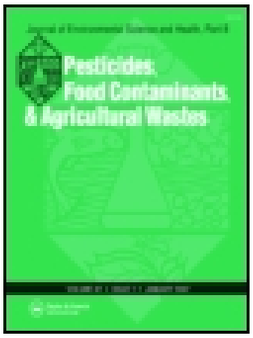

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Journal of Environmental Science and Health, Part B
Pesticides, Food Contaminants, and Agricultural Wastes

ISSN: 0360-1234 (Print) 1532-4109 (Online) Journal homepage: http://www.tandfonline.com/loi/lesb20

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To cite this article: Lucía Emilia Iglesias, Carlos Saumell, Federica Sagüés, Juan Manuel Sallovitz & Adrián Luis Lifschitz (2017): Ivermectin dissipation and movement from feces to soil under field conditions, Journal of Environmental Science and Health, Part B, DOI: 10.1080/03601234.2017.1371554

To link to this article: http://dx.doi.org/10.1080/03601234.2017.1371554

Published online: 26 Sep 2017.

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JOURNAL OF ENVIRONMENTAL SCIENCE AND HEALTH, PART B
2017, VOL. 0, NO. 0, 1–7
https://doi.org/10.1080/03601234.2017.1371554

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Ivermectin dissipation and movement from feces to soil under field conditions

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ABSTRACT

The aim of this work was to evaluate the fate of ivermectin (IVM) at two concentrations in cattle feces and its movement to the nearby soil and plants. Feces were spiked with IVM at two levels: 3000 ng g^{-1} (high group, HG) and 300 ng g^{-1} (low group, LG). Artificial dung pats were prepared and deposited in an experimental field area. Feces and underlying soil were sampled up to 60 days post-deposition (dpd). As an additional analysis, grasses growing around the pats were sampled at 30 and 60 dpd. Ivermectin concentrations in all matrices were determined by HPLC. Mean IVM fecal concentrations were in the range between 3901.9 ng g^{-1} and 2419.2 ng g^{-1} (high group) and 375.3 ng g^{-1} and 177.49 ng g^{-1} (low group). Mean times for 50% and 90% dissipation were 88.23 and 293.03 days (HG) and 39.1 and 129.9 days (LG). Soil concentrations ranged from 26.1 ng g^{-1} to 71.1 ng g^{-1} (HG) and 3.4 to 5.9 ng g^{-1} (LG); in plants, concentrations were between 71.4 and 380.8 ng g^{-1} and 5.40 and 51.8 ng g^{-1} in HG and LG, respectively. These results confirm that IVM moves from feces to the underlying soil as well as to nearby plants. The potential risk of detrimental effects on soil organisms and the impact on herbivorous animals should be further evaluated.

ARTICLE HISTORY
Received 27 April 2017
Accepted 22 August 2017

KEYWORDS
Ivermectin; ecotoxicology; endectocides; cattle; feces; soil; plants

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Introduction

The expansion of agronomic and veterinary activities introduces chemicals into the environment from different sources and often without a complete knowledge of their environmental impact. Potential transfer of these molecules to other substrates (soil, water and organisms), as well as their bioaccumulation, are incompletely understood. In this context, public concern has been raised regarding safe and non-polluted food products.

Antiparasitic drugs are widely used to both prevent and treat animal parasitic diseases that result in economic losses in livestock. Since non-chemical parasite control strategies are not yet available for commercial use, parasite control in livestock still relies on the use of antiparasitic drugs. Due to their wide applicability, anthelmintic residues are expected to have possible effects on the environment. Several studies have evaluated the potential environmental risk of veterinary medicine residues.[1–3] Avermectins (i.e., ivermectin, doramectin and abamectin) are very effective drugs against endo/ectoparasites in cattle. These compounds are poorly metabolized in cattle, are largely secreted in the bile and excreted into the feces as unchanged parent drugs.[4] Many factors, such as the administration route, dose rate, animal species and pharmaceutical formulation may influence avermectin concentrations excreted in the feces.[5–7]

Despite the benefits of ivermectin (22,23-dihydroavermectin B_{1a} + 22,23-dihydroavermectin B_{1b}; IVM) use on livestock production systems, this compound may affect the environment. The persistence of this drug in dung could pose a risk for a wide variety of terrestrial insects colonizing and consuming dung pats.[2–12] Several aspects may influence the environmental impact of veterinary drug residues, such as the molecule's physicochemical properties, soil structure, climatological conditions and vegetation characteristics.[1–15] There is scarce information on the movement of IVM from the feces of treated cattle to soil and its relationship with drug persistence in the environment. Due to the high lipophilicity of IVM, this molecule tends to bind to soil particles.[16,17] The soil properties, climatic conditions and regional biota may modify the degree of sorption and dissipations rates of IVM.[17] The sorption and desorption of IVM to soil under laboratory conditions has been recently described.[18] Importantly, the persistence of low concentrations of IVM in the environment may not only affect non-target organisms, but also can trigger resistant strains of parasites.[19] In this circumstance, it is important to characterize the relationship between IVM concentrations in feces and soil and the potential passage of the drug to plants. The aim of this work was to characterize the fate of IVM at two concentrations in cattle feces under environmental conditions and its movement to underlying soil.

Materials and methods

Study area

This trial was carried out in an experimental area (20 m × 20 m) of the Faculty of Veterinary Sciences, Tandil, Argentina (37°17′34″S, 59°5′W) in the period from November 8, 2012 to January 7, 2013. The soil was characterized as a

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typical Argiudoll and the pasture composition was *Trifolium repens, T. pratense*, *Lolium perenne* and *Dactylis glomerata.*

Fecal samples

Feces were obtained from four naturally infected Holstein calves, about 2 years old (300 kg) without a history of previous anthelmintic treatment in the last 8 months.

Meteorological data

Daily temperature and precipitation data during the study were provided by the Plains Institute of Hydrology station at the Faculty of Veterinary Sciences (Li-1200S, Li-Cor Inc. Lincoln, Nebraska).

Experimental design

Prior to beginning the experiment, soil characterization was made at the Soil Analysis Laboratory, Faculty of Agronomy (UNCPBA), Azul, Argentina. Soil properties are summarized in Table 1.

Twenty-two kg of fresh cattle feces were homogenized for 10 min using a small concrete mixer. From this total fecal mass, three aliquots of 6 kg each were taken to prepare experimental pats. The remaining feces were stored at −20°C and used for method validation. Two working solutions of IVM in ethanol were prepared: 100 μg mL^{-1} (high) and 10 μg mL^{-1} (low). These solutions were used to spike the fecal masses to a final concentration of 3000 ng g^{-1} (high) and 300 ng g^{-1} (low). The lowest IVM fecal concentration reflects the in vivo concentrations obtained after the subcutaneous administration of a commercial formulation to cattle.[4] The feces were thoroughly mixed after the addition of the relevant level of the drug. One aliquot of feces remained without drug addition, as a control group. Following drug addition and mixing, the feces were left to stand at room temperature for 30 min to allow for solvent evaporation. Three samples of each aliquot were then collected from different parts of the mix to obtain the sample at day 0.

Ten samples of each aliquot were taken to prepare the experimental dung pats (600 g, 20 cm diameter, 3 cm high), which were immediately deposited in the experimental field area. Experimental dung pats were 1 m apart from each other. Fecal and underneath soil samples (2 cm depth) were collected at 1, 7, 14, 30 and 60 days post deposition (dpd) in a clockwise direction, starting from the south. Samples were maintained at −20°C until HPLC analysis for drug quantification. At each dpd, the amount of total dry matter was determined in fresh feces and soil samples. Dry matter was estimated as the weight loss of feces and soil samples dried at 60°C in the oven until a constant weight. The mean percentage of dry matter in feces was between 8.84% (1 dpd) and 59.83% (60 dpd). The mean percentage of dry matter in soil was 80%. On day 14 post-deposition, some grasses were observed growing in the neighborhood of the dung pats. Even though it was not part of the original objective of the present work, these plants were collected on days 30 and 60 post-deposition, carefully rinsed with distilled water and stored for later analysis. The mean percentage of dry matter in plants was 24%.

Table 1. Soil characteristics (0–20 cm) of the experimental plot at the Faculty of Veterinary Medicine, Tandil, Argentina (37°17′34″S, 59°5′W).

				Texture%			
Soil type	OM[a]	pH[b]	BR[c]	Sand	Clay	Silt	Textural class
Typical Argiudol	5.42	6.4	0.080	40.55	39.81	19.64	Clay loam

[a]OM: organic matter (Walkley and Black).
[b](1:2,5) in water.
[c]BR: basal respiration (mg C-CO_2 g^{-1}day^{-1}).

Analytical procedures

Analytical standards of IVM and doramectin (internal standard) were obtained from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA). The standard purity was IVM B1a > 90% and IVM B1b < 5%. Methanol and acetonitrile (all HPLC grade) were purchased from J.T. Baker (Center Valley, PA, USA).

Ivermectin concentrations in feces, soil and plants were determined by high performance liquid chromatography (HPLC) with fluorescence detection, as reported by Lifschitz et al.[4] Whole plants were ground before HPLC analysis. The component measured in the different biological matrices was IVM B1a.

Briefly, 0.5 g of feces or soil and 1 g of plant samples were spiked with the internal standard (doramectin, 40 ng g^{-1}) and left to stand for 20 min. One mL of acetonitrile was then added and thoroughly mixed (Multi Tube Vortexer, VWR Scientific Products, West Chester, PA, USA) for 15 min. Samples were sonicated in an ultrasonic bath for 10 min (Transsonic 570/H, Laboratory Line Instruments Inc., Melrose Park, IL, USA) and centrifuged at 2600 *g* for 20 min. The supernatant was manually transferred into a tube and the procedure was repeated once. The supernatant was then placed on an appropriate rack of an Aspec XL sample processor (Gilson, Villiers Le Bel, France) to perform the solid-phase extraction.[4] The derivatization to render ivermectin fluorescent was performed with 100 μL of a solution of *N*-methylimidazole (Sigma Chemical, St Louis, MO, USA) in acetonitrile (1:1) and 150 μL of trifluoroacetic anhydride (Sigma Chemical, St Louis, MO, USA) solution in acetonitrile (1:2).[20] After completion of the reaction (<30 s), an aliquot (100 μL) of this solution was injected directly into the HPLC system (Shimadzu 10A HPLC System; Shimadzu, Kyoto, Japan). Drug detection was performed using a fluorescence detector (spectrofluorometric detector RF 10; Shimadzu) at an excitation wavelength of 365 nm and emission wavelength of 475 nm with an acetic acid (0.2% in water)-methanol-acetonitrile mobile phase (1.6:60:38.4; v/v/v). The flow rate was set at 1.5 mL min^{-1} through a reverse phase C18 column (Kromasil, Eka Chemicals, Bohus, Sweden, 5 μm, 4.6 mm × 250 mm) kept in an oven at 30°C.[4] Calibration curves were established using least-square linear regression analysis and correlation coefficients (r) and coefficient of variations (CV) were calculated. Calibration curves were prepared using the following concentration ranges: 100 to 4000 ng g^{-1} (feces), 1 to 1000 ng g^{-1} (soil) and 5 to 2000 ng g^{-1} (plants). The IVM/doramectin peak area ratio was used to estimate the IVM concentration in the experimental samples. Calibration curves were established using least-square linear regression analysis and determination coefficients (r^2) and coefficient of variations (CV) calculated. The linear

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regression lines for IVM in the different matrices showed determination coefficients > 0.99. The mean recoveries of IVM were in a range between 70 and 79%. The precision of the analytical procedures obtained after HPLC analysis of IVM showed CV < to 15%. The limit of quantification was established at 5 ng g^{-1} (feces), 1 ng g^{-1} (soil) and 5 ng g^{-1} (plants). The limit of detection was established at 1 ng g^{-1} (feces), 0.25 ng g^{-1} (soil) and 1 ng g^{-1} (plants).

Statistical analysis

The concentrations of IVM in fecal, soil and plant matrices are reported as mean ± SD.

IVM fecal concentration data were fitted to a first-order exponential decay model:

$$C_{(t)} = C_0 * e^{-kt},$$

where C_0 and $C_{(t)}$ are the initial concentration and concentration at time t, and k is the rate constant. Fitting was performed by using PK Solutions 2.0 software (Ashland, Ohio, USA). A weighting scheme of predicted squared was assigned. Ivermectin soil data were analyzed as the fraction that moved (FM) from the corresponding fecal pat to the underlying soil. Hence, soil concentrations were referred to as the percentage of the corresponding fecal concentration and analyzed as the percentage of drug moving from feces to soil at each sampling time point. These fractions were fitted to a simple first-order exponential model:

$$\frac{\%FM}{\%FMmax} = \mathbf{1} - e^{-kt}$$

where % FM is the percentage or fraction in soil at time t; % FM_{max} is the maximum percentage or fraction in soil and k is the rate constant. A weighting scheme of observed squared was assigned. The constant of the equation was the fecal-soil movement rate (as a percentage).

For both fecal and soil data, the dissipation (DT) or appearance (AT) times 50 and 90 (i.e., the times required to reach the 50% and 90% of dissipation from feces or of the maximum percentage in soil) were estimated.

Models were selected based on the adjusted R^2 and Akaike information criterion (AIC). Correlations between concentrations obtained in the different matrices and statistical analysis were performed with Instat 3.0 (GraphPad software Inc., San Diego, USA). IVM concentrations were statistically analyzed to confirm that the data obtained after both treatments had the same variances. Consequently, data were compared using Student's t-test with Welch's correction or a non-parametric Mann–Whitney test if significant differences among standard deviations were observed (GraphPad software Inc., San Diego, USA). A value of $P < 0.05$ was considered statistically significant.

Results

IVM was detected in the spiked fecal samples placed in the environment throughout the entire trial period. The results (observed concentrations) are presented from the first sampling time (day 1) to the last sampling time (day 60) of the experiment. IVM was not detected in fecal and soil control samples. The chromatograms of control and experimental samples are shown in Figure 1. Mean IVM fecal concentrations in the period between 1 and 60 days post-deposition of the high group and low group were in the ranges of 3901.9 ng g^{-1} and 2419.2 ng g^{-1} (high group) and 375.3 ng g^{-1} and 177.5 ng g^{-1} (low group), respectively (Table 2, Fig. 2). The IVM fecal concentration patterns and the models fitted to data of both groups (simple first-order decay model) are shown in Figure 3. No statistically significant differences in the first-order rate constants of both groups were observed (Table 2). The DT50s were 88.2 ± 62.6 days (high group) and 39.1 ± 16.7 days (low group), respectively. The DT90s were 293.1 ± 208.1 days (high group) and 129.9 ± 55.4 days (low group), respectively (Table 2). The differences between the DT50s and DT90s were not statistically significant. There was a strong correlation between the IVM concentrations in feces in the high group and the low group (r: 0.9211).

Ivermectin was also detected in soil samples between 1 and 60 days post-deposition of fecal samples (Fig. 2). Mean concentrations of IVM in soil were between 26.0 ± 27.3 ng g^{-1} and 71.1 ± 41.2 ng g^{-1} (high group) and 3.4 ± 1.7 ng g^{-1} and 5.9 ± 3.5 ng g^{-1} (low group), respectively (Table 3). The percentage of the IVM that moved from feces to soil was very similar in both groups (ranges 0.11%–5.17% and 0.38%–6.01% in high and low groups, respectively) (Table 4). The fractions moved and fitted models are shown in Figure 4. The mean movement rate constant values were 0.04 ± 0.02 and 0.05 ± 0.05 day^{-1} for the high and low group, respectively. Neither movement rate constants nor AT50 and AT90 times showed statistically significant differences between treatment groups (Table 4).

Interestingly, as an offshoot of this experiment, IVM was detected in the plants that grew close to fecal pats. Whole plants were sampled on days 30 and 60 post-deposition of the fecal pats and IVM concentrations were quantified. IVM concentrations in plants near high group pats were between 71.4 and 380.8 ng g^{-1}. IVM concentrations in plants near low group pats were between 5.4 and 51.8 ng g^{-1}. The mean concentrations of IVM in plants of both groups are given in Table 5 and compared in Figure 5. Due to the small number of samples collected, no statistical analysis was performed.

Discussion

Characterization of the environmental fate of IVM is relevant to understanding its ecotoxicological impact. In the current study, the fate of IVM at two concentrations in cattle feces under environmental conditions was evaluated. There was a high correlation between IVM fecal concentrations in the high and low groups. This indicates that IVM dissipation follows first-order kinetics, as reported by other authors.[21] Regarding the variation in IVM fecal concentrations, feces in the high group showed a greater coefficient of variation (CV, expressed as a percentage) compared to those in the low group. The CVs ranged between 8.51%–41.03% and 5.68%–28.03% in the high and low groups, respectively (Table 2). This may be explained by the different environmental factors that affect the dissipation behavior of avermectins.

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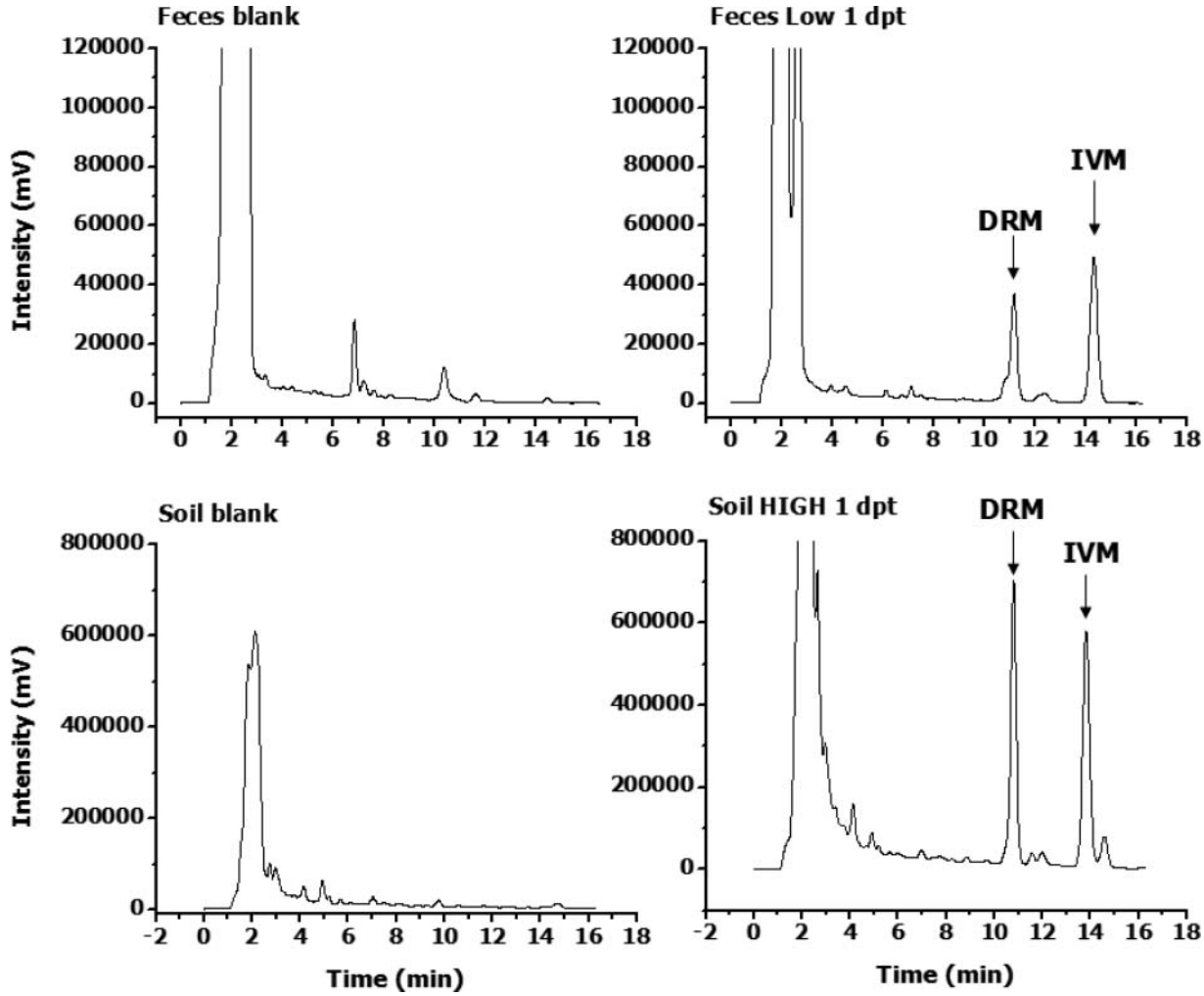

Figure 1. Chromatograms of control and experimental samples showing the baselines and ivermectin (IVM) and internal standard peaks in feces and soil.

The maximum mean concentration of IVM in spiked feces was observed on day 1 post-deposition in both the high and low experimental groups (3901.86 ng g^{-1} and 375.27 ng g^{-1} dry weights, respectively). The minimum mean concentration of IVM was recorded on day 7 post-deposition in the high group (2295.0 ng g^{-1}) and on day 60 post-deposition in the low group (177.5 ng g^{-1}). These minimum values represent 76.5% and 59.2% of the initial fecal drug concentrations in the high and low group, respectively. Previous regional studies have corroborated IVM persistence in the cattle dung under different climatic conditions.[11,12] According to these studies, minimal and maximal mean fecal concentrations observed in the low group of the present study were in the range reported as responsible for reducing the abundance and diversity of arthropods in feces.[11] While previous studies evaluated the

Table 2. Ivermectin (IVM) concentrations (dry weight) (after field deposition of feces containing 3000 ng g^{-1} (high group) and 300 ng g^{-1} (low group) of IVM.

	Group high			Group low		
Days post-deposition	Mean	SD	CV	Mean	SD	CV
1	3901.9	355.7	9.1%	375.3	47.3	12.5%
7	2295.0	941.7	41.0%	185.7	10.5	5.7%
14	3215.7	273.8	8.5%	272.1	66.8	24.5%
30	3717.5	723.8	19.5%	304.8	85.4	28.0%
60	2419.2	606.4	25.1%	177.5	41.7	23.5%
Parameters						
$k_{dissipation}$ (1/day)	0.011	0.008		0.021	0.009	
DT50 (days)	88.2	62.64	71.0%	39.1	16.7	42.6%
DT90 (days)	293.1	208.10	71.0%	129.9	55.4	42.6%

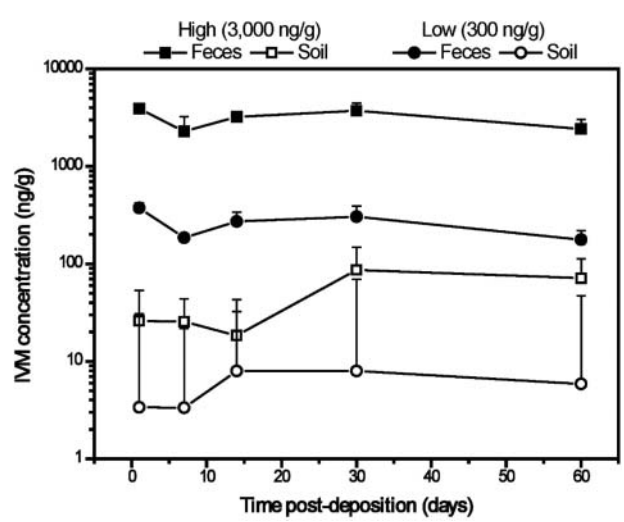

Figure 2. Ivermectin (IVM) concentrations (dry weight) in feces and underlying soil after field deposition. IVM was spiked at two levels: High (3000 ng g^{-1}) and Low (300 ng g^{-1}).

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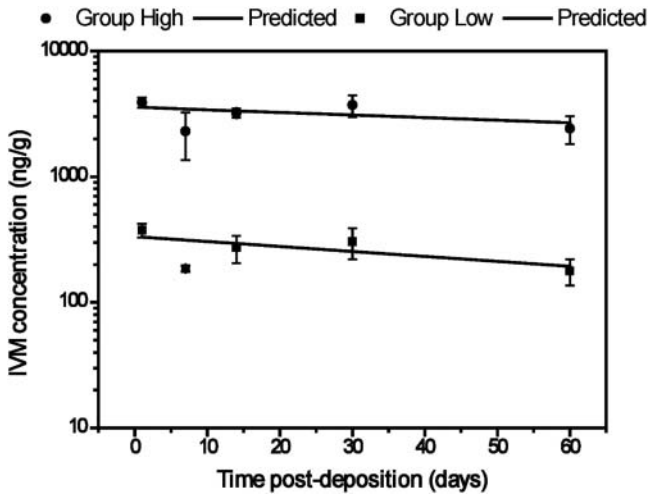

Figure 3. Ivermectin mean concentrations (dry weight) in spiked feces after field deposition (High group: 3000 ng g^{-1}; Low group: 300 ng g^{-1}). Data were fitted to a single first-order decay model.

toxicity of ivermectin on some species under laboratory conditions,[22–26] the ecotoxicological effects within a given trophic level have been only recently taken into consideration.[21,27] Similarly, the current work simultaneously determined the IVM concentrations in feces and soil at different experimental time points. The DT50 values for IVM in dung were similar to those observed by Wohde et al.[21]

The present study corroborated the movement of IVM from fecal masses to subjacent soil. IVM was detected in all underlying soil samples (up to 2 cm deep) throughout the experimental time period (60 dpd). IVM was measured in soil on day 1 post-deposition, suggesting that rapid drug movement takes place. The concentrations of IVM in soil agreed to those detected by Wohde et al.,[21] particularly in the low group.

The highest concentrations of ivermectin in soil were detected on day 30 post-deposition. Interestingly, it rained the day before taking this sample. Celestina et al.[28] observed a significant increase in avermectin concentrations in the dung of sheep after a massive rainfall. Additionally, arthropod abundance also increases in rainy weather.[11] Therefore, the physical effect of rain and coprophilous arthropods on dung pats may have contributed to the enhanced IVM concentrations in soil on day 30.

Considerable variation was also observed between the concentrations of IVM in soil. Coefficients of variation, expressed as percentages, ranged from 57.9% to 133.4% in the high group and from 50.6% to 101.6% in the low group, which were higher than those observed in the fecal samples. This variation did not allow us to observe a pattern in IVM soil concentrations during

Table 3. Ivermectin concentrations (dry weight) in underneath soil after field deposition of ivermectin-spiked feces (high group: 3000 ng g^{-1} and low group: 300 ng g^{-1}).

	High group			Low group		
Days post-deposition	Mean	SD	CV	Mean	SD	CV
1	26.1	27.3	104.8%	3.4	1.7	50.6%
7	25.5	18.4	72.2%	3.3	2.2	66.2%
14	18.4	24.6	133.4%	7.9	8.1	101.6%
30	86.6	61.6	71.2%	7.9	6.8	85.9%
60	71.1	41.2	57.9%	5.9	3.5	59.6%

Table 4. Ivermectin (IVM) fraction moved (as percentage) from feces to the corresponding underlying soil. IVM was added to feces at two levels: High (3000 ng g^{-1}) and low (300 ng g^{-1}). Percentages are expressed as mean ± SD.

	High group		Low group	
Days post-deposition	Mean%	SD	Mean%	SD
1	0.7	0.7	0.9	0.6
7	1.3	1.1	1.8	1.3
14	0.6	0.8	2.8	2.6
30	2.2	1.6	2.4	2.5
60	3.1	1.9	3.2	1.6
Parameters				
k (1/day)	0.040	0.02	0.05	0.05
AT50 (days)	19.0	5.4	25.0	14.5
AT90 (days)	63.0	17.8	83.1	48.1

the experimental period and thus we were not able to fit a model to describe IVM soil dissipation. However, when soil concentrations were related as a percentage of their corresponding IVM fecal concentrations, a simple first-order model that describes the rate of IVM movement from feces to its underneath soil could be fitted; this permitted the determination of the movement rate as well as the 50% and 90% movement times (Fig. 4, Table 4).

IVM metabolites were not detected in the present study, as was also described by Wohde et al.[21] Therefore, the ecotoxicological effect of IVM is attributed to the activity of the parent compound.[21] However, an important amount of transformation products was detected after the addition of radio-labeled IVM to an aerobic sediment/water system.[29] The relevance of IVM metabolites to the environmental impact of these drugs should be further assessed under field conditions.

After deposition by the animals, dung is naturally incorporated into the soil due to degradation and coprofauna activity. Thus, IVM may be translocated to the soil by biological perturbation or leaching. Furthermore, restorative options that include the use of cattle manure to improve soil fertility have increasingly turned attention to ameliorate eroded land by adding organic

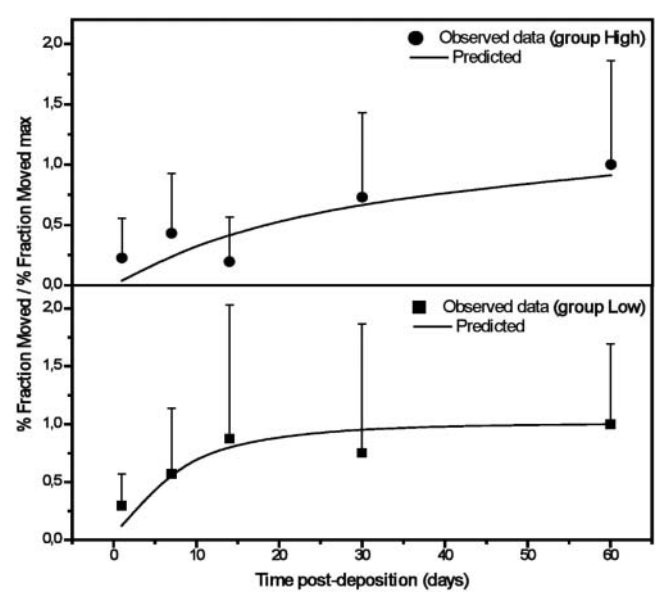

Figure 4. Ivermectin movement from feces to underlying soil as a percentage of the corresponding fecal concentration. Each point is the mean percentage of drug that moved referring to the maximum percentage moved (%max). Data were fitted to a single first-order model.

Table 5. Ivermectin (IVM) concentrations (wet weight) in plants growing close to IVM-spiked feces (high group: 3000 ng g^{-1} and low group: 300 ng g^{-1}) after field deposition.

	High group			Low group		
Days post-deposition	Mean	SD	CV	Mean	SD	CV
30	205.1	137.0	66.6%	34.3	16.4	47.9%
60	199.2	70.4	35.4%	16.8	11.4	67.9%

matter. Some researchers have evaluated the effect of cattle manure on soil productivity,[30] on the restoration of urban soils [31] and on the nutrient input to soil.[32] However, there is scarce information on the effects when veterinary drug residues such as IVM are present in these manure treatments.

In addition to feces and soil samples, a pilot study was carried out on the plants growing close to the experimental dung pats. These plants were collected and the IVM concentrations were analyzed. Even though the number of plant samples was not sufficient to draw any conclusions, it is worth noting that the mean IVM concentrations in the plants of both experimental groups were higher than those observed in their corresponding soil samples (Tables 3 and 4). Moreover, the plants collected from the area of dung pats in the high group showed higher IVM concentrations compared to those sampled in the area of the low group feces. This could suggest that IVM moved from feces to both the underlying soil and neighboring plants. Further investigations are needed to characterize IVM uptake by plants. There is scant information relating to the uptake of veterinary medicines by plants, but it is well-known that different sorts of plants can be used for the phytoremediation of polluted soil and water.[33] Plant metabolization of xenobiotics has been reviewed [34] and compared with liver enzymatic pathways.[35] The toxicity of avermectins to plants has not been observed previously.[36–38] Neither, to the best of our knowledge, is there any report of avermectin metabolism in plants. The presence of IVM in plants may have an impact on animal health, as plants could serve as a depot and convey these drugs to animals, contributing to the development of anthelmintic resistance.

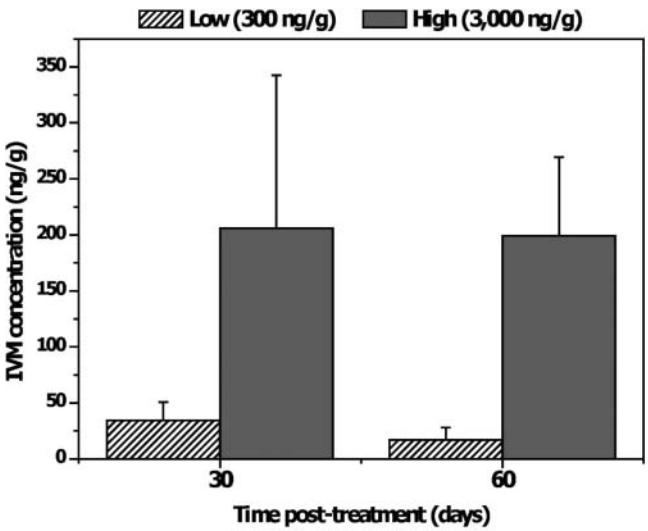

Figure 5. Ivermectin (IVM) concentrations (wet weight) in plants growing close to the IVM-spiked dung pats deposited in the field.

Conclusion

Currently, the main tool in antiparasitic programs for food-producing animals is the administration of pharmacological products, containing a single drug or a combination of them. Unfortunately, long-term studies on the environmental effects of those treatments are scarce and thus the regulation of these practices by environmental agencies is limited. The current study shows that IVM moves from feces to the underlying soil as well as to nearby plants. In this scenario, the environmental and biological monitoring of drugs and especially the evaluation of their long-term effects beyond the period of their presence in feces may minimize the ecotoxicological effect.

Acknowledgment

The authors are grateful to Dr. Peter P. Purslow and Prof. Cecilia Stambuk for revising the manuscript.

Funding

This work was supported by the Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CICPBA), La Plata, Buenos Aires, Argentina. Proyecto Nro. 813/13 and Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET).

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Supplementary materials

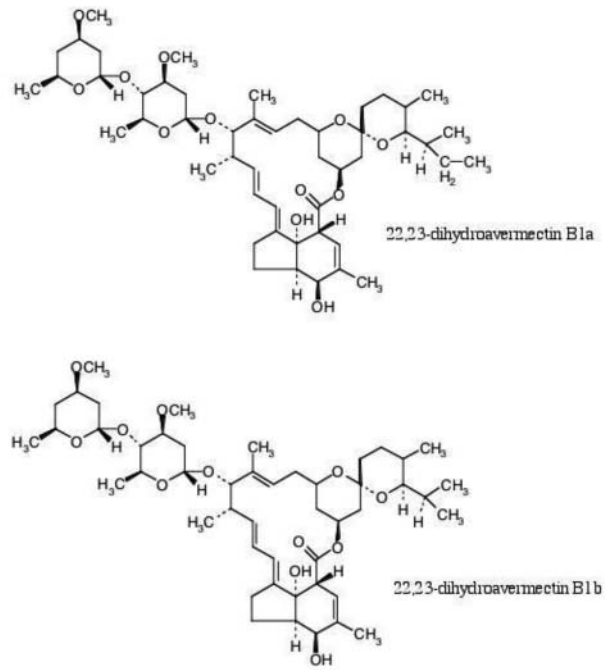

Figure 1S.