unlike artificially induced *T. tenuis* infections in domestic chickens, where young domestic chickens develop a resistance to the nematodes resulting in worms being actively expelled and the infection rejected (Watson *et al.*, 1988), the number of worms present in the caeca of red grouse increases throughout the life of the bird (Wilson, 1983).

Secondly, competition between worms for resources, for example space, may also be an important mechanism influencing egg production. Adult *T. tenuis* worms are found only in the caeca of red grouse, which is thought to play an important role in the absorption of water and proteins and the digestion of cellulose (Moss & Parkinson, 1972; Gasaway *et al.*, 1976). The length of the caeca is known to vary amongst herbivorous birds, probably in relation to food digestibility, the length of the caeca increasing as the diet becomes more fibrous (Leopold, 1953). The diet of red grouse is composed mainly of heather (*Calluna vulgaris*), a high-fibre, low-protein food and, accordingly, the caecum of this bird is relatively long (Moss & Parkinson, 1972). Although colonizing *T. tenuis* favour the proximal regions of the caeca (Shaw, 1988b), worms are distributed throughout the whole gut. Thus, at the level of worm intensities observed in this study, it may be that space was not a limited resource.

Egg counts derived from two different methods were both highly correlated with worm intensity, although the smear method consistently gave a higher eggs per gram concentration than the McMaster method. This is a similar finding to that of Sinniah (1982), who compared direct smear methods with a dilution egg count technique. As the exact form of the relationship between egg count and worm intensity differed significantly between the two methods, care should be taken when using regression equations to estimate worm intensity, to ensure that the relevant equation is used. The decision as to which method to use may be influenced by factors such as time and the relative ease of carrying out different techniques. In our case, the smear count was the more time consuming of the two methods. This was especially true where faecal samples contained bits of grit and heather from the collection process, which ultimately affected contact between the slide and coverslip, making the actual counting of eggs under the microscope more difficult.

Previous studies have demonstrated that egg counts may be carried out on caecum material (Hudson & Dobson, 1997) and have only used caecal material from the proximal end of the gut. The research conducted here showed that the spatial concentration of worm eggs varied significantly throughout the gut, with the concentration of eggs falling as the distance from the opening of the caecum (the proximal end) increased. This is consistent with the finding that worms are unevenly distributed throughout the gut, and tend to inhabit the proximal section (Shaw, 1988b). Despite this, egg counts from distinct sections of the gut were significantly related to worm intensity. However, caution should again be applied if using gut section EPG counts to estimate worm intensity, as the exact form of the relationship to worm intensity differed between the egg counts from different sections of the gut. If using caeca to estimate worm intensity, it would therefore be more reliable to use egg counts from thoroughly mixed caecum contents, which were also found to strongly correlate with worm intensity.

Where this technique may be of particular use, is where time is a limiting factor. Direct worm count method, can be very time consuming, whereas McMaster egg counts are relatively quick. Depending on the concentration of eggs in the sample, each direct worm count may take between 40 and 60 minutes to complete, whereas McMaster egg counts routinely take only a third of that time. Using egg counts from the caecum may also be of use where birds are shot or have been killed by predators and their guts have been broken. In this case a direct worm count is inaccurate due to loss of caecal material. By removing some material from an unaffected section of the caecum and using the EPG count in conjunction with the correct regression equation instead, it may be possible derive a reliable worm intensity estimate from an otherwise unusable sample.

Frozen guts are regularly used to carry out direct worm counts in red grouse. However, in this study freezing at a temperature of -40°C appeared to cause a deterioration of eggs within the gut material. Thus, the possibilities for using caecum egg counts as an estimate of worm intensity, are strictly limited to unfrozen gut samples. Further investigations are required to examine whether eggs may be preserved at freezing temperatures closer to 0°C.

Finally, even when storing faecal samples at an optimum temperature of 5°C to inhibit the hatching of eggs, we have shown that egg concentration in faecal samples decreases with increased storage time. In the fourth week of repeated sampling, this egg loss resulted in a significant difference between the estimate of worm intensity and actual worm intensity. We conclude therefore that, in order to maximize the accuracy of our worm intensity estimates, faecal egg counts should

be conducted as soon as possible after collection and within a period of three weeks maximum.

Acknowledgements

We are grateful to the owners and gamekeepers from the Edinglassie, Invermark and Invercauld estates for allowing us to conduct this study on their grouse moors; to R. Moss for providing us with his original data and for comments on the manuscript; and to D. Barr from Overshiels estate, Berwickshire for providing additional samples. We thank J. Irvine, A. Stien and A. Amar for helpful discussions of the results and D. Elston for help with the statistical analyses. We thank L. Money, for help with work in the field and in the laboratory. We also thank K. Wilson for comments on previous drafts of the manuscript. This work was funded by NERC and was completed under Home Office Licence PPL 80/1437.

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(Accepted 4 August 2003)