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Field effects of faecal residues from ivermectin slow-release boluses on the attractiveness of cattle dung to dung beetles

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Abstract. A 2-year study was performed in two sites in southern France to assess the effect of ivermectin residues on the attractiveness of cattle dung to colonizing insects. Insect captures were compared between pitfall traps baited with dung from untreated cattle and dung from cattle that had been treated with a slow-release (SR) bolus of ivermectin. Cattle dung was collected at different times after treatment (4, 14, 42, 70 and 98 days). Excretion showed a plateau, with levels ranging between 0.688 µg and 1.123 µg ivermectin per gram of wet dung. Faecal residues affected insect captures at both sites. Effects were independent of the time dung was collected after treatment, except for one result subsequent to a severe drought during the baiting period. Ivermectin-contaminated dung showed a significant attractive effect, with increased captures regardless of the guild to which beetles belonged. This study demonstrates the attractiveness of ivermectin residues over a long period after the treatment of animals. It draws attention to the danger of widespread use of this endectocide-based SR bolus, which is attributable to the preferential attraction of insects to treated dung, which potentially puts at risk the survival of their offspring.

Key words. Aphodiidae, attraction, dung faecal residues, Geotrupidae, ivermectin, non-target effects, Scarabaeidae.

Introduction

Dung beetles are common in pastures, where they accelerate the degradation of dung pats and maintain pasture productivity by enhancing the activity of the microorganisms in soil that participate in the mineralization of animal waste (Bornemissza & Williams, 1970). Dung pats have been considered as insular microecosystems (Mohr, 1943), in which insects converge in several waves of colonization according to the time of day and the nature or concentration of volatile compounds emitted by dung (Desière, 1973; Dormont *et al*., 2007). Although many scarabs are opportunistic and use a wide variety of dung types without much discrimination, field trapping as well as bioassays clearly suggest that dung beetles are capable of making choices between odours from faeces of different herbivores (Dormont *et al*., 2004, 2007). Use of veterinary drugs can affect the attraction of beetles to animal dung. Cattle dung from animals treated with endectocides has been shown to be either more (Wardhaugh & Mahon, 1991; Holter *et al*., 1993a, 1993b; Lumaret *et al*., 1993; Floate, 1998, 2007) or less (Floate, 1998, 2007; Webb *et al*., 2010) attractive than dung from untreated animals to dung beetles in the field.

Avermectins are veterinary endectocides used worldwide for the control of nematodes and arthropods affecting cattle. These broad-spectrum parasiticides have been implicated as being harmful to the survival and development of various dung insect species and consequently to the ecology of pasture ecosystems. Residues are excreted in the faeces of

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treated animals and can be toxic to dung-breeding insects (Wall & Strong, 1987; Lumaret *et al*., 1993; Floate, 1998; Errouissi *et al*., 2001; Lumaret & Errouissi, 2002; Floate *et al*., 2005). Their insecticidal effects can also indirectly affect dung degradation (Wall & Strong, 1987; Madsen *et al*., 1990; Sommer *et al*., 1992; Herd *et al*., 1993; Herd, 1995; Strong *et al*., 1996; Floate, 1998; Dadour *et al*., 1999). Widespread administration of these treatments to all animals in a herd and the simultaneous treatment of entire herds in the same area could lead to a drastic reduction in the number of coprophagous insects, especially if the treatments occur at the time when sensitive instars (in general, larval instars) are in contact with dung or consume it.

The current study was performed to compare the attraction of dung beetles to dung from untreated cattle vs. dung from animals treated with an ivermectin slow-release (SR) bolus. The current study is the first to examine this effect of residues from cattle treated with a SR bolus. Because of the longer period of excretion (each bolus was designed to release 12 mg of ivermectin per day over 135 consecutive days), the use of boluses could have a much greater effect on beetle populations than the topical (pour-on) or injectable formulations of products reported in previous studies (which affect excretion for approximately 4 weeks). If an increase in the attractiveness of dung from treated animals was confirmed (see Floate, 2007), contaminated dung might progressively reduce the number of dung beetles in pastures, with clearly undesirable effects in terms of population levels.

Materials and methods

The experiment was carried out in southern France at two sites. Site 1 was located at Saint Martin-de-Londres, in the subhumid Mediterranean climate area, 35 km north of Montpellier (43°47′ N, 03°43′ E; elevation 250 m a.s.l.). This site is a 600-ha herbaceous garrigue dominated by *Brachypodium retusum*, *Quercus ilex* and *Thymus vulgaris*. The substratum consists of dry and fissured clay soil. The annual average temperature is 13.9 °C and annual rainfall amounts to 1060 mm. Approximately 250 cattle regularly grazed at the site. Site 2 was located at Saint Maurice-de-Navacelles on the Causse du Larzac (43°51′ N, 03°29′ E, 800 m a.s.l.), about 80 km north of Montpellier. The site is a 700-ha open garrigue on compact limestone, dominated by *Stipa pennata*, *Buxus sempervirens*, *Sorbus aria* and *Quercus pubescens*. The substratum consists of compact limestone. The annual average temperature is 10.5 °C and average annual rainfall amounts to 1300 mm. Approximately 600 ewes grazed regularly at this site.

Experimental design

Dung for the study was collected from 2-year-old Aubrac heifers (average weight 320 kg) at Site 1. The animals were not pregnant and were not treated with any pharmaceuticals for at least 100 days before faeces were collected. In March 1999, 26 animals received an Ivomec® SR bolus. According to the manufacturer, the Ivomec® SR bolus device (MSD AGVET, Paris, France) delivered ivermectin at a rate of 12 mg/day over 135 days. It contained 1.72 g of ivermectin and was designed for cattle weighing 100–300 kg. The ivermectin SR bolus was administrated via a gun provided by the manufacturer and was designed to deliver the specific bolus under investigation. The animals were observed closely during the first 24 h after treatment to ensure that the bolus was not regurgitated. Twenty-five untreated animals were used as controls. No additional feed other than hay was supplied. Fresh water was provided via automatic drinkers. During the whole of the trial period, the two groups grazed in separate fields. The trial was conducted between March and August 1999.

Rectal faecal samples were collected prior to treatment and from treated and untreated animals on days 4, 14, 42, 70 and 98 after treatment. Dung collected on the same day from animals in the same group (treated or control) was mixed thoroughly. A portion of this dung was used immediately to bait pitfall traps at Site 1. The remainder was stored in sealed containers at −18 °C and subsequently used to bait pitfall traps at Site 2 in 2000.

Previous work conducted in the laboratory (Alvinerie *et al*., 1998) has demonstrated the importance of faecal elimination of ivermectin administered as an intraruminal bolus in cattle. Faecal ivermectin concentrations gradually increased to reach a peak concentration of 4.1 μg/g (wet dung) at 4 days post-administration. Subsequently, the level dropped to a steady-state concentration of 1.18 μg/g. The faecal steady-state level was maintained up to 120 days post-treatment and ivermectin was detected in the dung pats (2.67 ng/g) at up to 160 days. For this project, assays were conducted with dung collected at different times after the treatment (4, 14, 42, 70 and 98 days) and ivermectin concentrations in dung pats were measured 2 weeks after they had been deposited in the field. The concentration in pats collected on day 98 was not measured. Levels of ivermectin (μg ivermectin/g wet dung) in samples from days 4, 14, 42 and 70 were determined by M. Alvinerie [Institut National de la Recherche Agronomique (INRA), Toulouse, France] to be 0.688 ± 0.099, 1.123 ± 0.025, 0.839 ± 0.009 and 1.056 ± 0.015, respectively. These results are in agreement with the plateau in faecal excretion previously described by Alvinerie *et al*. (1998). Ivermectin was not detected in the controls.

The pitfall traps used in the study were of the Cebo-Suspendido-Rejilla (CSR) model (see Lobo *et al*., 1988; Veiga *et al*., 1989). Each trap consisted of a plastic basin (210 mm in diameter) buried to its rim in the soil and containing a water and formalin liquid soap mixture. Fresh cattle dung (1 L) was supported on a wire grid on the top of a bucket. Traps were placed along a transect, baited with dung from untreated and treated animals alternately, and separated by a minimum distance of 10 m. Trap contents were collected 1 week later.

At Site 1, four traps (two treated, two controls) were operated for five 1-week periods between 29 March and 1 July 1999, which corresponded to the 5 days of faecal collection (days 4, 14, 42, 70 and 98). At Site 2, eight traps (four treated, four controls) were baited with dung collected on day 70 and operated for a 1-week period in April 2000. This design was used only to check whether the results obtained in Site 1 could be generalized by choosing a different location and a different composition of fauna and if the dung stored in the freezer for 1 year retained its attractive properties. The use of the dung collected on day 70 was independent from the results obtained in Site 1 with the same batch.

Statistical analysis

All beetles were identified to species level and counted. Dung beetles include three families: Scarabaeidae, Aphodiidae and Geotrupidae. Geotrupidae and most of the Scarabaeidae (except Scarabaeini) species are tunnellers: they dig vertical tunnels below the dung pat and carry dung into the bottom of the burrow. Scarabaeini (*Scarabaeus* and *Sisyphus* genera) are rollers: they make and roll away balls of dung and dig tunnels outside the dung pat. The small-bodied Aphodiidae belong to a third guild (dwellers), members of which feed and preferentially oviposit inside the dung pats. In assemblages, species with a relative abundance of $\geq$10% in either numbers or biomass represented the core species (Stiernet & Lumaret, 1993). Because variations in trap catches could not be normalized by transformation, results were analysed using non-parametric tests to compare the numbers of beetles caught in pitfall traps baited with dung from untreated vs. treated cattle. Calculations were made for those species which reached 10 or more individuals for a treatment. Rare or sporadic species ($<$10 individuals) were not considered for calculations. The effect of treatment on dung beetle attraction was tested with the Mann–Whitney U-test. For Site 1, the U-test compared the results of the five successive deposits (days 4, 14, 42, 70 and 98 post-treatment, respectively). which generated five independent pairwise comparisons. For Site 2, only one pairwise comparison was performed using the U-test to compare the numbers of beetles attracted to dung from control vs. treated cattle. The non-parametric chi-squared test was used to compare the relative abundances of the core species attracted most frequently by control and treated dung.

All statistical analyses were performed using STATISTICA 6.0 (StatSoft, Inc., Tulsa, OK, U.S.A.) and MINITAB 13 (Minitab, Inc., State College, PA, U.S.A.) software.

Results

Dung preferences at Site 1

A total of 1957 beetles (24 species) were recovered at Site 1 (Table 1). Dung from treated animals attracted significantly more beetles than dung from control animals ($U = 197.5$; $P = 0.01$) for data combined across trapping periods. Pairwise comparisons of the number of beetles attracted by control vs. treated dung showed significant differences in total numbers regardless of how long the dung was collected after treatment (4, 14, 42 and 70 days) [U-values ranged from 36.0 for dung collected on day 70 (D70) to 155.5 for dung collected on day 4 (D4); P-values ranged from 0.004 (D70) to 0.05 (D14)], except for D98 ($U = 25.0$; $P = 0.46$). Both the most abundant dweller and tunneller species were significantly more attracted by treated dung (dwellers: $\chi^2 = 19.1$, d.f. $= 5$, $P = 0.002$; tunnellers: $\chi^2 = 53.4$, d.f. $= 8$, $P = 0.00$).

Eleven species were qualified as core species (Table 2). According to trapping periods, they represented between 48.9% and 93.0% of beetles in numbers and between 61.5% and 93.9% in biomass (Table 2). Most of them were dwellers and tunnellers and all of them were significantly more attracted by dung from treated animals. This was true for the dweller *Aphodius constans* (Coleoptera: Aphodiidae) during its period of activity ($\chi^2 = 6.6$, d.f. $= 2$, $P = 0.04$) and the tunneller *Onthophagus lemur* (Coleoptera: Scarabaeidae) ($\chi^2 = 12.6$, d.f. $= 3$, $P = 0.006$). Both Geotrupidae (tunnellers) and rollers (Scarabaeidae) were always scarce for both treated and control dung, regardless of trapping period and ivermectin concentration, and thus were not considered in calculations even if their biomass exceeded 10%.

Dung preferences at Site 2

A total of 990 beetles (24 species) were recovered at Site 2 (Table 1). Results were comparable with those for Site 1, with more beetles attracted to dung from treated cattle ($U = 138$; $P = 0.04$): numbers amounted to 187 vs. 20 dwellers, 681 vs. 95 tunnellers and five vs. two rollers attracted to dung from treated and untreated cattle, respectively. All the core species, which represented 75.5% and 70.7% of beetles in the control and treated dung catches, respectively (Table 2), were significantly more attracted to treated dung. All species were tunnellers; the species of other guilds remained in the minority at this site.

Discussion

Faecal residues of ivermectin SR bolus significantly affected the responses of dung beetles to dung pats. This effect was observed independently at the two sites, which differed in terms of both elevation and dung beetle communities. This suggests that ivermectin therapy enhanced the attractiveness of dung from treated cattle, as reported previously (Wardhaugh & Mahon, 1991; Holter *et al*., 1993a; Lumaret *et al*., 1993; Bernal *et al*., 1994; Floate, 2007). At Site 1, where the experiment was replicated five times (using deposits from days 4, 14, 42, 70 and 98 after treatment), treated dung had a significant attractive effect in four of five cases. The difference was not significant for dung collected on day 98 (D98), although traps baited with treated dung attracted more beetles than control traps (228 and 127 individuals, respectively). The lower number of beetles attracted to D70 (for both the control and treated baits, 176 and 17 individuals, respectively) probably reflected the occurrence of severe drought at that time, which reduced beetle activity because of the hardness of the soil and caused the rapid formation of a crust on dung pats, thereby reducing their release of volatile attractants (Lumaret & Kirk, 1987).

In addition, at Site 2, where eight traps were baited with D70 and operated for a 1-week period only, dung from treated cattle

Table 1. Numbers of beetles collected at Sites 1 and 2 in pitfall traps baited with dung from treated and control cattle.

Species	Individual dry weight, mg	Site 1												Site 2	
		D4		D14		D42		D70		D98		Total Site 1		Total Site 2	
		T	C	T	C	T	C	T	C	T	C	T	C	T	C
Aphodiidae															
Acrossus luridus (Fabr. 1775)	12.1	20	5	7	0	13	5	—	—	—	—	40	10	50	1
Agrilinus constans (Duft. 1805)	4.6	183	107	98	46	37	8	—	—	—	—	318	161	74	9
Aphodius fimetarius (L. 1758)	9.9	5	2	—	—	—	—	1	—	—	—	6	2	17	—
Aphodius scybalarius (Fabr. 1781)	6.8	—	—	—	—	1	—	—	—	—	—	1	—	1	—
Biralus satellitius (Herbst 1789)	22.7	14	7	12	1	10	4	6	—	—	—	42	12	1	—
Calamosternus granarius (L. 1767)	3.4	6	—	—	2	3	—	—	—	—	—	9	2	25	3
Chilothorax distinctus (O.F. Müller, 1776)	2.8	—	—	—	—	—	—	—	—	—	—	—	—	1	—
Colobopterus erraticus (L. 1758)	8.0	—	—	12	2	26	5	8	2	8	4	54	13	—	—
Esymus merdarius (Fabr. 1775)	0.9	4	—	2	2	—	1	—	—	—	—	6	3	1	—
Esymus pusillus (Herbst, 1789)	1.3	—	—	—	—	—	—	—	—	—	—	—	—	1	2
Eudolus quadriguttatus (Herbst 1783)	2.2	3	—	—	2	1	—	—	—	—	—	4	2	—	—
Euorodalus paracoenosus (Balth. & Hrub. 1960)	2.3	1	—	39	13	57	38	—	—	—	—	97	51	1	2
Melinopterus prodromus (Brahm, 1790)	4.9	—	—	—	—	—	—	—	—	—	—	—	—	7	—
Oxyomus sylvestris (Scop., 1763)	1.1	—	—	—	—	—	—	—	—	—	—	—	—	1	—
Phalacronothus biguttatus (Germ., 1824)	0.6	—	—	—	—	—	—	—	—	—	—	—	—	8	2
Otophorus haemorrhoidalis (L. 1758)	4.0	2	—	—	—	20	2	34	2	109	41	165	45	—	—
Trichonotulus scrofa (Fabr. 1787)	1.4	1	—	7	2	1	2	—	—	—	—	9	4	—	1
Scarabaeidae															
Bubas bubalus (Oliv. 1811)	197.6	16	4	8	5	5	4	—	—	1	1	30	14	—	—
Caccobius schreberi (L. 1758)	7.1	35	—	—	—	5	0	—	—	1	1	41	1	—	—
Euoniticellus fulvus (Goeze 1777)	25.1	—	—	—	—	13	3	37	3	103	76	153	82	—	—
Euonthophagus amyntas (Oliv. 1798)	27.0	—	—	—	—	9	2	—	—	1	—	10	2	—	—
Onthophagus coenobita (Herbst 1783)	21.8	—	1	2	2	—	—	1	—	—	—	3	3	68	30
Onthophagus emarginatus (Muls. & Godart 1842)	7.6	4	8	3	—	6	9	9	1	—	—	22	18	—	—
Onthophagus furcatus (Fabr. 1781)	3.7	—	—	—	—	5	—	1	—	—	—	6	—	—	—
Onthophagus fracticornis (Preyssl., 1790)	10.0	—	—	—	—	—	—	—	—	—	—	—	—	2	16
Onthophagus grossepunctatus (Reitt., 1905)	5.0	—	—	—	—	—	—	—	—	—	—	—	—	209	18
Onthophagus joannae (Goljan, 1953)	6.2	—	—	—	—	—	—	—	—	—	—	—	—	102	24
Onthophagus lemur (Fabr. 1781)	13.4	51	3	56	16	35	13	22	1	—	—	164	33	241	4
Onthophagus maki (Ill. 1803)	10.5	1	2	6	—	4	2	2	1	2	—	15	5	6	—
Onthophagus ovatus (L. 1767)	5.7	35	3	—	—	1	—	—	—	—	—	36	3	29	1
Onthophagus vacca (L. 1767)	41.2	4	1	28	13	38	23	23	4	3	4	96	45	17	—
Onthophagus verticicornis (Laich. 1781)	18.7	—	1	4	4	19	9	20	2	—	—	43	16	—	—
Scarabaeus laticollis (L.1767)	172.9	—	—	3	1	—	—	—	1	—	—	3	2	—	—
Sisyphus schaefferi (L. 1758)	29.0	13	1	3	1	11	3	12	—	—	—	39	5	5	2
Geotrupidae															
Geotrupes mutator (Marsham, 1802)	350	—	—	—	—	—	—	—	—	—	—	—	—	3	1
Trypocopris vernalis (L., 1758)	119.5	—	—	—	—	—	—	—	—	—	—	—	—	4	1
Total beetles		398	145	290	112	320	133	176	17	228	127	1392	565	873	117
Total species		18	13	16	16	22	18	13	9	8	6	26	24	23	16

At Site 1, dung was collected on days 4, 14, 42, 70 and 98 after treatment (ivermectin sustained-release bolus) and used as bait immediately in the field (two traps for each modality). At Site 2, dung used as bait was collected on day 70; eight pitfall traps were baited with dung from treated and control animals (four traps for each modality).

T, treated; C, control.

Individual dry weight: values were calculated as a mean of the weight of 30 individuals previously dried in an oven for 24 h.

Table 2. Frequencies of core species (percentage of biomass in brackets) attracted by dung from treated and control animals collected at intervals after the treatment (Sites 1 and 2).

Species	Site 1 Guilds	Day 4	Day 14	Day 42	Day 70	Day 98	Site 2 Day 70
	Control						
Aphodiidae							
Agrilinus constans	D	73.8 (27.8)	41.1 (9.0)	—	—	—	—
Colobopterus erraticus	D	—	—	—	11.8 (3.2)	—	—
Euorodalus paracoenosus	D	—	11.6 (1.3)	28.6 (3.2)	—	—	—
Otophorus haemorrhoidalis	D	—	—	—	11.8 (1.6)	32.3 (4.7)	—
Scarabaeidae							
Bubas bubalus	T	2.8 (49.6)	4.5 (42.0)	3.0 (29.1)	—	—	—
Euoniticellus fulvus	T	—	—	—	17.7 (14.9)	59.8 (77.1)	—
Onthophagus coenobita	T	—	—	—	—	—	25.6 (38.7)
Onthophagus fracticornis	T	—	—	—	—	—	13.7 (9.5)
Onthophagus grossepunctatus	T	—	—	—	—	—	15.4 (5.3)
Onthophagus joannae	T	—	—	—	—	—	20.5 (8.8)
Onthophagus lemur	T	—	14.3 (9.1)	—	—	—	—
Onthophagus vacca	T	—	11.6 (22.8)	17.3 (34.9)	25.5 (32.6)	—	—
Onthophagus verticicornis	T	—	—	—	11.8 (7.4)	—	—
Scarabaeus laticollis	R	—	—	—	5.9 (34.2)	—	—
Geotrupidae							
Geotrupes mutator	T	—	—	—	—	—	0.3 (20.7)
Total		76.6 (77.4)	83.1 (84.2)	48.9 (67.2)	84.5 (93.9)	92.1 (81.8)	75.5 (83.0)
	Treated						
Aphodiidae							
Agrilinus constans	D	46.0 (13.2)	33.8 (8.5)	11.6 (3.1)	—	—	—
Euorodalus paracoenosus	D	—	13.5 (1.7)	17.8 (2.4)	—	—	—
Otophorus haemorrhoidalis	D	—	—	—	19.3 (4.1)	47.8 (12.6)	—
Scarabaeidae							
Bubas bubalus	T	4.0 (49.6)	2.8 (29.8)	1.6 (18.3)	—	—	—
Euoniticellus fulvus	T	—	—	—	21.0 (27.7)	45.2 (74.7)	—
Onthophagus coenobita	T	—	—	—	—	—	7.8 (17.4)
Onthophagus grossepunctatus	T	—	—	—	—	—	23.5 (10.0)
Onthophagus joannae	T	—	—	—	—	—	11.5 (6.3)
Onthophagus lemur	T	12.8 (10.7)	19.3 (14.2)	11.0 (8.7)	12.5 (8.8)	—	27.1 (31.0)
Onthophagus vacca	T	—	9.7 (21.8)	11.9 (29.0)	13.1 (28.3)	—	—
Onthophagus verticicornis	T	—	—	—	11.4 (11.2)	—	—
Sisyphus schaefferi	R	—	—	—	6.8 (10.4)	—	—
Geotrupidae							
Geotrupes mutator	T	—	—	—	—	—	0.8 (10.1)
Total		62.8 (73.5)	79.1 (76.0)	53.9 (61.5)	84.1 (90.5)	93.0 (87.3)	70.7 (74.8)

D, dwellers; T, tunnellers; R, rollers.

attracted more beetles than dung from untreated animals. The level of attraction was strong for both the control and treated baits, despite the fact that the dung had been frozen at −18 °C for 1 year, which supports the hypothesis that the low attraction recorded 1 year earlier at Site 1 with manure of the same origin (D70) reflected climatic conditions at the time of trapping (drought) rather than the quality of the sample itself. Because the standardization of the deposits (all traps were baited at the same time with dung from the same origin and date), we assumed that the differences recorded were associated with the application of ivermectin, as already noted by Floate (1998), because ivermectin is apparently stable in frozen dung (Payne *et al*., 1995).

Beetles attracted to pitfall traps are unable to escape and insect abundances recorded with this method reflect the pattern of initial colonization by adult beetles based on the emission of volatile compounds from dung. This suggests that dung beetles are very sensitive to small differences in the concentrations of volatile chemicals between faeces and are capable of making choices between odours from faeces and thus can preferentially exploit a particular dung type (Dormont *et al*., 2007). The trapping method allows for a cumulative assessment of the absolute attractiveness of dung from treated vs. untreated animals, by contrast with experiments in which the dung is simply deposited on the ground and the number of insects found at time T are counted. The presence of dung beetles in the dung is density-dependent (Hanski, 1991) and insects attracted to dung leave quickly if the density is too high (competition), which may explain the results of some experiments. For example, Strong *et al*. (1996) did not notice any difference in the numbers of adult beetles found in pats from different treatment groups, whereas pitfall trap data suggested that dung from treated animals may have been more attractive, although this is not significant at a probability level of 5%. Webb *et al*. (2010) showed that beetles preferentially colonized dung from untreated vs. doramectin-treated cattle. In Canada, a 3-year study was performed to assess the effect of endectocide residues on the attractiveness of cattle dung to colonizing insects (Floate, 2007). Insect captures were compared between paired pitfall traps baited with dung from untreated cattle vs. traps baited with dung from treated cattle. Ivermectin showed a strong attractive effect with increased captures in 17 of 21 cases. This effect was observed yearly, in spring and autumn, and treatment effects were documented for 25 insect taxa representing 12 families in three orders. Wardhaugh & Mahon (1991) suggested that changes in the gut flora of livestock following avermectin treatment could alter dung quality, whereas Bernal *et al*. (1994) demonstrated that the aminoacid profiles of dung from cattle treated with ivermectin differed from those of dung from untreated animals, which may explain the differences in attractiveness.

It is unlikely that insects respond directly to the presence of a parent compound. The differences in volatile chemicals may result from differences between animal groups in diet or metabolism. However, in the present experiment, the cattle's diet was exactly the same for treated and untreated animals, the dung was taken from the same farm at the same time and the deposits were simultaneous. The possible differences between treated and control baits are likely to reflect subtle differences in volatile compounds emitted by the dung. Floate (1998) found significant differences in catches obtained between baits separated by 5 m and even by 3 m (Floate, 2007). Traps placed 1 m apart from one another and baited with different dung also showed significant differences (Dormont *et al*., 2007). In the present study, traps were separated by 10 m, thus reducing the possibility of confusion between the odours of different traps.

Ivermectin therapy enhanced the colonization of treated dung by most core species, with no difference between dwellers and tunnellers. These results must be considered in light of the role played by dung beetles in grazed ecosystems and their traits of life. If large numbers of dung-feeding beetles are attracted significantly by dung from treated animals and breed in treated and untreated dung in the same way, treated dung pats would seem likely to disappear more quickly than or in a manner comparable with (short-term effect, mechanical degradation) untreated pats. We might conclude that the risks related to treatments were limited (Barth, 1993). Wratten & Forbes (1996) concluded that, even under conditions of relatively high levels of avermectin use in cattle, the impact on non-target insect populations and their ecological function is limited. In fact, it may be the next step of the colonization process that is most affected by drug residues because this is when larvae develop inside the dung pats or in pedotrophic nests under the pats. The reduced numbers of eggs in pats from ivermectin-treated animals would suggest that colonizing females laid fewer eggs in these pats than in control dung, even if they were more attracted by treated dung (Strong *et al*., 1996). This finding can be explained by the fact that ivermectin could induce reproductive deficiencies in dung beetles (Wardhaugh & Rodriguez-Menendez, 1988; Ridsdill-Smith, 1993; Wardhaugh *et al*., 1993). Wardhaugh *et al*. (1993) also noted that adults of *Euoniticellus fulvus* Goeze (Coleoptera: Scarabaeidae) failed to feed normally after ingesting dung from sheep drenched with ivermectin 1 day previously. There are also reports that ivermectin has anti-feedant properties, but it is possible that insects contacting ivermectin are simply incapable of feeding (Strong, 1992, 1993). However, ultimately it is the larvae of dung-feeding insects that are mostly affected by ivermectin. The administration of ivermectin SR bolus to cattle is highly effective in killing dung beetle larvae for approximately 143 days after treatment (Errouissi *et al*., 2001). The ability to maintain the attraction of insects for several weeks (as in the present work) after initial treatment may have significant effects on the fauna because these insects cannot have offspring. This gradually reduces the number of emerging adults in successive generations so that, in a few generations, the decline in numbers of dung beetles should be obvious, as has been demonstrated by several models designed to assist in the evaluation of the impact of parasiticide-contaminated dung on the abundance and distribution of dung insect populations (Sherratt *et al*., 1998; Wardhaugh *et al*., 2001; Vale & Grant, 2002). Reductions in the feeding and tunnelling activities of dung-dwelling insects may delay dung degradation, and non-degraded dung pats can provide sites in which pest flies can complete development, and may harbour parasitic nematodes in livestock, reduce available grazing areas, and represent a loss of soil nitrogen in pastures (Fincher, 1981). The colonization of dung pats and their use involves a very complex ecological process in which many invertebrates and microorganisms take part. Any change in one of the sequences of recycling is likely to have effects on the overall ecosystem of the pasture.

The SR bolus formulation of ivermectin for cattle is no longer sold in various parts of Europe. It was discontinued in both the U.K. and France because of its overly lengthy release of ivermectin, which extended to 156 days post-administration (Errouissi *et al*., 2001). It was also discontinued in Canada in June 2004 (Floate, 2006). However, this preparation is still sold elsewhere in the world. One of the aims of our study is to draw attention to the need to conduct environmental impact assessments of veterinary medicinal products (VMPs) in the medium and long term, particularly if they cause differences in the attractiveness of dung from treated and untreated animals, regardless of the active substance. When new VMPs are registered, those with adverse effects in single-species laboratory trials require further 'higher-tier' testing to assess the extent of these effects on the entire dung community under more realistic field or semi-field conditions (VICH, 2004). Assuming that field studies comparable with that described in the present paper might be required for the purposes of registering a product in the foreseeable future, we should consider that the preferential attraction of beetles to either control or treated dung pats might introduce a bias in the evaluation of risk if only short-term measurements are required. Differences in the rate of dung degradation become significant only in the medium and long term because they take into account the action of offspring inside the dung, which may be null if the larvae cannot survive (Lumaret & Kadiri, 1995). Attraction cannot be evaluated as a single endpoint, but must be taken into account when assessing a new VMP in order to avoid the risk of failing to consider an effect on degradation. Such a lack of effect may occur in some cases because adults are often much less sensitive than larvae to VMPs and there is often no difference between treated and control dung in terms of its rate of disappearance in short-term measurements. Dung pat degradation must be considered from medium- and longterm perspectives, which extend beyond the completion (or not) of the larvae's biological cycle.

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