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1 **TITLE: Dung beetles reduce livestock gastrointestinal parasite availability**

2 **on pasture**

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Summary

26   1. Anthelmintics are commonly used to control gastrointestinal parasites of
27   livestock. However, the residues of these compounds, particularly the
28   macrocyclic lactones, are excreted largely unmetabolised in faeces where
29   they may have toxic effects on dung colonising insects. Impoverishment of
30   the coprophagous beetle community impairs the process of dung recycling
31   and, as a result, may enhance the persistence of dung-dwelling helminth
32   parasitic stages.
33   2. To test this possibility, a large-scale field trial was conducted in SW England.
34   The availability of infective parasite helminth larvae (L_3) was investigated
35   on the herbage around 240 artificial 1 kg dung pats that had been
36   constructed from the faeces of beef cattle with naturally acquired strongyle
37   infections. Herbage up to 15 cm surrounding each pat was sampled at 2, 4,
38   6, 8 and 10 weeks after deposition. Pats were subject to enhanced, natural
39   or no dung beetle colonisation and uncontrolled or enhanced rainfall.
40   3. Under uncontrolled rainfall conditions, 2 weeks after pat deposition,
41   significantly more L_3 were recovered from around pats that were exposed
42   to beetle colonization than from pats that were not colonised. However, by
43   week 8, significantly fewer L_3 were recovered from around pats that were
44   exposed to beetle colonization compared to uncolonized pats.
45   4. Under conditions of enhanced rainfall, pats yielded significantly more L_3
46   than under uncontrolled rainfall conditions, and there were no differences
47   in recovery from herbage around pats with enhanced, natural, or no beetle
48   colonization.
49   5. The data suggest that over the duration of a summer grazing season,
50   temperate habitat dung colonizing insect communities, which include
51   mainly small endocoprid dung beetles of the genus *Aphodius,* can reduce the
52   development and survival of livestock gastrointestinal parasites on
53   pastures, but that this can be overridden by the effect of high rainfall.
54   6. *Synthesis and applications*. The work demonstrates that conservation of
55   dung beetle populations in temperate climates is important in livestock
56   management, not only for their essential role in dung degradation and
57   nutrient cycling, but because their activity can also reduce the survival and
58   availability of gastrointestinal parasites on pastures.

2

Introduction

The management of gastrointestinal parasite infection is one of the most common and economically important challenges in livestock production (Charlier *et al.* 2009). Helminth eggs are shed from a parasitized host and then hatch and develop in the faeces until the infective third stage larvae (L_3) migrate away from the dung to the surrounding herbage, where they are ingested by a ruminant host, thereby completing the cycle (Smith & Grenfell 1985).

The successful development, survival and migration of helminth larvae depends on environmental factors such as temperature and moisture (Stromberg 1997). For example optimal conditions for development of the cattle strongylids *Ostertagia ostertagi* (Stiles) and *Cooperia onchophora* (Railliet) are 23 °C and a 60-65 % faecal moisture content (Rossanigo & Gruner 1995). Any factors that make environmental conditions within a dung pat less suitable for larval survival are likely to reduce parasitic helminth populations and contribute to their management. Such effects have been attributed to the burrowing and burying activity of coprophagous beetles; beetle activity in surface faeces may speed up desiccation and aeration, making the dung unfavourable for L_3 development and migration (Houston, Craig & Fincher 1984). Indeed, the contribution of beetle activity to the ecosystem service of gastrointestinal parasite management has been estimated to save UK farmers £188 million per year in conventional cattle farming systems, at almost £20 per cow per year (Beynon, Wainwright & Christie 2015). However, while some work has supported the assumptions on which this calculation was based, the inherent complexity of interacting environmental and ecological factors make the role of beetles in helminth control difficult to demonstrate clearly.

In laboratory studies, the presence of endocoprid (dung dwelling) beetles was associated with a significantly greater recovery of *C. oncophora* and *O. ostertagia* L_3 from cattle faeces after 12 days, compared to beetle-free control dung (Chirico, Wiktelius & Waller 2003). However, over the subsequent 12 days, this study found that L_3 recovery from the beetle-free dung increased significantly, whereas recovery from beetle colonised dung did not. An average reduction in faecal egg counts of 54% was recorded in 5-30 g sheep dung after 22-49 h of activity by 12-24 *Aphodius* spp. in the laboratory (Bergstrom, Maki &

98   Werner 1976). However, laboratory studies that sample L_3 directly from dung

99   may be of limited value, since ingestion by the host and hence parasite

100 transmission, is dependent on the availability of infective stage larvae on the

101 herbage. Field studies, undertaken during an Australian summer, showed 60%

102 reductions in L_3 recovery from herbage around strongyle-infected horse faeces;

103 naturally colonised 1 kg faecal masses were compared to insect-free faeces and

104 the difference was attributed to the activity of the paracoprid (dung burying)

105 beetle, *Onthophagus gazella* (English 1979). Similar results were reported by

106 Mfitilodze & Hutchinson (1988). Studies with paracoprid beetles are similarly

107 difficult to interpret because strongyle larvae may protect themselves from

108 desiccation by migrating into the soil, only moving to the herbage when

109 conditions are adequately moist, which may be many months after faecal

110 deposition (Bryan 1973, Krecek & Murrell 1988). Thus, the burial of

111 contaminated dung by beetles may result in greater numbers of parasitic larvae

112 being available in the long-term (Houston, Craig & Fincher 1984, Bryan & Kerr

113 1989). Any role of dung beetles in contributing to reduced parasite challenge

114 depends therefore on the habitat, climate and the species of beetles available.

115 Understanding these relationships is important in efforts to promote the

116 sustainable management of grazed pastures. This is of particular current

117 concern given that many of the anthelmintics administered to livestock,

118 particularly the macrocyclic lactone compounds, are excreted almost unaltered

119 in the faeces where they continue to exert an insecticidal effect, threatening dung

120 beetle populations (Floate *et al.* 2005).

121   The aim of the present work, therefore, was to examine the effect of

122 colonisation of cattle faeces by dung insects, particularly beetles, on the

123 development of strongyle eggs within dung and the availability of infective

124 larvae for migration onto pasture in a temperate habitat pasture system. In this

125 environment, the majority of the beetles colonising pats are endocoprid dung-

126 dwellers. The work compared the numbers of strongyle larvae in the herbage

127 surrounding dung pats that were colonised naturally by dung insects, pats where

128 dung insect colonisation was excluded and pats where the numbers of

129 endocoprid beetles were artificially enhanced, under conditions of natural or

130 enhanced precipitation.

Materials and methods

BEETLES

Dung beetles were collected in May and June 2015 from farmland in SW England, using dung-baited pitfall traps: for this 15 cm diameter buckets were buried flush with the ground, and 2 cm aperture wire mesh was placed on top. Artificial pats were placed on top of the wire mesh using a 20cm diameter pat former and fresh faeces from organic South Devon and Red Poll cattle. A 20 cm diameter rain shield was placed approximately 20 cm above each trap. Captured beetles were identified to genus in the field and *Aphodius* and *Onthophagus* spp. were collected, and stored in well-ventilated plastic containers with washed sand as a substratum and fresh organic cattle and horse faeces for food. Beetles were also collected by hand-searching naturally deposited pats from the same herd.

FAECES

Faecal egg counts were performed on dung samples from a commercial herd of 60 organic cattle in SW England to confirm natural infection, using the mini-FLOTAC® method, which is accurate to 5 eggs per gram (Godber *et al.* 2015). None of the animals had been treated with anthelmintics for at least the previous 6 months. In early June 2015, 400 kg of fresh faeces was collected over a period of 2 days. At the end of each day the faeces was transported to the University of Bristol and stored in six 80 L plastic bins in a walk-in cold room (Cold Control Services, Ropley, UK) at 5°C. The following day all dung was combined and thoroughly mixed using a hand-held industrial plaster mixer (Silverline, Yeovil, UK). A 10g sample was taken from each bin of mixed dung for faecal egg counts.

Larval cultures were performed on the dung to identify the strongyle species present. Three 50 g samples were taken at random and placed in 14 cm diameter Petri-dishes in an incubator (Sanyo, London, UK) at 25 °C for 7 days. To prevent anaerobic conditions, which had been observed to prevent egg hatch in preliminary observations, 10 g vermiculite was mixed into each sample. Third stage larvae (L_3) were harvested from the dung using a modified Baermann technique (Gruner 1986). Faecal samples were suspended in muslin over 250 mL inverse conical flasks filled with water, and left to stand for 24 h. The muslin and faeces were then removed and the supernatant was siphoned off leaving 20

mL of sediment. The sediment was agitated and transferred to 50 mL polypropylene centrifuge tubes (Fisher Scientific, Loughborough, UK) along with washings from the beaker to minimise the loss of larvae and placed in a centrifuge (IEC CL10, Thermo Scientific, Loughborough, UK) for 2 min at 1500 rpm. The supernatant was siphoned off to leave 5 mL of sediment, which was agitated and transferred to a 17 mL test tube (Beckman Coulter, High Wycombe, UK) along with washings from the centrifuge tube. The test tubes were centrifuged for 2 min at 1500 rpm and the supernatant was drawn off leaving 1 mL of sediment. This was agitated and transferred into plastic sample tubes (30 mL) (Fisher Scientific, Loughborough, UK) along with washings from the test tube and made up to 10 mL with water.

The suspension was placed on a glass slide with a drop of Lugol's iodine solution. The first 100 parasitic larvae observed were identified to genus and where possible species. Identification was carried out under a microscope at 200 x and 400 x total magnification by measuring total length, length of sheath tail extension, proportion of sheath tail extension comprising a filament, and using the morphological identification guide of van Wyk & Mayhew (2013). The shape of the head and presence of refractile bodies in the head, as found in *Cooperia* spp., were also used for identification.

EXPERIMENTAL FIELD TRIALS

Immediately after mixing, the dung was transported to a 180 x 20 m plot of grassland in SW England, which had not been grazed by cattle for at least the previous 5 years. Nematode extractions were performed on grass samples from across the site to confirm that no parasitic larvae were present. The faeces was used to form 260 artificial pats that were placed on the pasture in rows 3.5 m apart with 3.5 m between each pat. Pats were created using a 20 cm diameter plastic former that held 1 kg of faeces. A 10 g sample of faeces was taken after forming every 26th pat for faecal egg counting, to confirm that the distribution of strongyle eggs was consistent between pats.

Three factors were manipulated in the experimental design: dung colonisation (no colonisation, natural colonisation, enhanced beetle colonisation), rainfall (natural, enhanced), and dung disturbance (mechanically

disturbed, undisturbed). Twenty pats were allocated to each of the 12 unique treatment groups at random across the entire site. A further 20 pats (10 with natural colonisation and 10 with enhanced beetle colonisation) were created and then selected at random and removed after 10 days; they were then placed in emergence traps to allow insect colonisation to be quantified.

Five of the beetles that had been collected in the pit-fall traps were added to each of the pats in the enhanced beetle treatment group. Each pat received 1 *Aphodius fossor* (Linnaeus), 3 other *Aphodius* spp. from a pool of *A. fimetarius* (Linnaeus)*, A. pedellus (*De Geer)*, A. sphacelatus (*Panzer)*, A. prodromus* (Brahm), *A. obliteratus* Panzer*,* and *A. contaminatus* (Herbst) and 1 *Onthophagus* similis (Scriba). This equated to adding approximately 67 mg dry beetle biomass per pat, an increase of 25% of the expected beetle biomass colonising pats under natural field conditions (Beynon *et al.* 2012).

In the mechanically disturbed treatment group a 2mm diameter rod was inserted into the pat to make 5 evenly spaced vertical 'tunnels' every day for the first 10 days. For the enhanced rainfall pats, water was applied to each pat three times per week for the duration of the entire experiment. A watering can and rose was held 1.5 m above the ground so that a 0.5 m diameter rain shadow was created over each 20 cm pat and surrounding 15 cm sampling area; 1 L of water was applied to each pat, over the 0.2 m^2 area; this equated to 5 mm of rainfall per application.

Immediately after formation of the 'no colonisation' pats, 30 cm diameter cages of 0.5 mm insect mesh were firmly pegged over them to prevent access by larger insects. In this treatment group, where pats were also to be disturbed mechanically and/or watered, the cages were removed, the treatment was administered and the cage was immediately replaced taking care that no beetles entered during this time. On day 10 all cages were removed to allow natural weathering of pats, as beetle colonisation was considered to be relatively unlikely after this time (Lee & Wall 2006). Pats to be placed in emergence traps were removed from the pasture on day 10 along with the underlying first 3 cm of soil. A weather station (OneCall, Leeds, UK) was placed at the field site to monitor temperature and precipitation throughout the trial.

GRASS SAMPLING

231 On day 14 after the construction of the pats, all the herbage in the surrounding

232 15 cm area was cut to ground level taking care that no root mass, soil, or dung

233 were included; the majority of infective strongyle larvae are expected to migrate

234 up to 15 cm from the faeces (Williams & Bilkovich 1973). In the afternoon of the

235 days prior to grass sampling, 2 L of water were applied to the pat and

236 surrounding sampling area to encourage migration of available L_3 onto the

237 herbage. Herbage was placed in grip seal polythene bags and returned to the

238 laboratory. Sixty pats, selected at random, were cut each day over a period of 4

239 days so that all 240 pats were sampled. Nematode extractions were immediately

240 set up on return to the laboratory, since the recovery of strongyle larvae from

241 herbage samples has been shown to decline over time (Fine *et al.* 1993). This

242 was repeated every 2 weeks for 10 weeks.

243

244 NEMATODE EXTRACTION FROM HERBAGE

245 A modified version of the Baermann technique was used to extract the third

246 stage parasitic nematode larvae from herbage (Gruner 1986). A 20 cm length of

247 rubber tubing, with a 2 cm internal diameter (Fisher Scientific, Loughborough,

248 UK), was attached to each of 60, 18 cm diameter plastic funnels and sealed with

249 silicone sealant. Plastic specimen pots (2 cm diameter, 30 mL) (Fisher Scientific,

250 Loughborough, UK) were pushed in to the other end of the rubber tubing. Grass

251 samples were wrapped in 30 x 30 cm squares of muslin cloth to form a loose ball

252 and secured with a rubber band. A wooden rod was passed through the band and

253 the samples were suspended over the funnels. Funnels were filled with water

254 that contained 2 ml of detergent per 12 L water, until 0.5 cm below the rim so

255 that the entire muslin bag was submerged. Funnels were left to stand for 24 h;

256 muslin bags were agitated by squeezing after 15 hours to encourage larvae to

257 exit the bag. Herbage samples were then removed from the funnels, the tubes

258 containing sedimented larvae were carefully collected from the ends of the

259 rubber tubing, and placed in a refrigerator (Liebherr, Biggleswade, UK) at 5 °C

260 for 1.5 h.

261 Water was siphoned off the top of each tube leaving 5 mL of sediment.

262 The sediment was disturbed and transferred to a 17 mL test tube (Beckman

263   Coulter, High Wycombe, UK) along with washings from the original tube to

264   minimise the loss of larvae. Test tubes were centrifuged for 2 mins at 1500 rpm.

265   The supernatant was siphoned off leaving 1 mL of sediment, which was

266   disturbed, transferred to 30 mL plastic specimen pots (Fisher Scientific,

267   Loughborough, UK) along with washings from the test tube, and made up to 10

268   mL with water. The resulting larval suspensions were stored at 5°C until

269   counted.

270

271   NEMATODE COUNTING AND IDENTIFICATION

272   Aliquots of 1 mL were taken from each larval suspension and transferred into a

273   Sedgewick Rafter nematode counting chamber with one drop of Lugol's iodine,

274   under $40 \times$ total magnification. Counting was repeated three times for each

275   larval suspension and the average used for analysis. Parasitic larvae were

276   differentiated from free-living larvae by the presence of a sheath, presence of a

277   tail filament, staining of the intestinal cells; shape of the head, refractile bodies in

278   the head and the absence of reproductive organs, using the morphological

279   identification guide of van Wyk & Mayhew (2013).

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281   STATISTICAL METHODS

282   All statistical analysis was performed using RStudio (Version 0.99.489, 2009-

283   2015) (R Core Team 2015). A generalized linear mixed model with a negative

284   binomial error distribution was performed using the glmer.nb function of

285   package 'lme4', with colonisation (none, natural, enhanced), dung disturbance

286   (disturbed, undisturbed), and rainfall (natural, enhanced) as fixed effects, and L_3

287   count as the dependent variable. Experimental week (2, 4, 6, 8, 10) nested within

288   individual pat was a random factor. Interactions between colonisation level,

289   dung disturbance, rainfall, and experimental week were included. The model was

290   simplified by stepwise removal of non-significant factors and the resulting

291   minimal model contrasted with Akaike's Information Criterion (AIC) to the

292   global model, as well as the conventional criterion of a statistically significant

293   change in deviance between the models indicating a poorer fit, until the best

294   fitting model was found (see Table S1 in Supporting Information).

295   T-tests were performed on the $\log_{10}$-transformed number (+1) of
296 *Aphodius* spp. recovered from emergence traps containing pats that were
297 exposed to either natural or enhanced beetle colonisation, and on faecal egg
298 counts of dung samples after the dung was homogenised, and after it had been
299 formed into pats. All means are presented ± their 95% confidence intervals,
300 unless otherwise stated.

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Results

FAECES

The dung collected from organic cattle and used to construct the artificial pats yielded an overall average of 106±12 strongyle eggs per gram (epg). Samples removed from each bin had an average of 102±16 epg and samples taken from every 26th pat in the field had an average of 109±17 epg; there was no significant difference between these groups (t_{14}=-0.6, P=0.57). Hence, mixing of the 400 kg of collected dung appeared to have adequately homogenised the sample and it can be assumed that each of the 260 pats created contained a statistically similar number of strongyle eggs.

After laboratory incubation for 7 days at 25°C, larvae recovered from the cattle faeces used in this experiment consisted of 66% *Cooperia oncophora,* 29% *Ostertagia ostertagi* and 5% *Cooperia* spp.; the latter were not identified to species level. It must be noted that this is only an indication of the relative abundance of the species present, since optimal culture conditions for different species of gastrointestinal parasite larvae may differ (Whitlock, 1956; Dobson *et al.*, 1992).

RAINFALL

Over the 10 week experiment the field site received 178.6 mm of rainfall, averaging 2.55 mm per day. The pats experiencing enhanced rainfall conditions received an extra 150 mm over the 10 weeks, giving them a combined average of 4.70 mm per day. Overall, the herbage surrounding pats in the enhanced rainfall group yielded significantly more L_3 than herbage surrounding pats in the natural rainfall group ($z=6.3_{1194}$, P<0.001) (Fig. 1).

WEEK

The number of L_3 recovered from the herbage around pats increased significantly over the first 6 weeks of the experiment ($z=5.0_{1184}$, P<0.001) then remained relatively high until week 10 (Fig. 2).

DUNG DISTURBANCE

360   There was no significant difference between the number of L_3 recovered from

361   herbage around pats that were disturbed or undisturbed, and dung disturbance

362   was not included in the final model.

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364   DUNG COLONISATION

365   There was a significant interaction between colonisation treatment, rainfall and

366   experimental week ($z=-3.6_{1184}$, $P<0.001$). At week 2, there was a significant

367   interaction between colonisation treatment and rainfall ($z=2.4_{232}$, $P<0.05$). Under

368   natural rainfall conditions, pats with no colonisation yielded significantly fewer

369   L_3 than pats with natural colonisation or enhanced beetle numbers ($t=-3.1_{2,\,117}$,

370     $P<0.05$) (Fig. 3). Under enhanced rainfall conditions, there was no significant

371   difference in the number of L_3 recovered from herbage around pats that had no

372   or natural dung insect colonisation, or enhanced beetle numbers (Fig. 3). At

373   weeks 4 and 6 there were no significant differences in the number of L_3

374   recovered from herbage around pats with no or natural insect colonisation, or

375   enhanced beetle numbers (Fig. 3). At week 8 there was a significant interaction

376   between colonisation level and rainfall ($t=-2.6_{2,\,234}$, $P<0.05$). Under natural

377   rainfall conditions, pats with no insect colonisation yielded significantly more L_3

378   than pats with natural colonisation or enhanced beetle numbers ($t=4.4_{2,\,117}$,

379     $P<0.001$) (Fig. 3). Under enhanced rainfall conditions there was no significant

380   difference in the number of L_3 recovered from herbage around pats that had no

381   or natural colonisation or enhanced beetle numbers. At week 10, under natural

382   rainfall conditions pats with no insect colonisation also yielded significantly

383   more L_3 than pats with natural insect colonisation or enhanced beetle numbers

384   ($t=2.1_{2,\,234}$, $P<0.05$) (Fig. 3). As at week 8, under enhanced rainfall conditions at

385   week 10, there was no significant difference in the number of L_3 recovered from

386   herbage around pats that had no or natural colonisation or enhanced beetle

387   numbers.

388

389   BEETLE SPECIES RECOVERED FROM EMERGENCE TRAPS

390   Pats in this experiment were colonised by an average of 20 *Aphodius* spp. each,

391   and had an average insect abundance of 199 including all Coleoptera and

392   Diptera. A total of 407 *Aphodius* belonging to seven species were recovered from

393   the emergence traps and no *Onthophagus* spp. (Table. 1). There was no

394   difference in the abundance of *Aphodius* spp. recovered from the traps covering

395   the naturally colonised pats or the pats with enhanced beetle numbers (t=-

396   0.33_{17}, *P*= 0.75). Pat colonisation approached a negative binomial distribution

397   pattern (Fig. 5).

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Discussion

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427 Most of the field studies on the impact of beetles on strongyle abundance have

428 focused on dung-burying paracoprids (Bryan, 1973; Fincher, 1973; English,

429 1979; Bryan & Kerr, 1989; Nichols & Gómez, 2014). The present study examined

430 a temperate system, where endocoprid dung-dwellers predominate (Jessop,

431 1986). In the present study, larvae had begun to migrate out of the pats in low

432 numbers after 2 weeks in the field, increasing over weeks 4 to 6 and reaching a

433 maximum by 8 weeks. Migration remained high for at least 10 weeks, as has

434 been reported previously in the UK (Ogbourne 1972). The results show that the

435 impact of dung colonising insects on the numbers of strongyle larvae available to

436 emerge on to pastures is complex, changes over time and is strongly affected by

437 environmental conditions. Under conditions of natural rainfall, in the first two

438 weeks after dung pat construction, naturally colonised pats and those with

439 additional beetles gave rise to a significantly greater number of *C. oncophora* and

440 *O. ostertagi* L_3 on pasture herbage than uncolonised dung. Similarly, laboratory

441 studies in Sweden by Chirico, Wiktelius & Waller (2003), using 500 g aliquots of

442 cattle faeces naturally infected with trichostrongylids, mainly *C. oncophora* and

443 *O. ostertagi,* to which forty endocoprid beetles (20 *Aphodius rufipes* and 20 *A.*

444 *scybalarius*) had been added found that after 12 days a significantly greater

445 number of L_3 were recovered from the dung with beetles than from beetle-free

446 control dung. Strongyle egg hatch is dependent on oxygen availability (Albrecht

447 1909, Brown 1928, Nielsen *et al.* 2010), so it is possible that beetle activity

448 initially provides aeration within freshly deposited faeces, preventing

449 unfavourable anaerobic conditions from developing in the pat. This is especially

450 likely in moist temperate climates. However, over time in the present study the

451 initial pattern reversed; by week 8 significantly more L_3 were recovered from

452 herbage around pats that were not colonised, compared to naturally colonised

453 pats and those with additional beetles, under natural rainfall conditions. This

454 pattern persisted until the end of the experiment - at least 10 weeks after pat

455 deposition. A similar reverse was also seen by Chirico, Wiktelius & Waller

456 (2003). In Australia strongyle larvae continued to migrate out of pats that were

457 not colonised by dung beetles after 28 weeks but this was reduced to 24, 18 and

14

458   1.6 weeks for pats with minimal, moderate, and maximum natural beetle

459   colonisation (Bryan and Kerr 1989).

460   The mechanism through which dung colonising insects might impact

461   strongyle larvae remains unclear. Parasite egg hatch and larval development in

462   the dung requires moisture, and migration of L_3 onto pasture herbage is

463   dependent on adequate moisture to provide a film to facilitate movement

464   (Stromberg 1997; Genever & Davies 2011). It is possible therefore that

465   colonised pats undergo more rapid desiccation than uncolonised pats, leading to

466   high mortality. This has been demonstrated for example in Australia; 1 kg pats

467   of infected cattle faeces had a significantly lower water content after 7 days

468   exposure to natural colonisation than faeces protected by insect mesh

469   (Mfitilodze & Hutchinson, 1988). In temperate climates such as the UK, this

470   process is likely to occur over several weeks rather than days. It was in an

471   attempt to demonstrate this mechanism that some of the pats were disturbed by

472   hand with a rod in an attempt to speed up desiccation in a similar manner to

473   beetle 'tunnels'. No effect of this treatment was observed. However tunnels were

474   only created for the first 10 days after pat construction, whereas colonised pats

475   began to yield fewer L_3 than uncolonised pats only after eight weeks. So it is

476   possible that the mechanical disturbance applied was insufficiently prolonged.

477   Several authors have suggested that the ingestion of strongyle eggs and larvae by

478   beetles may be responsible for some mortality (Miller, Chi-rodriguez & Nichols

479   1961; Bergstrom, Maki & Werner 1976; Grønvold *et al.* 1992). However, the

480   maximum particle size ingested by species of large paracoprid, *Copris amyntor*

481   and *Copris elphenor* was shown to be 20-45 μm and <5-50 μm by small

482   endocoprids such as *Aphodius* (Holter, Scholtz & Wardhaugh 2002). Strongyle

483   eggs are approximately 90 x 40 μm in size (Cuomo, Lawrence & White 2012).

484   Hence, ingestion seems unlikely. Dung beetles probably avoid larger particles,

485   which are mostly indigestible plant remains, and remove the smaller more

486   nutritious particles which include bacteria and dead epithelial cells from the

487   mammal gut (Holter 2000; Holter, Scholtz & Wardhaugh 2002).

488   The rate of development of gastrointestinal parasite larvae on pastures

489   depends on temperature, optimal conditions being around 25°C for *O. ostertagi*,

490   but without moisture larvae do not develop (Stromberg 1997). Similarly the

491   migration of L_3 away from faeces on to the herbage is dependent primarily on

492   moisture, with temperature as the second most important factor (Krecek,

493   Murrell & Douglass 1990; Stromberg, 1997). Splash dispersal by rain also has a

494   role in the movement of infective larvae from faeces to the surrounding herbage

495   (Grønvold 1984; Grønvold *et al.* 1992). L_3 release is therefore highly dependent

496   on weather conditions. The field site received an average of 2.6 mm rain per day

497   over the 10-week trial and the enhanced rainfall pats received 4.7 mm per day.

498   Average rainfall (1981-2010) over the same June, July, August period for this

499   area is approximately 1.9mm per day (Metoffice.gov.uk, 2016). The data

500   presented here therefore suggest that under normal rainfall conditions, dung

501   beetles will significantly reduce the availability of the infective stages of livestock

502   gastrointestinal parasites on pastures, but that during periods of very high

503   rainfall L_3 numbers are likely to be high regardless.

504   Under conditions of natural rainfall the differences between the colonised

505   and uncolonised dung in the median numbers of L_3 counted in the herbage at 8-

506   10 weeks were approximately 29%, supporting the figure of 31% used by

507   Beynon, Wainwright & Christie (2015) in their calculation of the estimated

508   reduction in strongyle numbers by dung beetles. However, effects of colonisation

509   on L_3 availability was a dynamic process and the overall difference was lower in

510   total; the difference in the total L_3 population between colonised and

511   uncolonised dung over the entire 10 weeks was 19%. It should also be noted that

512   the difference of 29% in strongyle numbers between colonised and uncolonised

513   dung pats at 8-10 weeks was the effect of the entire dung colonising insect

514   community; the inset mesh also excluded Diptera, the direct impacts of which on

515   strongyle larvae are unknown. Hence estimates of the economic value of the

516   decomposition ecosystem service provided by dung beetles in temperate

517   climates need to reflect the subtlety of this effect.

518   A total of 407 dung beetles of seven species in the genus *Aphodius* were

519   recovered from the 20 pats placed into emergence traps, and colonisation

520   showed a highly aggregated distribution; significant levels of aggregation have

521   been recorded previously in the majority of coleopteran and dipteran dung

522   colonising taxa in SW England (Wall and Lee 2010). High replication is therefore

523 important in studies that consider the effects of natural dung colonisation on the

524 development and transmission of strongyle larvae. In the weeks prior to the

525 trial, approximately 500 beetles were collected in pitfall traps, which allowed 5

526 beetles to be added to each of the enhanced beetle colonisation pats (n=80) and

527 the 10 enhanced colonisation pats to be placed in emergence traps, thereby

528 increasing their beetle abundance by 25% on average. Had greater numbers of

529 beetles been added to the pats, the impact on strongyle larval availability may

530 have been more pronounced. Clearly given the almost bimodal distribution of

531 beetle colonisation seen, beetle populations of up to 80 per pat would be within

532 the normal range, but testing different beetle densities was outside the scope of

533 this study. Nevertheless, the shape of the beetle colonisation distribution

534 emphasises the considerable heterogeneity that there is likely to be in the

535 impacts of beetles on strongyle availability within a single field.

536   The data presented here suggest that dung-colonising insect communities

537 in temperate climates, which mainly include small endocoprid dung beetles in

538 the genus *Aphodius*, will reduce the development and survival of livestock

539 gastrointestinal parasites on pastures over the summer grazing season. The use

540 of anthelmintics to control gastrointestinal parasites in livestock is

541 commonplace, however many of these chemicals are excreted in the faeces

542 largely unmetabolised, where they cause mortality in dung-colonising insects

543 and their larvae (Steel & Wardhaugh, 2002). The conservation of dung beetles in

544 temperate climates is therefore important in livestock management, not only for

545 their role in dung degradation and nutrient cycling, but because they can

546 contribute to the reduction in abundance of economically deleterious

547 gastrointestinal parasites. Livestock management practices should focus on

548 reducing reliance on anthelmintics to minimise damage to natural dung beetle

549 populations.

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559 **Data Accessibility**

560 Experimental data: Dryad Digital Repository (Sands & Wall 2016).

561 **Supporting Information**

562 Additional supporting information may be found in the online version of this
563 article.

564 **Table S1.** Model simplification using AIC (Akaike's Information Criterion) and
565 Chi Squared P values for change in deviance, for identification of the best fitting
566 negative binomial generalized linear mixed model for statistical analysis.

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702

Table 1. Insect abundance recovered from pats that had been removed from the field 10 days after deposition and placed in emergence

traps. There were ten replicates of each of naturally colonised and enhanced beetle pat.

705

Treatment	Natural colonisation										Enhanced beetle colonisation										
Repeat	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	
Aphodius fossor	–	2	–	–	47	–	–	–	4	–	88	33	1	–	–	1	2	16	–	–	
Aphodius fimetarius	–	2	3	1	–	1	2	1	3	1	4	2	6	2	–	–	2	–	2	–	
Aphodius pedellus	–	–	–	1	–	2	–	1	–	–	–	1	4	2	–	–	–	–	2	–	
Aphodius ater	–	2	–	–	–	–	–	2	–	–	–	–	–	–	–	1	–	–	–	–	
Aphodius haemorrhoidalis	90	14	–	23	2	–	–	–	2	–	1	–	–	2	8	–	–	11	2	4	
Aphodius erraticus	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
Aphodius depressus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	
Aphodius sp. (head only)	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
Sphaeridium scarabaeoides	4	10	4	–	–	2	4	3	3	5	2	6	6	–	2	1	6	5	6	6	
Sphaeridium bipustulatum	–	5	–	1	1	1	–	–	–	–	1	–	–	1	–	–	–	–	–	3	
Sphaeridium lunatum	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	1	3	–	
Megasternum obscurum	5	4	5	3	18	25	2	17	24	9	8	25	4	26	7	9	19	4	2	17	
Cercyon spp.	4	4	3	2	8	14	1	6	2	1	4	3	5	2	–	1	–	7	6	7	
Paralister spp.	2	–	–	–	–	–	3	3	–	–	–	–	2	–	–	3	–	–	4	–	
Staphylinidae	88	31	54	55	86	55	25	61	22	51	40	47	87	23	47	30	33	76	29	26	
Carabaeidae	–	4	2	–	–	5	–	–	1	3	–	3	–	–	1	2	2	2	–	2	
Diptera	47	67	48	85	168	191	113	77	39	51	54	69	109	44	57	62	276	73	236	128	
Ptilidae	–	10	10	14	–	–	3	6	18	6	–	–	7	34	12	–	4	15	–	21	**Average (±CI)**
Insect abundance	240	155	129	185	332	297	154	177	118	127	202	189	231	136	134	111	344	210	292	214	198.85 ± 31.37
***Aphodius* spp. abundance**	90	20	3	25	51	4	2	4	9	1	93	36	11	6	8	3	4	27	6	4	20.35 ± 12.12

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Figure legends

710 **Fig. 1.** The numbers of infective strongyle larvae (L_3) recovered from the 15 cm area around pats that received natural rainfall or

711 enhanced rainfall. The median L_3 is displayed within boxes representing the first and third quartiles; whiskers show 95% confidence

712 intervals, outside of which fall the outlying points.

713

714 **Fig. 2.** The numbers of infective strongyle larvae (L_3) recovered from the 15 cm area of herbage around pats every 2 weeks over the 10-

715 week experimental period 11th June 2015 – 25th August 2015. Median L_3 is displayed within boxes representing the first and third

716 quartiles; whiskers show 95% confidence intervals, outside of which fall the outlying points.

717

718 **Fig. 3**. The numbers of infective strongyle larvae (L_3) recovered from the 15 cm area of herbage around pats under (a) natural and (b)

719 enhanced rainfall conditions, over the 10-week period from 11th June 2015 – 25th August 2015. Pats were exposed to no insect

720 colonisation (white box plots), natural insect colonisation (light grey), or natural insect colonisation plus enhanced beetle numbers

721 (dark grey). Median L_3 is displayed within boxes representing the first and third quartiles; whiskers show 95% confidence intervals,

722 outside of which fall the outlying points.

723

724 **Fig. 4** Frequency distribution of the number of dung beetles of the genus *Aphodius* recovered from 1 kg pats of naturally colonised cattle

725 faeces.

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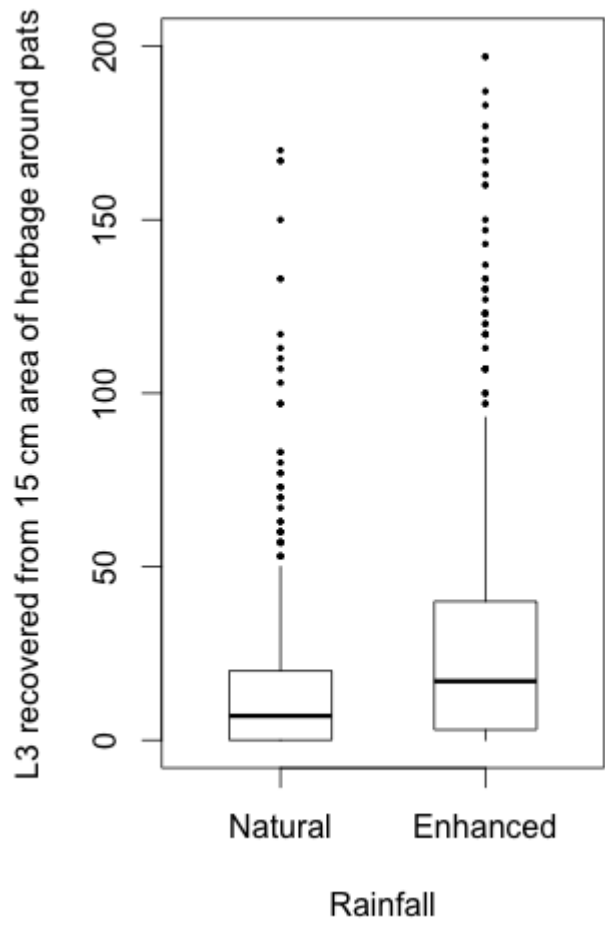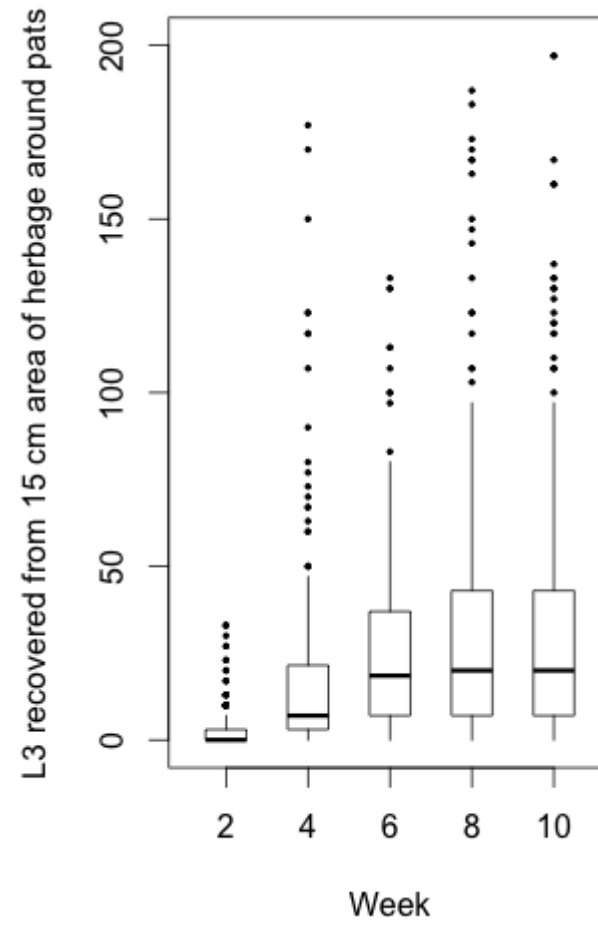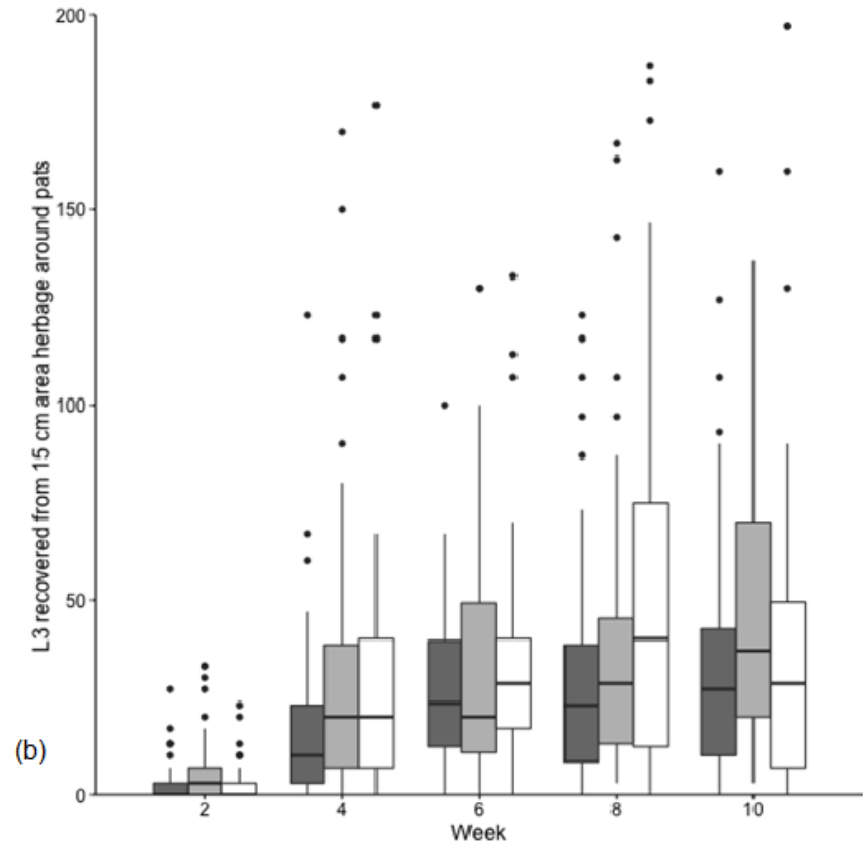731

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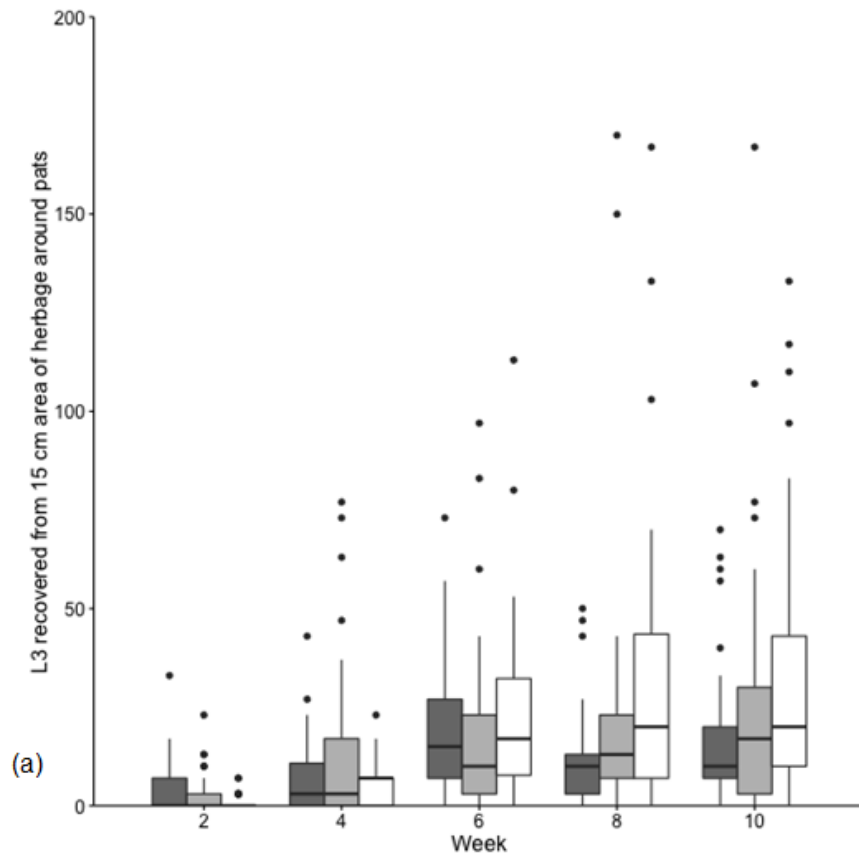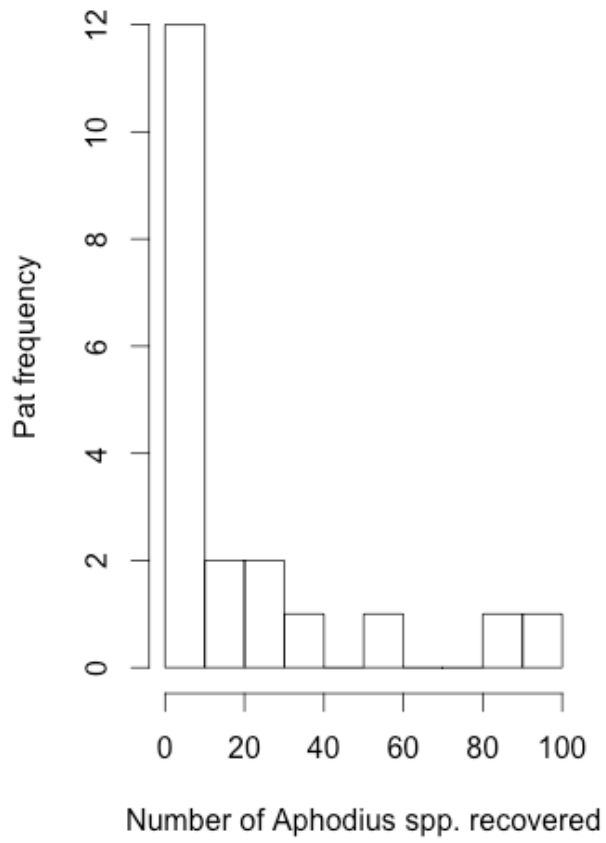734

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Fig. 1

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744   **Fig. 4**

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