Effects of ivermectin residues on dung invertebrate communities in a UK farmland habitat

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Abstract

1. Ivermectin is a broad-spectrum anti-parasitic drug, routinely administered to livestock worldwide, and concerns have been raised about its impacts on non-target dung fauna and pasture systems. This paper reports the effect of sward structure (long sward, short sward or bare ground) on ivermectin persistence and cowpat colonisation by invertebrates, during an on-farm experiment in the UK.

2. The levels of ivermectin in cowpats were high (21,899 µg/kg one day after treatment with a pour-on formulation) and remained detectable throughout the 47 day trial. Residue breakdown occurred, but levels persisted above those lethal to some invertebrates. Sward structure had no significant effect on ivermectin levels.

3. Ivermectin residues affected cowpat colonisation. Diptera were present in significantly lower numbers in treated cowpats, whereas coprophagous Coleoptera were present in significantly higher numbers in treated cowpats in the majority of cases. This attractive effect on coprophagous Coleoptera was most pronounced in the sward environments.

4. The non-target effects of pesticides are currently of concern to policy makers. The results of this research add further weight to these concerns, particularly with regard to the duration for which ivermectin persists *in situ* in UK pasture, and because of the preferential attraction exhibited by coprophagous Coleoptera.

Key words: Ivermectin; Breakdown, Biodiversity; Invertebrate diversity; Dung fauna; Habitat.

Introduction

Ivermectin, a macrocylic lactone belonging to the avermectin group of chemicals, is a broad-spectrum anti-parasitic drug, which is routinely administered to livestock throughout the world. While it offers an efficient and economical method for treating and controlling parasitic diseases, concerns have been raised about its possible impact on biodiversity in farmland systems (Floate *et al*., 2005; Wall & Strong, 1987). In particular, the long-term effects on non-target dung fauna and pasture systems remain unclear (Floate *et al*., 2005; Lumaret & Errouissi, 2002; Suarez *et al*., 2003), especially in temperate climates (O'Hea *et al*., 2010).

After administration, the breakdown of ivermectin by metabolism is generally moderate, and between 62 and 98% of the ivermectin used in treatment may be excreted unaltered in the faeces (Floate *et al*., 2005: Kryger *et al*., 2005). While few studies have quantified ivermectin levels in faeces, it is evident that ivermectin does not rapidly degrade, and remains at concentrations considered harmful to coprophagous fauna for long periods. Suarez *et al.* (2003) detected residues of 13 µg/kg in cowpats after 180 days in an Argentinian field, and Sommer & Steffansen (1993) reported that 84% of the ivermectin initially excreted remained in cowpats after 45 days in a Danish pasture.

Residues of ivermectin are known to affect dung colonisation by Coleoptera and Diptera, but relatively few studies have investigated this effect (O'Hea *et al*., 2010), and research to date has produced conflicting results (Floate, 2007). It has been demonstrated that certain insects may avoid dung from treated cattle (Floate, 1998a, 2007; Holter *et al*., 1993; Suarez *et al*., 2003; Wall & Strong, 1987; Webb *et al*., 2010), while others may preferentially colonize it (Floate, 2007; Holter *et al*., 1993; Lumaret *et al*., 1993; Wardhaugh & Mahon, 1991), both of which have implications for dung breakdown, insect survival and long-term pasture health.

Ivermectin has been shown to have a significant effect on temperate dung beetles. O'Hea *et al.* (2010) found that ivermectin residues significantly slowed the development of *Aphodius* species, with ivermectin levels as low as 200 μg/kg (wet weight) reducing the percentage of *A. ater* developing beyond larvae instar III to just 15%. Ivermectin has also been shown to cause mortality of *A. constans*, with the LC50 for first instar larvae being determined at between 420 and 692 μg/kg (dry weight) by Lumaret *et al.* (2007), and between 880 and 980 μg/kg (dry weight) by Hempel *et al.* (2006).

Diptera are particularly sensitive to ivermectin, and concentrations as low as 1 μg/kg are toxic to common species such as the yellow dung fly (*Scathophaga stercoraria*) (Strong & James, 1993). West & Tracy (2009) reported a significant reduction in *S. stercoraria* pupation in cowpats containing ivermectin, with only 28% of flies pupating when ivermectin levels were 0.2 μg/kg (wet weight). The median effective concentration (EC50) for 50% egg-to-adult mortality for *S. stercoraria* was determined by Römbke *et al.* (2009) as 20.9 $\pm$ 19.1 μg/kg (fresh weight).

Researchers have highlighted the need for studies into the effects of ivermectin under a wider variety of conditions (Suarez *et al*., 2003). Studies in the UK are particularly important, since there has been limited research in temperate climates, with the majority of recently published literature relating to tropical countries and tropical species, as noted by O'Hea *et al.* (2010) and Webb *et al.* (2010). There has also been a call for greater understanding of the effects that landscape features such as field size, boundary type and sward height, have on the persistence and impact of avermectins in the environment (Webb *et al*., 2007). Pastures are non-uniform environments, varying in terms of vegetation height and cover – factors that alter microclimate and insect activity, survival and reproduction (Hutton & Giller, 2003; Vessby, 2001), and possibly the persistence and breakdown of ivermectin (Halley *et al*., 1989b). Despite this, no studies have been carried out to determine the effect of sward structure on ivermectin persistence or cowpat colonisation.

The overall objective of this study was to assess ivermectin persistence, and how it affects cowpat colonisation, in three different sward structures commonly found in and around temperate pasture ecosystems in the UK.

Method

Study area

The experiment was carried out on a small mixed beef and dairy farm in Shustoke, east of Birmingham, England (SP 230901, 52°30'N, 01°39' W), between the 22nd April and 10th June 2005. The farm was selected since it was representative of a typical livestock farm which routinely administers ivermectin pour-on formulas for the treatment of internal and external parasites.

Livestock treatment

On the 22nd April 2005, ten young beef calves (continental cross – Limousin with Friesian) aged between 12 and 24 months were treated with 500 µg per kg weight with an ivermectin pour-on formula (Noromectin, produced by Norbrook, UK, which contains ivermectin 0.5% w/v), while ten calves remained untreated as the control group. Treatment occurred when local farmers were administering antiparasitic drugs to their cattle, to coincide with the time that cattle are turned out to grazing at the end of April or beginning of May (Webb *et al*., 2007). The calves' diet from early April was grass pasture.

Faecal collection and cowpat preparation

Approximately 60 litres of dung was collected from each treatment group the day after treatment (day zero). The dung collected from each group was carried to the field site where it was homogenised by group, on a large plastic sheet, to minimise the variation that would otherwise occur between animals within each treatment. For each treatment group, the dung was then made into 60 experimental cowpats, each with a wet weight of 1kg and diameter of 20 cm. A 20g sample of untreated and treated dung was collected and stored at -20°C for later analysis to determine the ivermectin level on day zero.

Experimental design

Three areas within a single pasture, bordered by hedges on all four sides, were selected to represent typical long sward, short sward and bare ground areas commonly found in pastures. In each sward type, two replicate plots of 3m by 7m were marked out using string and pegs, thus giving a total of six plots.

Within each plot, 10 cowpats from each treatment were spaced in an alternating sequence, one metre apart, across three rows. This layout was used to intersperse treatments in space, since cowpat colonisation by various insects is not uniform, and because Barth (1993) recommends that cowpats of the same treatment should not be placed in groups, but rather varied to minimise effects of location.

Cowpat collection

The experimental cowpats were collected from the field 1, 4, 10, 24 and 47 days after deposition day (day 0). These collection dates were chosen on the basis that more intensive sampling should be conducted during the early stages of the experiment because insect activity is greatest during this period (Skidmore, 1991). The experiment was terminated at day 47, because after 45 days in temperate pastures it is unlikely that coprophilous invertebrates will colonise any remaining dung (Skidmore, 1991).

On each collection date, two randomly selected cowpats were removed from each replicate of each treatment, and hence 24 cowpats were collected on each visit. Approximately 20g of dung was immediately removed from the centre of each cowpat, sealed in a small air tight polythene bag and stored at -20^{o}C to enable the later determination of drug residues. The remainder of each cowpat was transferred to the laboratory in a large polythene bag where it was stored at 5^{o}C overnight until analysed.

Invertebrate sampling

Invertebrates in the cowpats were hand sorted in a white tray and individuals were stored in a fridge until they could be identified and counted. Coprophagus Coleoptera species were identified to species level. Coprophagus Diptera larvae were also recorded, but could not be identified to species level, so were grouped prior to analysis.

Drug residue determination

High-performance liquid chromatography (HPLC) was used to determine ivermectin levels in the dung, based on the method developed by Payne *et al.* (1995) and Asbakk *et al.* (1999). Initial ivermectin levels were determined using dung collected on the day the experiment was set up (day zero), and mean levels at time points thereafter were calculated using the four cowpats retrieved from each treatment and sward structure on the subsequent collection days.

Sward structure features

Several physical parameters were monitored in the field since they influence dung degradation and insect activity. On each collection day, sward height, and temperature and humidity 1 cm above the ground were measured at three randomly determined points in each sward structure plot to capture the microclimatic conditions at the cowpat.

Statistical analysis

Sward height, air temperature and humidity measurements on each collection date were compared across sward structures using one-way ANOVA followed by Tukey's multiple comparison test.

Ivermectin residue levels were calculated as concentrations per 10g dry weight to remove the variability of the dung water content. Split plot analysis was carried out to determine the effect of sward type (main plots) and time (sub plots) on ivermectin levels.

To determine whether there was an attractive or repellent effect on each species of coprophagous Coleoptera, and on total Diptera recorded, the numbers recovered from treated and untreated cowpats were bulked and compared using Chi-square analysis assuming equal numbers. Species with less than 10 individuals were excluded from analysis.

Results

Sward structure

The average sward height, air temperature and humidity recorded in each sward structure, on each collection day, are presented in Table 1. As expected, sward height varied significantly between the three sward structures ($P < 0.01$). Temperature also varied significantly between sward structures ($P < 0.01$), with the long sward habitat being significantly warmer than the short sward and bare ground habitats. Humidity also varied significantly between sward structures ($P < 0.01$), with the bare ground habitat being significantly less humid than the long and short sward habitat.

Table 1. Mean (and range) sward height, and temperature and humidity one centimetre above ground level, on each collection day (n=6).

Sward structure	Day	Sward height (cm)	Temperature (°C)	Humidity (%)
Long sward	1	12.5 (10.0-14.0)	13.4 (12.9-13.6)	66.4 (66.1-66.6)
	4	10.0 (7.0-12.0)	11.4 (10.9-12.0)	92.2 (88.9-95.2)
	10	9.3 (4.0-12.0)	19.1 (17.6-20.5)	58.5 (49.0-65.0)
	24	12.8 (8.0-21.0)	17.8 (17.2-18.2)	60.4 (59.3-61.2)
	47	10.8 (8.0-15.0)	11.4 (11.1-11.7)	86.4 (85.8-86.2)
Short sward	1	2.5 (2.0-3.0)	12.4 (12.0-12.9)	71.7 (71.4-72.0)
	4	2.0 (0.0-3.2)	11.4 (10.9-11.7)	88.6 (87.9-88.9)
	10	3.3 (2.5-4.2)	16.1 (15.7-16.5)	63.1 (55.0-64.9)
	24	3.5 (2.8-4.0)	18.5 (17.8-19.4)	53.7 (52.9-54.9)
	47	2.9 (2.0-4.0)	11.8 (11.5-12.1)	80.6 (79.2-81.8)
Bare ground	1	0	11.7 (11.4-12.0)	72.2 (71.8-72.5)
	4	0	10.9 (10.6-11.1)	85.0 (84.7-85.3)
	10	0	16.5 (15.9-17.5)	61.3 (60.4-62.0)
	24	0	18.6 (18.4-18.7)	52.6 (51.9-53.1)
	47	0	11.6 (11.5-11.7)	74.5 (72.0-75.9)

Ivermectin residues

The ivermectin level in the faeces from treated cattle one day after treatment with Noromectin, averaged 21,899 µg/kg dry weight. There was no significant difference between sward structures on ivermectin levels ($F_{2,3} = 2.38$, $P > 0.05$). Ivermectin levels reduced significantly through time ($F_{4,36} = 16.73$, $P < 0.001$), and there was no significant interaction with sward structure ($F_{8,36} = 0.84$, $P > 0.05$).

The control cowpats, collected from the untreated cattle, contained no ivermectin residues (0 µg/kg dry weight) throughout the trial.

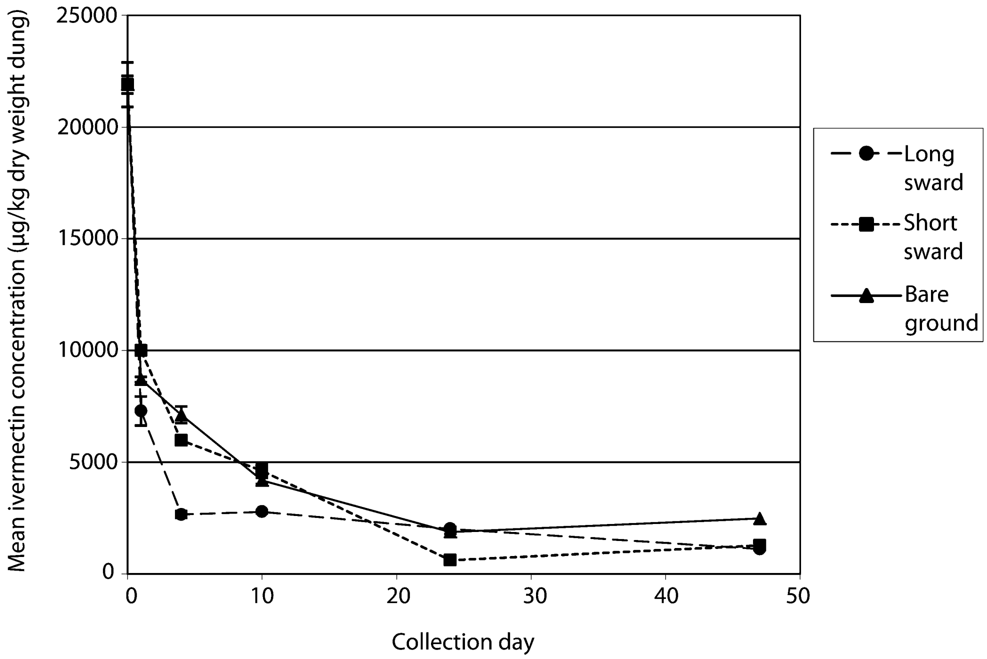

Figure 1. Mean ivermectin concentrations in cowpats (µg kg^{-1} dry weight) (n = 4).

Invertebrate presence

Non-dipteran invertebrates were found in higher numbers in treated cowpats at the majority of collection dates (Figure 2), and Diptera larvae were found in greater numbers in untreated cowpats in all sward structures (Figure 3).

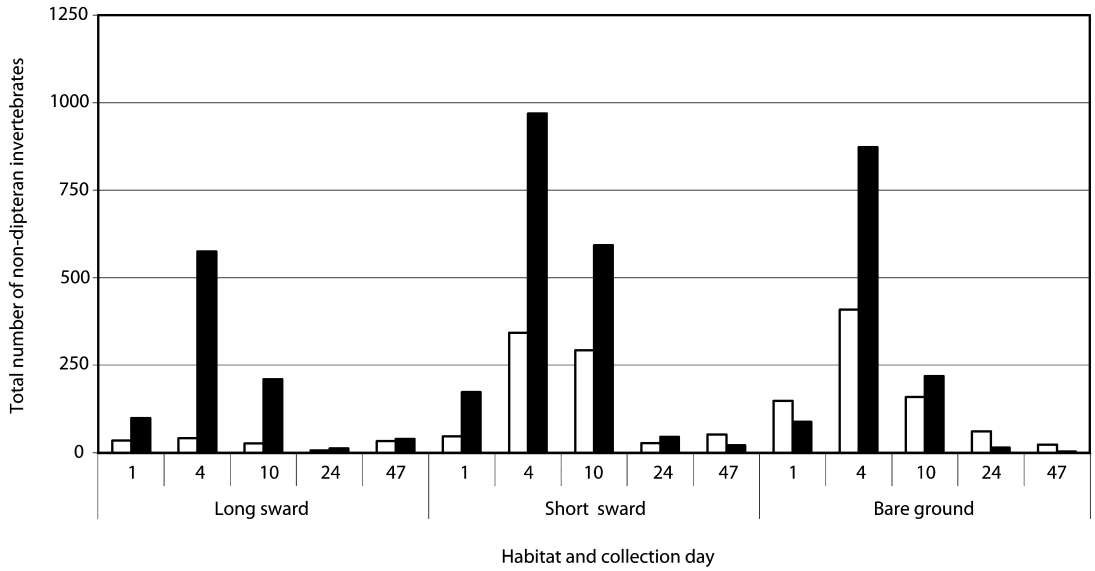

Figure 2. Total number of non-dipteran invertebrates collected from untreated cowpats (white bars) and treated cowpats (black bars) in the three sward structures after 1, 4, 10, 24, and 47 days in the field.

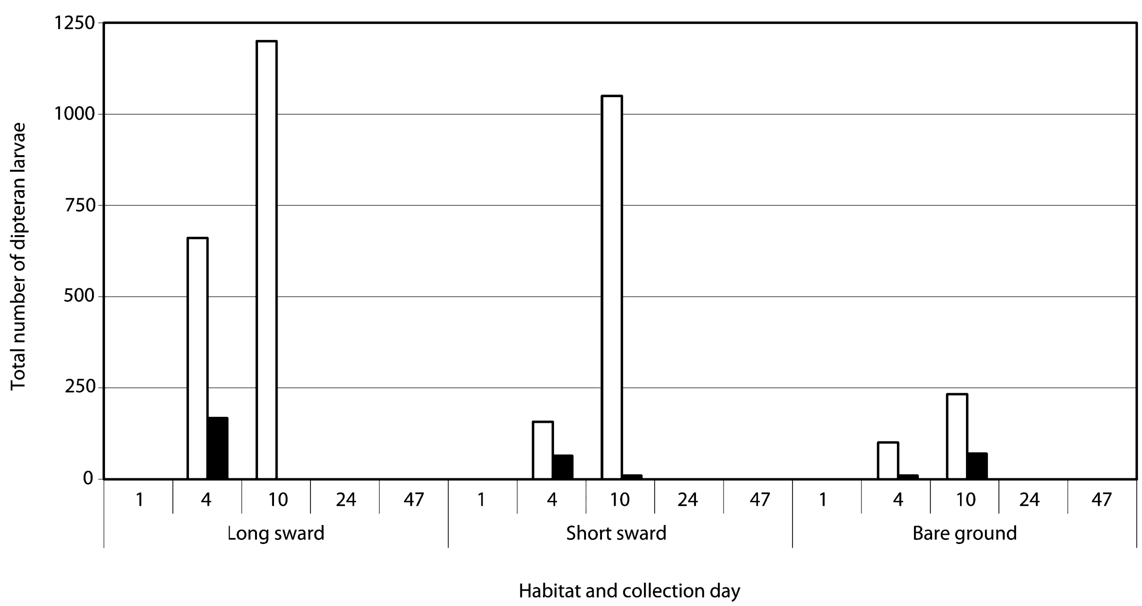

Figure 3. Total number of Diptera larvae collected from untreated cowpats (white bars) and treated cowpats (black bars) in the three sward structures after 1, 4, 10, 24, and 47 days in the field.

Coprophagous invertebrates

The coprophagous species collected from cowpats are presented in Table 2.

A total of 12 coprophagous Coleoptera species were present in this study. With the exception of one species (*Aphodius fossor*), represented by a singleton, species of Scarabaeidae were present in consistently higher numbers in cowpats from ivermectin-treated cattle in all sward structures. *A. ater* and *A. erraticus* were present in significantly higher numbers in treated cowpats in all sward structures (all $P < 0.01$), as was *A. prodomus* in the long and short sward habitats (both $P < 0.01$), and *A. fimetarius* in the short sward habitat ($P < 0.01$). While cowpats from treated cattle in the bare ground habitat contained more *Aphodius* species than the untreated cowpats, this was not always significant.

Members of the Hydrophilidae family were generally present in higher numbers in treated cowpats in the long sward habitat, but were found in higher numbers in untreated cowpats in the short sward and bare ground habitats. Low numbers prevented data analysis for several species, but *Cercyon haemorrhoidalis* ($P < 0.05$) and *Sphaeridium scarabaeoides* ($P < 0.01$) showed a significant preference for treated dung in the long sward habitat.

There were significantly more Diptera larvae in the untreated cowpats, in all three sward structures (all $P < 0.01$).

Table 2. Total coprophagous invertebrates recovered from cowpats from untreated (U) and treated (T) cattle in each sward structure (n=20).

	Long sward			**Short sward**			**Bare ground**		
	U	**T**	**Effect**	**U**	**T**	**Effect**	**U**	**T**	**Effect**
Histeridae									
Paralister purpurescens	2	0		4	3		3	2	
Hydrophilidae									
Cercyon atomarius	0	8		8	2	↓	8	0	
Cercyon haemorrhoidalis	2	10	↑*	2	3		3	3	
Cercyon melanocephalus	1	0		1	0		0	1	
Cercyon pygmaeus	0	3		1	0		1	0	
Sphaeridium scarabaeoides	1	10	↑**	9	6	↓	6	2	
Scarabaeidae									
Aphodius ater	13	77	↑**	460	824	↑**	388	481	↑**
Aphodius erraticus	5	114	↑**	18	150	↑**	20	231	↑**
Aphodius fimetarius	0	5		8	22	↑**	10	15	↑
Aphodius fossor	1	0		0	2		0	0	
Aphodius prodromus	39	311	↑**	204	556	↑**	185	209	↑
Aphodius sphacelatus	0	3		0	0		35	47	↑
Diptera larvae	1860	165	↓**	1207	74	↓**	333	80	↓**

Arrows indicate whether ivermectin treatment increased or decreased insect abundance in each sward structure (where samples were large enough for statistical analysis). * $P < 0.05$; ** $P < 0.01$.

Discussion

The initial mean ivermectin concentration measured in faeces collected from treated cattle one day after treatment (day zero) was 21,899 µg/kg (dry weight). Similarly high concentrations have been reported following treatment with comparable pour-on ivermectin formulas; Herd *et al.* (1996) detected a peak excretion of 18,500 µg/kg (dry weight) in dung collected from cattle two days after treatment. The ivermectin residues in cowpats decreased through time, and followed a similar pattern of breakdown to that reported by Iglesias *et al.* (2006), with an initial rapid decrease in residues over the first three weeks, after which the concentration remained fairly constant.

Despite reductions in ivermectin concentrations, by the end of the 47 day trial, residues remained at relatively high concentrations in cowpats. This demonstrates that ivermectin can persist in temperate environments for more than six weeks, and is supported by similar research in other temperate environments e.g. O'Hea *et al.* (2010) and Sommer & Steffansen (1993). Indeed Suarez *et al.* (2003) reported concentrations of 13 µg/kg after 180 days in a temperate Argentinian pasture.

The persistence of ivermectin implies that residues have the potential to affect the dung community for the entire period that cowpats are utilisable by dung fauna in temperate climates (Hempel *et al*., 2006; Skidmore, 1991). Suarez *et al.* (2003) also reached this conclusion, stating that drug residues remained at levels high enough to adversely affect dung-colonising fauna throughout their entire 180 day trial. If farmers also adhere to the treatment regime recommended by manufacturers, whereby ivermectin administration is carried out three times, at five weekly intervals, then pastures in the UK would probably contain relatively high levels of ivermectin residues throughout the entire grazing season, between April and September (Floate, 1998a).

Sward structure did not have a significant effect on the ivermectin levels in cowpats. This is somewhat surprising since the three sward structures differed significantly in terms of mean sward heights, which resulted in significantly higher temperatures in the long sward habitat, and significantly lower humidity in the bare ground habitat. These relatively small differences were obviously not sufficient to differentially affect ivermectin breakdown, because higher temperatures are known to increase ivermectin breakdown rates (Halley *et al*., 1989a). It is anticipated that more extreme environments within grazing areas, for example those shaded by trees in wood pastures, may have a greater effect, since exposure to sunlight is one of the most important factors influencing the breakdown of ivermectin (Halley *et al*., 1989b).

Ivermectin residues had a clear effect on the presence of invertebrates in cowpats. Diptera larvae were consistently present in higher numbers in cowpats from untreated cattle, and non-dipteran invertebrates were generally present in higher numbers in cowpats from treated cattle. Similar findings have been reported in other studies (Floate, 1998b; Lumaret *et al*., 1993), and Wardhaugh & Mahon (1991) reported that the attractive effect continued to occur in faeces produced up to 25 days after treatment.

Diptera larvae were present in significantly higher numbers in untreated cowpats in all sward structures. This finding is supported by many researchers (Floate, 2007; Lumaret *et al*., 1993; Suarez *et al*., 2003), with some studies reporting that ivermectin residues eliminate certain dung-dwelling Diptera (Madsen *et al*., 1990; Strong & Wall, 1994). It seems most likely that the low number of Diptera larvae recovered from treated cowpats was a consequence of mortality, since Diptera larvae are highly sensitive to ivermectin residues (Lumaret & Errouissi, 2002), and because adult *S. stercoraria* are unable to detect even lethally high levels of ivermectin, meaning that ovipositing females cannot avoid it (Rombke *et al*., 2009).

The EC50 of ivermectin for egg-to-adult mortality of *S. stercoraria* was determined as 20.9 $\pm$ 19.1 µg/kg (fresh weight) (Rombke *et al*., 2009) and concentrations as low as 0.2 µg/kg (wet weight) have been found to reduce pupation to just 28% (West & Tracy, 2009). These effects occurred at ivermectin levels which were much lower than those detected at any time during this experiment, and this has significant implications for survival and cohort size, particularly because *S. stercoraria* colonise cowpats immediately at deposition and are therefore likely to be exposed to very high levels of ivermectin (Skidmore, 1991).

Coprophagous Coleoptera were present in higher numbers in treated cowpats in the majority of cases. Of the species sufficiently abundant for data analysis, most were found in significantly higher numbers in treated cowpats, and none were present in significantly higher numbers in untreated cowpats. Other studies have also reported increased numbers of Coleoptera in cowpats from treated cattle (Floate, 2007; Lumaret *et al*., 1993; Wardhaugh & Mahon, 1991), and Lumaret *et al.* (1993) noted that many species also spent more time foraging within these cowpats.

Members of the Scarabidae family were present in higher numbers in treated cowpats. The attractive effect was consistent across sward structures, although it was not always significant in the bare ground habitat. The most common species, *A. ater,* and *A. erraticus*, were present in significantly higher numbers in treated cowpats in all sward structures, and *A. prodomus* was present in significantly higher numbers in treated cowpats except in bare ground. Ivermectin clearly had a strong attractive effect on *Aphodius* species, a finding supported by Floate (2007) who reported this for all members of the Scarabidae family.

Hydrophilidae species were generally present in numbers too low for statistical analysis. However, although many species were present in greater numbers in untreated cowpats, *Cercyon haemorrhoidalis* and *Sphaeridium scarabaeoides* were found in significantly higher numbers in treated cowpats in the long sward habitat. This contradicts published findings, which have reported ivermectin to cause significant reductions in the adult populations of these species (Floate, 1998a; Holter *et al*., 1993), and the complete absence of their larvae (Strong *et al*., 1996).

The consistently and significantly larger numbers of Scarabidae from the treated cowpats in both the long and short sward habitats, and Hydrophilidae from the treated cowpats in the long sward habitat, suggests that the sward structures were accentuating the attractive effect of ivermectin. It is possible that this was related to the nutritional quality of the dung. Treatment with ivermectin is thought to increase the microbial flora and protein levels in cattle dung (Lumaret *et al*., 1993; Wardhaugh & Mahon, 1991), and sheltered environments in sward structures have been shown to increase microbial activity (Gittings *et al*., 1994). It is therefore possible that the already elevated nutritional content of the treated cowpats was increased further in the significantly more humid sward habitats, making them more attractive resources than the untreated cowpats. This possibility is supported by laboratory experiments that have revealed beetle colonisation preferences according to the nutritional quality of dung (Finn & Giller, 2002), the aggregation of beetles in high quality patches where resource quality differs between cowpats (Hutton & Giller, 2004) and increased growth rates of earthworms fed on dung from ivermectin-treated cattle (Svendsen *et al*., 2005).

The influence of sward structure means that ivermectin could have a greater impact on coprophagous Coleoptera in sward structures that promote microbial growth, such as the long sward areas typically found in field margins and meadows. Further studies are needed to determine whether ivermectin consistently increases the attractiveness of cowpats in these sward structures. If ivermectin is found to increase the attractiveness of cowpats in meadows, then the use of ivermectin may need to be restricted or prohibited in these sward structures in the UK, as they tend to be small and fragmented – characteristics that increase the risk of species extinctions (JNCC, 2006).

The increased attraction to cowpats containing ivermectin has significant implications for the survival of coprophagous Coleoptera because drug residues have been shown to inhibit the development of *Aphodius* larvae (Hempel *et al*., 2006; O'Hea *et al*., 2010), reduce adult emergence (Floate, 1998a) and cause mortality (Hempel *et al*., 2006). The ivermectin levels recorded in this study, even at the end of the 47 day trial, remained higher than the LC50 determined for *A. constans*, which was reported as 880 to 980 µg/kg (by dry weight) by Hempel *et al.* (2006), and 470 to 692 µg/kg (by dry weight) by Lumaret *et al.* (2007). If other species have similar LC50s, then this has severe implications for *Aphodius* populations. This is supported by O’Hea *et al.* (2010) who concluded that ivermectin caused significant reductions in cohort size which were sufficient to affect the next generation of beetles.

Conclusions

The impact of ivermectin on farmland biodiversity is currently of real concern to policy makers in the UK, to the extent that the question 'What are the impacts on biodiversity of prophylactic treatment of farm livestock with antibiotics, anti-fungal and anti-helmintic compounds?' was identified as one of 100 questions of relevance following a workshop of policy makers, advisers, lobbyists and members of the research community (Sutherland *et al*., 2006).

These findings help further our understanding of how ivermectin impacts on invertebrates in an *in situ* farm environment and thus improve the knowledge-base from which to develop grounded policy recommendations for conserving on-farm biodiversity in the UK. Of particular note from this research is the fact that ivermectin was able to persist at levels well above those reported toxic to dung fauna, even after 47 days in the field. This suggests that pastures will contain ivermectin at biologically significant levels for the entire grazing season, especially if the treatment regimes recommended by manufacturers are adhered to. Practical options for the reduction of ivermectin use include the application of targeted treatments in preference to broad-spectrum chemicals, or the selection of less toxic macrocyclic lactones such as moxidectin (Floate, 2006; Suarez *et al*., 2009).

Also of importance from this research is the finding that ivermectin residues significantly alter cowpat colonisation. Coprophagous Coleoptera were present in significantly higher numbers in treated cowpats in the majority of cases, and ivermectin had a strong attractive effect on *Aphodius* species. This means that Coleoptera, especially *Aphodius* species, are likely to be continually exposed to ivermectin as a result of their cowpat selection, and this has implications for beetle presence and cohort size. Moreover, these impacts will be compounded if dung beetles are preferentially attracted to farms using ivermectin. This has potential implications for current UK government schemes such as Environmental Stewardship (ES), which incentivise the conservation of on-farm invertebrate diversity, particularly where ES farms are located in close proximity to conventional farms. However, further research is required to determine the distances at which *Aphodius* species can detect ivermectin, and the spatial scales at which their resource selection occurs.

Although ivermectins were found to have a very significant impact on Diptera abundance in treated cowpats, this is less likely to have short term conservation implications for UK farmland due to the continued availability of ivermectin-free cattle dung in most areas. For example, it is likely that some ivermectin free dung will be present within the local landscape, since famers often graze untreated milking cows with the young treated cattle (Webb *et al*., 2010).

Above all, the current research highlights the potential for on-farm field experiments to improve understanding of the effects of ivermectin compounds on invertebrates, and the need for further field investigations in temperate environments to determine the long-term impacts on non-target dung fauna.

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