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Effects of faecal residues of moxidectin and doramectin on the activity of arthropods in cattle dung

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Abstract

Dung invertebrate colonization and degradation levels of faeces from cattle treated with endectocides were studied. Faeces of control and doramectin (DRM) (subcutaneous) and moxidectin (MXD) (subcutaneous and topical) treated animals were deposited on the field from 3 to 21 days post-treatment (pt). Pats were recovered after 6 to 42 days post-deposition (pd). Faecal weight, dry matter, arthropods number, and drugs concentrations were determined. Total arthropods number was higher in control ($P<0.0001$) than in the other groups from days 3 to 21 pt. Total number of insects recovered on days 3, 11, and 21 pt from control pats was significantly ($P<0.001$) higher than in treated-animal pats during all the trial. At day 21 pt, the insects' number in dung voided by DRM-treated cattle was ($P<0.05$) lower than in the other groups. Comparisons of dung degradation among treatments were inconclusive. A lower adverse effect was observed for MXD compared with DRM. No significant degradation of MXD or DRM was observed during the present trial.

Keywords: Cattle; Nematoda; Dung fauna; Moxidectin; Doramectin; Ecotoxicity; Pour-on; Endectocide; Avermectins

1. Introduction

The avermectins (ivermectin [IVM] and doramectin [DRM]) and milbemycins (moxidectin [MXD]) are potent anthelmintic compounds, known as endectocide molecules due to their activity against ecto- and endoparasites (Shoop et al., 1995). They have low mammalian toxicity and their formulations are convenient to use. Hence, they are extensively used worldwide in veterinary medicine. After their subcutaneous administration, large amounts of unchanged IVM, DRM, and MXD are excreted by bile and faeces, particularly during the first weeks after treatment (Lifschitz et al., 1999, 2000). These high IVM and DRM concentrations excreted by faeces account for their important efficacy against larvae and adults of insects in the dung pats (Miller et al., 1981); however, decaying concentrations have effects even when reaching low levels, since more susceptible insects, such as *Haematobia irritans*, are affected by low concentrations long after treatment (Anziani et al., 2001; Floate et al., 2001). The prolonged presence of IVM in faeces produces an adverse effect against other invertebrates of the dung that have a relevant role on the nutrients' recycling to the soil (Strong, 1993; Herd, 1995). Reductions in the feeding and tunneling activities of dung-dwelling insects may delay dung degradation. Undegraded dung pats provide sites for pest flies to complete development, harbor nematodes parasitic in livestock, reduce available grazing area, and represent a loss of soil nitrogen in pastures (Fincher, 1981). Reduced numbers of dung-breeding insect species can also affect other aspects of the pasture ecosystem, since coprophilous insects help pollinate plants and provide food for vertebrates. In the face of these complex interactions, the consequences of this toxicity, both direct and indirect, on the ecosystem are not fully understood (Floate et al., 2005). Most of the current knowledge about the non-specific effects against dung-dwelling fauna is referred mainly to

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IVM (Floate, 1998a, b; Floate and Fox, 1999, 2000) but scarce available information exists about the ecotoxicity of the other endectocide drugs (Dadour et al., 2000; Floate, 2006; Floate et al., 2001, 2002). As can be seen by the cited references, most of the research of the ecological impacts of endecotocides was performed in the northern hemisphere and few in the southern hemisphere, where the use of endectocides in cattle and small ruminants production systems has steadily increased due to favourable economic situations. In Argentina, these compounds, administered subcutaneously, are included in most of parasite control programmes (Suárez, 1992). The insect populations and, particularly, the sensitivity to endectocides on a species basis can differ among geographical locations. This determines the need for these studies in those locations. In Argentina, previous studies showed the effect of IVM and DRM against the dung fauna (Suárez, 2002a; Suárez et al., 2003), but not the probable effects of MXD. Moxidectin has been considered as a less harmful compound compared to other avermectin endectocides (Floate et al., 2001, 2002). In the last years, a novel pour-on formulation of MXD has appeared in the veterinary pharmaceutical market. As a different faecal excretion pattern was observed after the pour-on administration of endectocide compounds compared with the subcutaneous treatment in cattle, a possible different negative action of MXD against the dung fauna could be considered. The goals of the present work were: (a) to study the possible effects of faecal residues of DRM and MXD in dung voided from treated animals on dung fauna, (b) to compare the influence of the route of administration of the antiparasitic drugs on the effects of faecal residues on dung fauna, and (c) to determine, at different times post-treatment (pt), the degradation of DRM and MXD faecal residues in the field and to correlate their faecal concentrations with the effects observed on dung fauna.

2. Materials and methods

2.1. Treatments and animals

The experiment was carried out at the Agricultural Research Station (EEA) of Anguil in the Western Pampa region of Argentina. In March 19, 2002, forty steers ageing 15 months and weighing 330 ± 12 kg were randomly allocated into four groups of donor animals. A washout period of 8 months was allowed after the last antiparasitic treatment with a benzimidazole. Each group of animals was kept in separate paddocks after treatments and until the last faecal samples were collected. Group GD was treated subcutaneously with doramectin (DRM) (Dectomax®, Pfizer) with the dose of 200 μg/kg b.w. Group GM was treated subcutaneously with moxidectin (MXD) (Cydectin®, Fort-Dodge) with the dose of 200 μg/kg b.w. Group GMp was treated topically with MXD (Cydectin, Fort-Dodge) with the dose of 500 μg/kg b.w. Group GT remained untreated as the control group.

2.2. Faecal collection and pat preparation and deposition

Fresh faeces from each experimental group were collected on days 3, 11, and 21 pt, mixed thoroughly and divided into 550 g wet weight aliquots fashioned into experimental pats of 15 cm of diameter and 4 cm of height. A total of 144 pats were prepared for each experimental group. Twelve pats per combination of time (i.e., days pt vs. days in the field) were prepared and deposited in the field. For the control group, an additional row corresponding to time 0 post-deposition (pd) for each post-treatment time was prepared. These artificially formed pat replicates were kept refrigerated and placed within 18–22 h after collection. Experimental pats of each group were deposited in the field forming blocks of three columns and four rows. Columns corresponded to times pt (3, 11, and 21) and rows corresponding to time in the field (6, 14, 21, and 42). Blocks were placed in line, separated by a 2-m-wide alley. Adjacent pats within blocks were separated by 2 m. Pats were deposited on the bare spaces of sandy soil, between the plants of a representative alfalfa pasture, using separate equipment to avoiding possible cross contamination of the pats.

2.3. Pat determinations

At deposition day, weight, dry matter, and parasitological measurements from fresh faeces were determined. Ten grams of wet weight of the samples from each group was collected for estimating faecal moisture. This was done by drying samples for 48 h at 100 °C and expressed as percentage of wet weight. Nematode egg counts were done according to the method of Roberts and O'Sullivan (1949), and specific infective larvae (L_3) differentiated after culture of faecal samples (Suárez, 1997). Eight whole pats (replicate) per group were recovered and examined at 6, 14, 21, and 42 pd days. Collected pats were transferred to plastic bags and taken to the laboratory. Each pat sample was weighed and the dry weight was determined. The dung fauna was enumerated and identified. Coleoptera, Diptera, Hymenoptera, Collembola, and Acari were recovered using individual Berlese funnels for each pat sample (Berlese, 1904). However, an important limitation of this method is that only live insects would be recovered and can yield a low number of collected insects if samples present a high lethality. A 5-g dung sub-sample was processed through Baermann modified method (Suárez, 1997) to isolate nematode larvae.

An attempt to recover insects immediately under the pats and in the soil under the pat at the time of collection was made. However, since the number of organisms was very low, it is not reported here. Organisms recovered were ants, homoptera (Cicadellidae), crustacean (*Armadillidum vulgar*), earth worms, and collembola.

2.4. Measurement of faecal drug residue concentrations

Faecal sub-samples of 1.25 g, from each of the eight pat samples recovered per group, were collected and mixed for drug residue determination. Mixed faecal samples of 10 g of moxidectin- and doramectin-treated animals were kept in labelled vials and stored at −20 °C until analysed by high-performance liquid chromatography (HPLC). The extraction of MXD and DRM from faecal samples was carried out following the technique described by Lifschitz et al. (1999, 2000). Briefly, 1 g aliquots of faeces were combined with the internal standard abamectin, 1 mL acetonitrile, and 0.125 mL water. The mixture was mixed (Multi Tube Vortexer, VWR Scientific Products, West Chester, PA, USA) for 20 min. After mixing, the faecal samples were sonicated during 10 min (Transsonic 570/H, Laboratory Line Instruments Inc., Melrose Park, IL, USA) and the solvent–sample mixture was centrifuged at $2000 \times g$ for 15 min. The supernatant was manually transferred into a tube and the procedure repeated once. The pooled supernatants obtained were then placed on the appropriate rack of an Aspec XL autosampler (Gilson, Villiers Le Bell, France). The derivatization to convert MXD and DRM in fluorescent molecules was performed in accordance to the method proposed by De Montigny et al. (1990). Moxidectin and DRM faecal concentrations were determined by HPLC with fluorescence detection using a Shimadzu 10 A HPLC system (Shimadzu Corporation, Kyoto, Japan) following the technique described by Lifschitz et al. (1999, 2000). A reverse phase C18 column (Selectosil, Phenomenex, Torrance, CA, USA) (5 μm, 4.6 mm × 250 mm) kept in a column oven at 30 °C (Shimadzu Corp.) and an acetonitrile/methanol/acetic acid (0.2% in

water) (53:40:7) mobile phase at a flow rate of 1.5 mL/min were used. MXD and DRM were detected with a fluorescence detector (Spectrofluorometric detector RF-10, Shimadzu), reading at an excitation wavelength of 365 nm and an emission wavelength of 475 nm. The MXD or DRM/ABM peak area ratios were used to estimate the MXD and DRM concentrations in spiked (validation of the analytical method) and experimental faecal samples. The solvents (Baker, Phillipsburg, NJ, USA) used during the extraction process and drug analysis were of HPLC grade. A complete validation of the analytical procedures used for extraction and quantification of MXD and DRM was performed before starting the analysis of experimental samples. Calibration curves were prepared in a range between 0.5 and 100 ng/g using least squares linear regression analysis. Correlation coefficients (r) were >0.99. Drug recovery from faeces at different concentrations was 78.0% (MXD) and 73.0% (DRM). The interassay precision expressed as coefficient of variation was 7.90% (MXD) and 5.26% (DRM). The limit of quantification for MXD and DRM in faeces was established at 0.5 ng/g.

2.5. Weather data

Daily rainfall, evaporation, relative humidity, and daily temperatures were recorded from the Department of Meteorology of INTA Anguil, near to the experimental site.

2.6. Statistical analysis

The data of the counts of arthropods and nematodes recovered per 100 and 5 g, respectively, of dung fresh matter, weight, and dry matter percentages from eight replicated pats were analysed by analysis of variance with treatment and duration of field exposure as factors (STATITCF, 1988). A two-way ANOVA was used to analyze data, with treatment and time in the field as the two factors. Since the distribution of the arthropod data was not normal, data were previously rank transformed to perform the statistical analysis (Iman and Canover, 1979a, b). Results are reported as mean ± S.E.M. of raw data.

3. Results

The annual precipitation in this region ranged between 700 and 800 mm and the highest incidence was from October (spring) to April (autumn). During the whole study (March 19–May 20), the precipitation (253.6 mm) was very intense. The temperatures were moderate and averaged 17.8 °C.

Statistical analysis showed no interaction between treatment and time in the field. The infective larval genera of gastrointestinal nematodes recovered from the control cattle pats were *Ostertagia*, *Haemonchus*, *Cooperia*, and *Trichostrongylus*. Only negligible infective nematode larvae were detected in pats of MXD-treated groups and no larvae from DRM-treated group.

The number of insects recovered at days 3, 11, and 21 pt from the control pats was significantly ($P<0.001$) higher than those of the treated-animal pats during all the trials (Table 1). Statistical analysis showed that significant differences among groups were significant ($F=3.25$, $P<0.05$, d.f. 86). At day 21 pt, only the number of insects collected from dung voided by doramectin-treated cattle was ($P<0.05$) lower than the other groups. There was a significant ($P<0.01$) interaction between treatment groups and time in the field.

Table 1
Average ± S.E.M. no. of insects recovered from 100 g of fresh pats deposited 3, 11, and 21 days post-treatment (pt) and exposed for 6, 14, 21, and 42 days on the field

Group	Day 6 pd	Day 14 pd	Day 21 pd	Day 42 pd
Day 3 pt				
GD	12.4 ± 6.6 c	10.4 ± 2.9 b	8.8 ± 5.1 c	17.6 ± 8.0 a
GM	22.6 ± 15.1 b	16.6 ± 7.5 b	12.6 ± 6.9 bc	20.2 ± 11.6 a
GMp	15.3 ± 7.0 bc	19.2 ± 9.8 b	16.3 ± 9.9 b	13.5 ± 5.2 a
GT	48.4 ± 25.4 a	44.1 ± 17.3 a	48.4 ± 30.1 a	16.6 ± 4.9 a
Day 11 pt				
GD	3.9 ± 2.2 b	22.6 ± 8.9 b	21.8 ± 12.2 b	22.7 ± 13.0 b
GM	4.8 ± 1.7 b	31.7 ± 20.3 b	23.9 ± 11.4 b	19.2 ± 9.9 b
GMp	3.7 ± 0.9 b	32.2 ± 19.0 b	27.0 ± 16.9 b	23.3 ± 15.5 b
GT	8.4 ± 5.1 a	69.8 ± 25.8 a	45.0 ± 30.2 a	39.7 ± 21.7 a
Day 21 pt				
GD	3.4 ± 2.0 b	11.7 ± 7.1 b	8.8 ± 3.2 b	9.7 ± 3.9 a
GM	6.9 ± 2.6 a	22.5 ± 10.1 a	16.0 ± 5.0 a	6.8 ± 2.7 a
GMp	7.2 ± 2.1 a	20.6 ± 12.0 a	17.9 ± 6.1 a	7.8 ± 3.5 a
GT	7.9 ± 2.6 a	22.2 ± 9.9 a	17.6 ± 5.6 a	8.8 ± 4.6 a

Dung pats were from cattle treated subcutaneously with doramectin (GD), moxidectin (GM), topically with moxidectin pour-on (GMp), and untreated control (GT).
Column means values with different letters are significantly different at $P<0.05$.

The great variety of species collected were divided in large arthropods, mainly Coleoptera and Diptera larvae, and microarthropods such as Collembola and Acari. The classification is based on the work by King (1993), where the author outlines the methods and sampling techniques for micro and macro invertebrates, using the term microarthropod to name Collembola and Acari. For the analysis of the species diversity, we considered three categories: adult insect, insect larvae, and microarthropods, based on feeding behavior (dung-feeding). However, to study adult insects not only feeding behavior but migration should also be considered. The latter was not considered in the present work, since our main goal was to analyze the abundance and diversity of immature forms and microarthropods based on their dung-feeding behavior.

3.1. Coleoptera

The most numerous family of Coleoptera present in the pats were Scarabaeidae. The largest and active dung beetles found were *Sulcophanaeus menelas*, *Onthophagus hirculus*, and *Canthidium breve*, but the most frequent family recovered by the Berlese method were mainly Aphodiidae and Histeridae. Other species families such as Melolonthidae, Staphylinidae, and Elateridae were also recovered. No significant differences ($P=0.65$) were observed between the adult Scarabaeidae, Aphodiidae, and Histeridae. Only the Sthaphylinidae number ($P<0.061$) from the control pats collected at day 3 pt was higher than treated groups. Mean Sthaphylinidae numbers recovered from 100 g of

fresh faeces were 4.84±3.29, 3.30±1.92, 2.50±2.01, and 1.11±1.02 for GT, GM, GMp, and GD, respectively.

The Coleoptera larvae counts (mainly Aphodiidae) in the dung pats from treated groups were significantly ($P<0.05$) lower than those from the control group only at day 3 after treatment and after 21 days in the field. The mean number of Coleoptera larvae collected per 100 g of pat fresh weight is shown in Table 2. The number of larvae recovered from GD group was significantly ($P<0.05$) lower than from the other groups. No statistical differences ($P<0.64$) were seen at days 11 and 21 pt.

The predominant families of Diptera larvae recovered were Calliphoridae, Sarcophagidae, and Muscidae. Table 3 shows total mean Diptera counts recovered from dung pats examined at days 6, 14, 21, and 42 pd. Control group pats excreted from the 3rd to 21st pt day contained the highest ($P<0.018$) counts (total mean: 30.3±32.5 larvae), but at day 21 pt there were no differences between moxidectin-treated groups and control group. The mean number of total larvae obtained from samples of the GM (11.0±11.5 larvae) and the GMp (11.5±13.2 larvae) groups were higher ($P<0.05$) than those from the GD (5.4±6.6 larvae) group. At day 3 after treatment, there were no differences between endectocide-treated groups, but at day 11 pt more larvae were obtained ($P<0.05$) from the faeces of the groups treated with MXD pour-on (GMp 0.44±0.43, GM 0.22±0.28, GD 0.20±0.26), and at day 21 pt, the Diptera larvae counts of GM (0.27±0.25 larvae) and GMp (0.22±0.24 larvae) groups were higher ($P<0.05$) than those of the GD (0.13±0.15 larvae) group.

Table 2
Average±S.E.M. larvae of Coleoptera recovered from 100 g of fresh pats deposited 3, 11, and 21 days post-treatment (pt) and exposed for 6, 14, 21, and 42 days on the field

Group	Day 6 pd	Day 14 pd	Day 21 pd	Day 42 pd
Day 3 pt				
GD	0.39±0.33 a	1.24±1.09 a	3.50±3.22 c	1.19±1.10 a
GM	0.47±0.41 a	1.76±1.50 a	9.96±4.11 b	6.55±7.16 a
GMp	0.35±0.30 a	2.29±1.18 a	6.13±4.28 b	4.75±6.63 a
GT	0.40±0.34 a	2.19±2.01 a	15.61±7.57 a	4.74±5.05 a
Day 11 pt				
GD	0.01±0.02 a	2.00±1.01 a	4.92±3.22 a	3.44±3.25 a
GM	0.08±0.09 a	1.55±1.23 a	4.71±4.10 a	2.96±3.11 a
GMp	0.06± 0.05 a	2.89±2.00 a	4.00±3.88 a	2.78±2.91 a
GT	0.02±0.02 a	2.63±1.75 a	4.18±3.57 a	2.57± 2.70 a
Day 21 pt				
GD	0.90±1.01 a	0.97±1.10 b	3.89±3.71 a	1.07±1.19 a
GM	0.36±0.63 a	1.56±1.21 a	3.51±3.50 a	1.25±1.08 a
GMp	1.02±0.97 a	3.00±3.90 a	4.08±3.93 a	2.97±3.86 a
GT	0.88±1.15 a	2.57±2.49 a	5.18±5.03 a	2.10±2.07 a

Dung pats were from cattle treated subcutaneously with doramectin (GD), moxidectin (GM), topic with moxidectin pour-on (GMp) and untreated control (GT).
Column means values with different letters are significantly different at ($P<0.05$).

Table 3
Average±S.E.M. of Diptera larvae recovered from 100 g of fresh weight from pats exposed 6, 14, 21, and 42 days post-deposition (pd) on the field

Group	Day 6 pd	Day 14 pd	Day 21 pd	Day 42 pd
GD	0.30±0.40 b	0.83±0.98 c	0.51±0.58 c	0.22±0.70 b
GM	0.22±0.35 b	1.54±1.80 b	1.64±1.80 a	0.50±0.67 a
GMp	0.41±0.47 b	1.13±1.42 bc	1.71±2.12 a	0.48±0.51 a
GT	1.12±1.05 a	4.43±3.81 a	4.23±4.11 a	0.37±0.31 a

Figures are the mean Diptera larvae of the three times post-treatment (3, 11, and 21) for each time post-deposition.
Dung pats were from cattle treated subcutaneously with doramectin (GD), moxidectin (GM), topically with moxidectin pour-on (GMp), and untreated controls (GT).
Column means values with different letters are significantly different ($P<0.05$).

3.2. Microarthropods

Collembola specimens numbers showed significant ($P<0.05$) differences between treated groups and the non-treated group after 21 days in the field. Mean total Collembola numbers recovered from 100 g of fresh faeces were 15.5±7.27, 5.8±3.84, 5.6±3.65, and 5.8±4.80 for GT, GM, GMp, and GD, respectively. Numbers of Acari recovered from the control group pats were significantly ($P<0.003$) higher than those of treated groups. Mean total Acari numbers recovered from 100 g of fresh faeces were 51.20±37.11, 18.90±11.14, 15.17±10.50, and 15.4±9.97 for GT, GM, GMp, and GD, respectively. There were no differences in the number of Collembola and Acari between endectocide-treated groups at days 3 and 11 pt, but only GD showed lower ($P<0.05$) numbers of microarthropods than those of the other groups at day 21 pt.

About dung-specific nematodes recovered, only on days 3 and 11 after treatment, total counts occurred in significantly ($P<0.05$) reduced numbers in pats from treated cattle compared with controls. This drug adverse effect was observed in treated cattle pats from 14 days of field exposure. Mean total nematode numbers recovered from 5 g of fresh faeces were 80.3±65.02, 37.78±21.36, 39.95±23.31, and 36.3±21.47 for GT, GM, GMp, and GD, respectively. No further characterization was done of the dung-specific nematodes, since the aim of the present work was the arthropods involved in dung recycling.

There was a very high percentage (41.3%) of pat depositions totally destroyed by the great dung beetles (*Sulcophanaeus*, *Onthophagus*). Total pat destruction was rapid at the beginning of the deposition and put out for any determination. There were no significant differences in dung pat wet and dry weight (moisture content) and in the number of pats destroyed or buried in the four groups at any time. The appearance between treated and control group pats was not different throughout this study. The mean dry weight of entire or partially formed pats during the whole trial was 122±65.2, 136.0±70.4, 112.5±65.0, and 108.3±49.9 g for GD, GM, GMp, and GT group, respectively.

The methodology used for determining drug concentration in faeces (HPLC) presents a higher sensitivity (limit of detection) than that used in other works (TLC) (Floate et al., 1997). This allowed for a more precise determination of drug degradation and concentrations present at different times. Tables 4–6 show the endectocide residue concentrations expressed as ng/g of dry matter of faecal pat. Large concentrations of DRM and MXD were observed. DRM concentrations measured at day 3 pt varied from 1277 ng/g (at the deposition time) to 559 ng/g (after 42 days in the field). Doramectin faecal concentrations obtained at 21 days pt varied between 291 and 113 ng/g from days 0 to 42 pd, respectively. No differences of MXD residues between both formulations were observed in the pats exposed in the field. However, higher initial MXD residues at day 3 pt in MXDp group (782–490 ng/g) than in the MXD group (645–160 ng/g) were observed. This can be explained by the effect of the licking behaviour of the animals. After topical treatment, animals lick themselves and between them (allolicking), ingesting topically administered drug and determining high concentrations in the gastrointestinal content during the first days post-treatment (Laffont et al., 2001; Sallovitz et al., 2005).

Table 4
Mean moxidectin (MXD) ($n = 3$) concentrations (ng/g dry weight) in dung pats obtained at different days post-administration from cattle treated with MXD subcutaneously (200 μg/kg b.w.)

Days post-deposition	Days post-treatment (MXD, ng/g)		
	3	11	21
0	645±21.6	76.3±6.72	42.9±3.18
6	455±114	24.7±17.3	11.7±2.93
14	511±43.6	45.3±11.5	14.3±1.76
21	160±20.8	38.9±9.00	9.93±2.11
42	160±12.0	42.2±5.40	9.74±5.67

After the deposition, the pats were exposed in the field for different periods (6–42 days). pt: days post-treatment. pd: days post-deposition.

Table 5
Mean moxidectin (MXD) ($n = 3$) concentrations (ng/g dry weight) in dung pats obtained at different days post-administration from cattle treated with MXD pour-on (500 μg/kg b.w.)

Days post-deposition	Days post-treatment (MXD, ng/g)		
	3	11	21
0	782±24.7	94.5±6.36	25.0±6.33
6	766±22.4	16.3±4.38	7.60±3.58
14	349±105	38.6±18.7	7.96±0.52
21	481±317	35.7±19.2	7.82±6.13
42	490±128	29.2±16.2	4.67±0.64

After the deposition, the pats were exposed in the field for different periods (6–42 days). pt: days post-treatment. pd: days post-deposition.

Table 6
Mean doramectin (DRM) ($n = 3$) concentrations (ng/g dry weight) in dung pats obtained at different days post-administration from cattle treated with doramectin subcutaneously (200 μg/kg b.w.)

Days post-deposition	Days post-treatment (DRM, ng/g)		
	3	11	21
0	1277±53.0	312±11.3	291±21.2
6	577±256	170±14.1	131±42.6
14	341±70.7	254±59.8	124±30.3
21	363±44.7	213±6.36	99.8±30.9
42	559±18.3	244±89.9	113±15.8

After the deposition the pats were exposed in the field for different periods (5–42 days). pt: days post-treatment. pd: days post-deposition.

4. Discussion

The current trial corroborates the high dispersion of faeces deposited in early autumn. This phenomenon could be explained by the action of the large beetles and the high incidence of rainfall that occurred between March and April in the Pampa region. Therefore, a high number of deposited pats were destroyed at the beginning of the trial and, thus, the initial measures planned were performed until the 42nd day post-deposition of faeces in the environment. In contrast with Wardhaugh and Mahon (1991), in the present study, there was no difference in dung mass dispersion among the experimental groups and in the attraction of the adult Scarabaeidae to the pats deposited from the endectocide-treated animals. These results agree with others obtained from trials performed in the same area (Suárez, 2002a; Suárez et al., 2003). However, a recent work, using pitfall traps baited with treated and untreated cattle dung, shows that the presence of endectocide residues affects the insect attraction to dung (Floate, 2007). This work shows that MXD presents a lower repellency of insects than DRM.

A lower number of total arthropods were obtained from the faeces of cattle treated with MXD and DRM compared with the control group. These negative effects were markedly observed in the dung containing DRM, and it was in agreement with previous studies performed with IVM and DRM (Suárez, 2002a, Suárez et al., 2003).

The mean Coleoptera larvae recovered was Aphodiidae, because this experiment design does not account for the effect of drug residues on the larvae of dung burying beetles as *Sulcophanaeus*, *Onthophagus*, or *Canthidium*. Coleoptera larvae counts were lower in dung pats obtained at 3 days post-administration of DRM, containing concentrations between 559 and 1277 ng/g (dry weight). Dadour et al. (2000) observed an adverse effect of DRM against *Onthophagus binodis* up to 18 days post-administration with a concentration in faeces above 60 ng/g (wet weight), while Suárez et al. (2003) observed toxicity up to 21 days pt with faecal concentrations of 200 ng/g dry weight. These differences may be explained by the rapid pat destruction observed in the current trial and the possible

interaction with other species of dung-decomposing fauna under different climatic conditions.

The effect of MXD residues showed interesting results. The higher MXD faecal concentrations obtained during the first days after the pour-on administration account for a greater negative effect against Coleoptera larvae in the pats obtained after 3 days post-administration compared with the MXD subcutaneous treatment. After this period, the pour-on treatment of MXD resulted in a lesser harmful effect compared with the subcutaneous administration. These results are in agreement with the effect of the licking behaviour on the pattern of faecal elimination of MXD described for the animals treated with pour-on formulations (Laffont et al., 2001; Sallovitz et al., 2003). The licking behaviour facilitates the oral ingestion of the topically administered drug resulting in higher concentrations excreted by faeces during the first 3 days post-administration compared with the subcutaneous treatment.

Other studies reported a lower adverse effect for MXD compared with DRM. However, it should be noted that although MXD produced the lower reduction in the number of insects, these are results combined for all the species (Doherty et al., 1994). More detailed works showed that this reduction in the number of insects is not due to a negative effect on all the dung-dwelling species, but only some species are affected (Floate et al., 2001, 2002). The residues of IVM and abamectin in faeces not only affect the development and reproduction of Coleoptera adults but also are toxic against larval stages between 2 and 4 weeks after administration of the injectable formulation (Strong and Wall, 1994; Herd, 1995). A significantly longer period (12 weeks) was observed with IVM for certain species of flies (Floate, 1998a).

Diptera larvae were affected by DRM during 21 days pt while MXD exert its deleterious action during 6 and 14 days post-administration of the pour-on and injectable formulation, respectively. These results accord with other experiments that indicate that MXD is ecologically safer than other endectocides (Herd, 1995; Lumaret, 1997; Floate et al., 2002). The faecal residues of IVM prevented the development of Cyclorrhapha, *Musca vetustissima*, *Musca domestica*, and *H. irritans* during 35 days in the environment (Madsen et al., 1990; Wardhaugh and Mahon, 1998). Guglielmone et al. (1998) reported an efficacy of IVM against adult stages of *H. irritans* of approximately 2 weeks. Anziani et al. (2001) evaluated the activity of the DRM in treated cattle against field population of *H. irritans* and observed in the faeces no adult emergence during 35 days after treatment. Although, dung residues from steers treated with injectable MXD had no significant effect on larvae of *M. vetustissima* and *M. domestica* (Wardhaugh et al., 1996), other experiences showed that MXD had larvicidal activity against the immature stages of *H. irritans* in the manure of treated cattle. Lumaret (1997) evidenced that MXD residues in sheep and horse faeces were toxic to the fly *Neomyia cornicina* stages during few days post-treatment with an oral formulation, and Doherty et al. (1994) found toxic effects of MXD against *Haematobia* larval survival around concentrations of 128 ppb, but without effects on the eclosion of the adult flies. Nevertheless, MXD has always showed shorter toxic effect than IVM. The lower faecal concentrations obtained from cattle after the MXD treatment compared with those obtained after IVM and DRM administration (Lifschitz et al., 1999, 2000) may influence on its minor deleterious effect against dung colonizing fauna. Considering the total drug excreted in faeces, the percentage of inactive metabolites was two-fold higher after the subcutaneous administration of MXD compared to those obtained after the IVM and DRM subcutaneous treatment (Lifschitz, 2000). In the present trial, endectocide concentrations declined along the experimental time. A reduction ranging between 66% (MXD) and 50% (DRM) of the initial concentrations was observed. Despite this difference in degradation percentages, a non-statistically significant tendency in the slope degradation ($P>0.05$) was observed between both drugs. As drug concentration in the dung declined, the number of larval forms recovered increased. This could be explained because insects arrive to the dung at different times and the later they arrived, the lower the drug concentration and the lesser the effect on larval development. This does not explain the difference in the insect number obtained for each drugs. A differential insect sensitivity to drugs is a more plausible explanation, as suggested by Floate et al. (2001). However, low drug concentrations may allow larval development, but this does not imply that there is no negative effect, which could be seen in later stages of development (Floate et al., 2005).

A possible explanation for the few dipteran families recovered in the present work may be that sampling 6 days pd is a too long time. This neglects the recovery of Diptera larvae such as Brachycera and Nematocera, which present a fast development and early emergence. Also, the heavy rainfalls and subsequent low temperatures at the fall beginning could have reduced the Diptera number by day 6 pd.

Microarthropods were affected by DRM and MXD treatments. The data obtained in the current trial in addition with previous reports from the same area (Suárez, 2002a; Suárez et al., 2003) and from Brazil (Iglesias, 1998) confirm the detrimental action of the macrocyclic lactones against these microarthropods. A deleterious action up to 11 days pt was observed against dung-specific nematodes in pats containing DRM or MXD without significantly differences between drugs.

Results testing the effect of treatment on the rate of pat degradation were inconclusive, due to confounding activities of dung-degrading beetles and intense rainfalls. Other techniques, such as luminiferous traps or baits, are needed to differentiate between these confounding factors. Contradictory information over this topic exists, while some trials show a low rate of dung dispersion in faeces from cattle treated with IVM (Barth et al., 1993; Strong, 1993; Suárez,

2002b, Iglesias et al., 2006), others report that there is no delay in dung destruction (Iglesias, 1998; McKeand et al., 1988; Suárez et al., 2003). The differences between trials may reflect the regional or seasonal diversity of dung fauna or the differences of the experimental designs applied. The exclusion of beetles and flies from fresh dung pats decrease the rate of degradation (Lumaret and Kadiri, 1995).

Previous results obtained in the West Pampa region (Suárez, 2002a, Suárez et al., 2003) show that avermectin residues are high and toxic to non-target dung-breeding fauna. The present results evidence a lower detrimental effect of MXD, mainly against some beetles and flies, independently of the route of administration used. Currently, different evaluation trials under real production system have been performed to understand the long-term consequences of the toxic effect of these compounds and the probable damage on pastureland ecology. Further research is needed to clarify the biology of the dung beetles and their importance on the dispersion of faeces from cattle.

In conclusion, the use of antiparasitic endectocide drugs for treating cattle affect the dung degradation owing to their non-target effects on dung-dwelling fauna. Faecal residues of both drugs presented a similar pattern of degradation after environmental exposure of the dung. However, a lower adverse effect was observed for moxidectin than for doramectin and this was not affected by the route of administration. Currently, in Argentina, studies of the environmental impact of antiparasitic drugs for cattle is not requested by regulatory agencies; however, this should be considered in the future if parasite control programmes will still rely on the administration of chemical compounds to cattle and the preservation of the soil and pasture ecosystems is desired.

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Animal welfare

The present experiment was approved by the Comisión de Bienestar Animal (Animal Welfare Committee) according to the Acta de Bienestar Animal (Animal Welfare Act, Res. 087/02, Consejo Académico, Facultad de Ciencias Veterinarias, UNCPBA).

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