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ORIGINAL PAPER

Environmental monitoring of ivermectin excreted in spring climatic conditions by treated cattle on dung fauna and degradation of faeces on pasture

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Abstract The effect of ivermectin excreted in faeces of cattle treated in late winter on the arthropods and the degradation of faeces on pasture were evaluated. Four calves of similar age and weight were allocated to two groups, one group was treated subcutaneously with ivermectin and the other group remained as untreated control. From faeces collected from both groups at 3, 7, 14, 21 and 28 days post-treatment (dpt), three faecal pats of 1 kg each were made and deposited on a mixed paddock. One quarter of each faecal pat was removed at 10, 20, 30 and 60 days postdeposition (dpd) to determine the concentration of ivermectin, the organic matter content, and to collect colonising dung arthropods. Concentrations at days 3 and 7 pt were significantly higher than at the other dpt ($p<0.05$). The highest ivermectin concentrations were found in samples from 3 dpt ($p<0.05$). The organic matter percentage was not significantly different between treatments. An edaphic fauna characterised the colonisation of the faeces by organisms. Although arthropods' abundance differences were not significant except for the 28 dpt at 30 dpd ($p<0.0003$), fewer organisms were collected from the ivermectin group at all times.

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Introduction

Since its introduction into the veterinary market, ivermectin became a practical and accessible tool for parasite control in the cattle production. The endectocide properties, such as the broad spectrum, has allowed the optimisation of the control of a great variety of harmful organisms. Also, the faecal elimination in elevated quantities (Halley et al. 1989; Chiu et al. 1990; Alvinerie et al. 1998) warrants the control of other free-living biological stages of parasites that utilise the dung as developmental environment. However, the lack of adequate utilisation programs and low cost led to a massive, frequent and indiscriminate administration of endectocides without a simultaneous test about the ecotoxicity risk, which has determined disturbing environmental effects (Wall and Strong 1987; Madsen et al. 1990; Sommer et al. 1992; Lumaret and Erroussi 2002; Lumaret and Martínez 2005; Floate 2006) and an increase of parasite resistance (Fiel et al. 2001; Anziani and Fiel 2005).

Even though different evaluation methodologies under varying geographic and climatic conditions were used, the harmful effects of ivermectin on the development of nontarget dung organisms have been reported by several authors (Wall and Strong 1987; Floate 1998; Iglesias 1998; Floate and Fox 1999; Floate et al. 2002, 2005; Wardhaugh and Rodriguez Menendez 1988; Gover and Strong 1995a, b; Wardhaugh et al. 2001; Iglesias et al. 2006). The level of damage depended on the biological stage or the particular

susceptibility of the species under study. Furthermore, the environmental drug impact also included reports on delayed faecal degradation, which, in turn, would affect the natural soil nutrients recycling (Floate 1998; Iglesias et al. 2006). Accordingly, experiments conduced in Tandil (Argentina) showed reduced coprophilous fauna abundance and diversity as well as delay of the organic matter degradation in dung pats from treated animals deposited in autumn (Iglesias et al. 2006).

Moreover, the ivermectin environmental effects can vary depending on the seasonal and biological conditions as well as on the particular dung coloniser organism complexity.

One feature scarcely considered is the diversity of organisms that collaborate on dung degradation and further nutrients integration into the soil. It is possible that, in some regions where the ivermectin use has become a routine as an effective endectocide, the ecological role and the colonisation pattern of the coprophilous organisms are yet poorly known. This, in turn, limits the determination of deleterious biological effects.

The purpose of this work was to continue previous assays performed to evaluate the local effects of ivermectin on the coprophilous arthropods and faecal degradation of cattle dung deposited on the identical pastures and soil, but under different meteorological and biological conditions in the same geographical region.

Materials and methods

Place

This trial was carried out on an experimental area of the Faculty of Veterinary Sciences, Tandil, Argentina (37°17′ 34″S, 59°5′W) throughout the period from August 17, 2007 to November 14, 2007. The soil was characterised as a typical Argiudol and the pasture composition by *Trifolium repens*, *T. pratense*, *Rye grass* and *Dactylis glomerata*.

Animals

Four naturally parasited male calves, two Holando Argentino (Holstein) and two Aberdeen Angus, about 1 year old and 178±69.8 kg body weight were used. The animals were allocated to one of the two experimental groups, ivermectin-treated (IVM group) and untreated (Control group).

Methodology

Both animals in the IVM group were treated subcutaneously with ivermectin (IVOMEC®, Merial) at a dose of 0.2 mg/kg of body weight, while calves in the control group remained untreated.

Faecal sample collections

At 3, 7, 14, 21 and 28 days posttreatment (dpt), every group was allocated into similar stockyard for 24 h. At this time, the animals were fed with balanced food and oat bales and received water ad libitum. Faeces from each group were taken from the ground prior cleaning, collected in identified plastic bags and were thoroughly homogenised in the laboratory. Five 1-kg masses from each group were elaborated and placed on a mixed paddock where animal access was prevented. The voided faecal pats were duly identified with plastic tag stakes. A quarter of three faecal pats were removed from each group at 10, 20, 30 and 60 days postdeposition (dpd).

Laboratory tests

Two subsamples of each sampling pt and pd day were pooled and ivermectin concentrations were determined by high-pressure liquid chromatography (HLPC) with a Shimadzu 10 A fluorescence detector (Lifschitz et al. 2000). Another set of pooled samples was used for determining organic matter content. This was estimated as the weight loss of faeces dried at 60°C in a forced-circulation oven until a constant weight was achieved. Subsequently, inorganic content was burned in a muffle furnace at 550°C for 6 h. All the other samples were used for collecting arthropods for 3 days by Tullgren's funnel method (Tullgren 1918).

Meteorological data

Meteorological data throughout the study were provided by the Plains Institute of Hydrology station at the Faculty of Veterinary Sciences by using a Li-1200S Data Set Recorder (Li-Cor, Lincoln, NE) and Meteo-4 Mini (Li-Cor, Lincoln NE and Inc.; C Cavadevices).

Statistical analysis

The concentration of the drug in faecal matter and the percentage of organic matter were analysed with a model including the dpt effect and the dpd effect; the latter was considered as repeated measures of the faecal matter. PROC MIXED of SAS V.9.1.3 (SAS Institute 1989) was used for the analysis. The variable degradation of organic matter that arises from the difference between the final organic matter weight minus the initial one (OM 60–OM 10) was analysed with the PROC GLM procedure of SAS V.9.1.3.

To evaluate the abundance of arthropods, the variable total abundance was analysed by a model with treatment effect (control, IVM), dpt and dpd effects; the latter was considered as repeated measures of the faecal matter

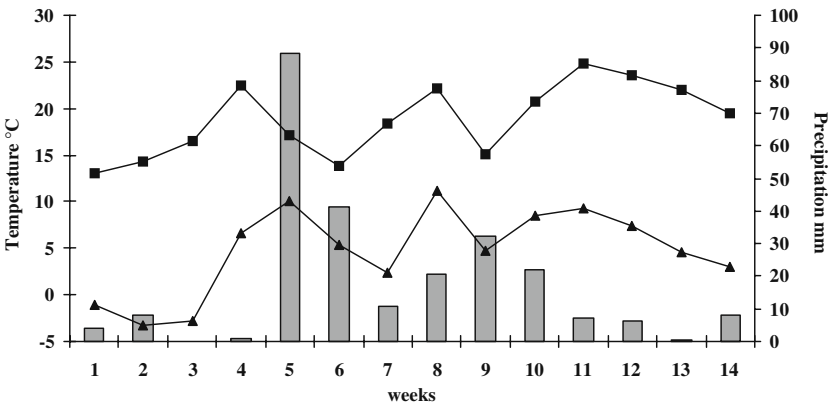

Fig. 1 Meteorological data during the experimental period (August 17, 2007–November 14, 2007). *Bars* Cumulative weekly precipitation, ■ average maximum weekly temperature, ▲ average minimum weekly temperature

Results

The meteorological data were recorded weekly during the entire study and are shown in Fig. 1.

Concentration of ivermectin in faeces

The profile of faecal (dry weight) ivermectin concentration throughout the dpd time revealed significantly higher drug concentrations ($p<0.05$) in samples obtained 3 and 7 dpt (Fig. 2a). The highest IVM concentrations were found in samples at 3 dpt ($p < 0.05$) after 30 days under environmental conditions (0.779 ppm), whereas the lowest concentrations were found in samples from 28 dpt at 60 dpd (0.002 ppm; Fig. 2b).

Organic matter

The percentages of organic matter from faecal samples collected from nontreated cattle (control) and from cattle treated with IVM at 0.2 mg/kg during the experimental period are shown in Fig. 3.

For the variable percentage of organic matter, no interaction was detected between treatments and dpt ($p=0.4034$) or between treatments ($p=0.9505$).

Organisms in faecal pats

The mainstream of the collected arthropods in the samples corresponded to collembolan insects prevailing the Poduridae species and the Entomobrydae and Sminthuridae specimens being the less represented.

Taking into account that, at 60 dpd, most of the pats was disorganised and integrated into the ground (due to the activity of earthworms, crustaceans and arachnids) and considering samples with complete data (10, 20, and 30 dpd), the number of arthropods observed in samples from animals treated with IVM compared to control group was lower (Table 1). Despite these facts, differences were not statistically significant.

In comparison control group samples revealed a pattern with a higher abundance towards the 30 dpd even as samples of the IVM group maintained a similar abundance to those samples of 20 dpd.

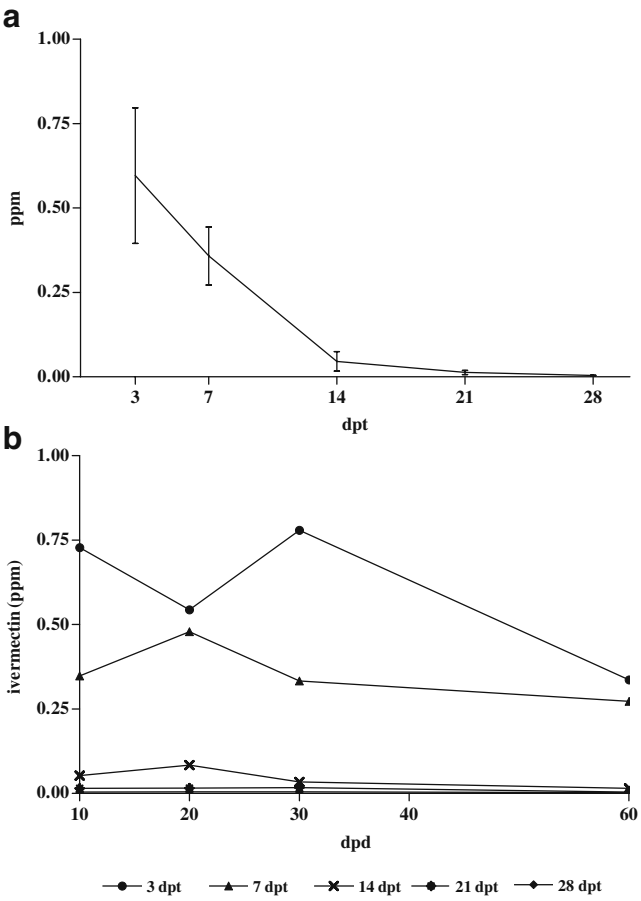

Fig. 2 **(a)** Concentration of ivermectin (ppm dry weight) in faecal samples from treated animals at different dpt times throughout the entire period of environmental exposure (*SD bars*). **(b)** Concentration of ivermectin (ppm) in faecal samples from treated animals. Faeces were deposited on pasture 3, 7, 14, 21 and 28 dpt and collected at 10, 20, 30 and 60 dpd

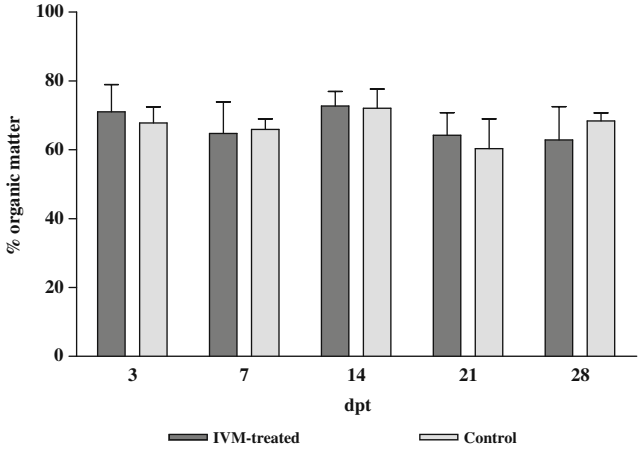

Fig. 3 Percentage of organic matter in faecal samples from ivermectin-treated and untreated control animals at 3, 7, 14, 21 and 28 dpt throughout the entire period of environmental exposure

How the dung pats colonisation was?

In the colonisation of faecal masses exposed to environmental conditions during 10 days, the abundance of edaphic organism's population over the coprophile organisms was notable. The reduced presence of coprophile insects is emphasised, in particular, those of Brachycera Diptera larvae that initiate the colonisation of faecal masses very early, when chemical and physical conditions, especially humidity, enable their development in that environment.

With a few exceptions the majority of the adult insects (among those the young forms of hemimetobola insects included) corresponded to the Order Collembola, prevailing the Poduridae family, with scarce specimens of Entomobrydae. Coleoptera adult insects were represented by the Staphylinidae, Ptiliidae, Pselaphidae and Histeridae families and specimens of Cecidomyiidae family represented the Order Diptera.

Among the collected insect larvae, species found were dipterans of the Chironomidae and Cecidomyiidae families; while Coleoptera species were of the Staphylinidae family. As to mites, the greater part of them corresponded to oribatid mites (recognised genera *Scheloribates, Hemileius*), few species of the Order Gamasida (Macrochelidae, Parasitidae) and Acaridida (*Gliciphagus*).

In every sampling corresponding to the 20 dpd, the prevailing larvae were of Nematocera Diptera of the Cecidomyiidae family, with a few species of Chiromomiidae and, among the coleoptera insects, a few larvae of the Staphylinidae and Ptiliidae family. It has to be emphasised that the Nematocera larvae colonise late faecal masses in relation to Brachycera Diptera. In the mite populations, the Oribatida and Gamasida prevailed. A great number of hypopus forms (Acaridida) were observed.

In the samplings done since October 2007 (including samples of 30 dpd), a great part of the samples have showed only the superficial layer and a clear incorporation to the faecal matter to the ground with a very active participation of earthworms. The abundance of edaphic organisms, however, exceeds the previous samplings. The pasture reached a height of 20 cm, and it frequently acts as a mechanical disintegrator of faecal masses.

The 60 dpd samples (last quarter of faecal mass to be removed from the lot) showed the same situation, with more integration into the ground, in both control and treated group samples. Hence, it was not possible to process the whole sample, except for the necessary amounts to determine the organic matter and drug concentration. The pasture exceeded the height of 30 cm and a great soil organisms (worms, ants, pill bug) activity was observed.

Effect of ivermectin on abundance of organisms

As it was previously explained, data from samples corresponding to the 60 dpd were not included in the statistical analysis.

As mentioned before, the abundance of edaphic organisms registered in the samplings at 30 dpd was higher and showed a significant ($p<0.05$) dpt effect (Table 1). However, a treatment effect on arthropod abundance between control and IVM group was not detected except for the 28 dpt at 30 dpd samples ($p < 0.0003$; Fig. 4).

Table 1 Total abundance of arthropods (total no.) in faecal samples of treated cattle (IVM) and untreated cattle (control) during experimental time

Postdeposition days (dpd)	10 dpd	20 dpd	30 dpd	60 dpd[a]	Total
Group					
Control	2191	7801	15.413	1698	27103
IVM	2576	7682	7120	450	1782

[a] Incompletes dates by samples absence

Discussion

Although sampling times were different to those in the previous trials done in autumn in the same region (Iglesias et al. 2006) a general comparison among the obtained data can be made.

In agreement with this previous experiment, all faecal samples from the ivermectin-treated group have shown different concentrations of ivermectin throughout the time of exposition to environmental conditions. However, with the exception of the values recorded in collected samples at 60 dpd, drug concentrations detected in the present study

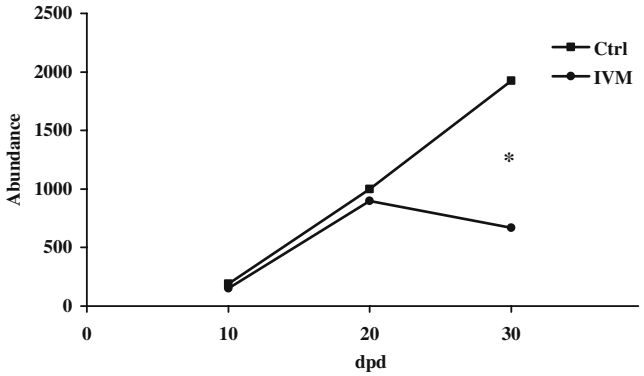

Fig. 4 Arthropods abundance at 28 dpt samples of control (*Ctrl*) and ivermectin (*IVM*) group and collect at 10, 20 and 30 dpd. $^{*}p<0.05$

were higher than those previously reported in the autumn assay and, in some cases, were twofold higher.

Concentrations of IVM in faecal mass at 3 dpt collected at 10 and 30 dpd (Fig. 2b) exceeded the maximum values recorded in previous work. Samples from 7 dpt at 30 dpd were two times the values showed in autumn as related by Iglesias et al. (2006) (0.333 ppm as dry weight in the present study, while in autumn, a value of 0.157 ppm as dry weight was observed).

Despite having no comparative data, IVM concentrations in samples at 28 dpt were similar or higher than those in samples at 21 dpt recorded in autumn assay.

Similarly, the highest concentrations were found in samples from 3 dpt, although samples from 7 dpt also presented drug concentrations significantly higher when compared with samples from 14, 21 and 28 dpt, maintaining a similar pattern observed in previous experiments (Iglesias et al. 2006).

Besides, spring samplings were performed on the same pat, and this may have influenced on the internal physicochemical proprieties of dung including drug concentration. Several authors have highlighted the effect of faecal humidity content and/or thickness of pat on drug concentrations (Sommer and Steffansen 1993; Krüger and Scholtz 1998a,b; Kolar and Kožuh Eržen 2007; Celestina et al. 2010). When we analyse the precipitation regime throughout experimental period, we conclude that the higher drug concentration in faecal samples in spring coincide with the most (accumulated) week rains, much more than the autumn data for the same time.

Undoubtedly, drug concentrations have lasted in the environment for the experimental time as was represented in Fig. 2b and will be incorporated into soil and edaphic food web where the drug will be metabolised or binded on the complex soil structure.

On the other hand, no statistical differences were found between organic matter content in samples from different treatments. In previous autumn, assay differences in this indicator were detected among treatment groups (Iglesias et al. 2006). The functional role of organisms that breed or eat on cattle dung was well established in different latitudes (Mohr 1943; Desière 1973; Valiela 1974; Floate and Gill 1998). We assume that burial behaviour or diet habits of coprophilous organisms collaborate on dung degradation reducing the organic matter percentage. Despite great soil dung integration observed at the end of experimental work, coprophilous insect abundance was scarce when compared to soil organisms in the present trial. The rain period was unusually preceded by a warm and dry weather, and this phenomenon could reduce the colonisation of the first species that arrive at dung. Consequently, other factors, as well as mechanical (e.g. grass growth through the pats or late spring rains) and chemical modifications of dung, take place and make possible the ultimate disintegration.

Accordingly, similar priority should be given to the understanding of the role played by regional organisms that colonise dung pats and to the evaluation of the importance of population composition and/or diversity. The latter is depicted when analysing fauna population in 30 dpd samples. Fauna population showed a rapid increment, but most of the organisms present (springtails and mites) were not relevant for faecal degradation, even though ivermectin levels were toxic for some of these organisms (Jensen et al. 2003). Therefore, the functionality of population diversity will have to be considered in future studies in order to provide accurate information about these complex interrelated factors, making available regional knowledge, which allows the development of tailored parasite control programs for each particular region.

In a thorough review, Lumaret and Martínez (2005) emphasised on the risk of disturbing pasture ecosystems and encouraged to raise the consciousness by all social sectors.

In spite of the lack of integral information on the frequency and chemical used in different regions of the world, ivermectin has been reported as the more extensively administered antiparasitic drug when compared with benzimidazoles, other macrocyclic lactones (both avermectins and milbemycins) and pyrethroids (Boxall et al. 2007).

In Argentina, the studies aimed to evaluate the long-term environmental effects of endectocide compounds are scarce, although these compounds are known to be of customary use. The environmental effects can be accurately determined by continuous field observations as showed by other authors (e.g. the works of Floate 1998; Floate et al. 2002). In turn, these field studies along with the knowledge of seasonal coprofauna colonisation patterns (Iglesias et al. 2004) and other factors affecting the rate of natural faecal degradation (Merritt and Anderson 1977) will allow the prediction and avoidance of possible environmental harm-

ful effects of residual quantities of veterinary chemical products administered for parasite control.

Long-term field observations will discriminate the impact of different interacting factors as was related by Krüger and Scholtz (1998a, b). Hence, determining both seasonal and climatic effects on the use of ivermectin will allow developing more rational uses of drug.

The regional use of macrocyclic lactones and more exactly the crescent administration of concentrated ivermectin at higher dose rates alerts about its ecotoxicological hazard as well as requires to perform standardised studies that avoid its residual presence on soils and food.

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