

Effects of eprinomectin on the survival, reproduction and feeding activity of the dung beetles, *Onthophagus lenzii* Harold, and rare species, *Copris ochus* Motschulsky (Coleoptera: Scarabaeidae)

I. Ishikawa* and M. Iwasa

Laboratory of Entomology, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080-8555, Japan

Abstract

Effects of the antiparasitic drug eprinomectin were studied on the dung beetles, *Onthophagus lenzii* Harold and the rare species, *Copris ochus* Motschulsky by pour-on administrations ($500 \mu\text{g kg}^{-1}$) in Japan. Eprinomectin was detected in cattle dung from 1 to 7 or 14 days after treatment, with a peak at 1 day after treatment in two experiments. In *O. lenzii*, adult survivals and numbers of brood balls constructed were significantly reduced in dung from eprinomectin-treated cattle at 1 and 3 days post-treatment in experiment 1, and adult emergence rates were extremely reduced in dung at 1, 3, and 7 days post-treatment. In *C. ochus*, adult survivals were significantly reduced in dung at 3 days post-treatment (experiment 1), and equivalent levels to the control were restored in dung at 7 and 14 days post-treatment (experiment 2). Numbers of brood balls of *C. ochus* were nil in dung at 3 days (experiment 1), and significantly reduced in dung at 7 days (experiment 2) post-treatment compared with control. Adult emergence rates of *C. ochus* were 100 and 71.6% in dung from control cattle in experiments 1 and 2, respectively. However, no oviposition was observed in dung at 3 days post-treatment, and all offspring died at egg or the first instar larval stage in dung from 7 and 14 days post-treatment. Feeding activities of *O. lenzii* and *C. ochus* were significantly inhibited in dung from treated cattle at 1–3 days and 3 days post-treatment, respectively, returning to levels of the control at 7 days post-treatment.

Keywords: eprinomectin, pour-on, *Onthophagus lenzii*, *Copris ochus*, Japan, ecotoxicity

(Accepted 9 April 2018; First published online 22 May 2018)

Introduction

Macrocyclic lactones for veterinary endectocides that are currently used worldwide include ivermectin (ivermectin, doramectin, and eprinomectin) and milbemycin (moxidectin). In particular, ivermectin has been used all over the world; it not only controls gastrointestinal nematodes and arthropod

parasites of livestock but also gets rid of pest flies by residual active ingredient in feces (Miller *et al.*, 1981; Benz, 1985; Drummond, 1985). However, parasiticide residues in dung are not selective. They kill non-target insects, which would otherwise accelerate degradation of the dung (Wall & Strong, 1987; Madsen *et al.*, 1990; Floate *et al.*, 2002, 2005). In particular, dung beetles provide important ecosystem services in soil fertility and pest control by transporting and decomposing dung in pastures (Bornemissza, 1970; Nichols *et al.*, 2008). Adverse effects of ivermectin residues on the larval survival and reproduction of dung beetles have been reported by Ridsdill-Smith (1988), Wardhaugh & Rodriguez-Menendez (1988), Houlding *et al.* (1991), Fincher (1992), Holter *et al.*

*Author for correspondence
Phone: +81 080 5389 0019
Fax: +81 155 49 5492
E-mail: s26277@st.obihiro.ac.jp

(1993), Lumaret *et al.* (1993), Sommer *et al.* (1993), Krüger & Scholtz (1997), Dadour *et al.* (1999), Errouissi *et al.* (2001), Iwasa *et al.* (2005, 2007), and Pérez-Cogollo *et al.* (2015). Interference with dung degradation throughout reduction of abundance and activity of dung beetles could damage the productivity of pasture.

Eprinomectin was recently introduced to the animal health industry, and it has a broad spectrum of activity and high efficacy for cattle, including lactating cows (Schoop *et al.*, 1996a, b). Furthermore, eprinomectin can be applied to milking cows with zero milk holding time because eprinomectin has lower transferability than other avermectins (Williams *et al.*, 1997; Reist *et al.*, 2011; Gokbulut *et al.*, 2012; Mason *et al.*, 2012). Effects of eprinomectin on non-target coprophagous insects have been studied in only a few papers in some countries (Wardhaugh *et al.*, 2001; Floate *et al.*, 2002; Lumaret *et al.*, 2005; Iwasa & Sugitani, 2014), and no information is available about its toxic effects on medium- and large-sized dung beetles. This study was performed to estimate the effects of eprinomectin by a pour-on application on the survival, reproduction, and feeding activity of the dung beetles *Onthophagus lenzii* Harold and *Copris ochus* Motschulsky (Coleoptera: Scarabaeidae), which are important dung-degrading species in Japan.

Materials and methods

Cattle used and dung collection

The experiments were conducted in the pasture of Yachiyo Farm southwest of Obihiro City, Hokkaido, Japan. Six Holsteins (breeding cows, aged 10–25 months) were selected. On 1 June 2015 (experiment 1) and 6 June 2016 (experiment 2), eprinomectin (Eprinex Topical; Merial, Haarlem, The Netherlands) was applied topically on the back from the withers to the tail head of cattle by pour-on formulation at the recommended dose of 500 µg kg⁻¹ of body weight. Ten Holsteins (breeding cows, aged 10–35 months) were used as controls. The cattle were fed mainly with green grass composed of Kentucky bluegrass, timothy, and ladino clover.

Fresh dung pats were collected from treated and control groups on the day prior to treatment and at 1, 3, 7, 14, and 21 days after treatment. During collection, each dung pat was thoroughly mixed and stored at –20°C until used.

Determination of eprinomectin concentration

The concentration of eprinomectin in the feces was determined using high-performance liquid chromatography (HPLC) following the method of Payne *et al.* (1995) with some modifications. A 5 g dung sample on each date after treatment was analyzed. The extraction was carried out with homogenization (Phycotron, Microtec Co., Ltd) at 10,000 rpm for 1 min (instead of sonication), and the concentration was calculated from the calibration curve of a standard solution. The determination limit of concentrations was determined at 0.05 ppm.

The effect of eprinomectin on survival and reproduction of O. lenzii

O. lenzii is a medium-sized dung-burying beetle, which is distributed throughout Japan. A 50 g dung sample collected from treated (1, 3, and 7 days after treatment in experiment 1)

and control cattle was placed on andosol (15 cm depth) in a plastic container (12 cm diameter × 18 cm depth). Three male–female pairs were placed on dung in each container, which was covered with gauze and maintained at 22°C and the light regime of 16L:8D. Dung samples were replaced with fresh ones once per week for 2 months. At that time, andosol from each container was sieved to collect and record the numbers of brood balls produced by the beetles. The balls were placed in a plastic cup (5 cm diameter × 3 cm depth) with andosol and kept at 22°C (light regime 16L:8D) until adult emergence. Rearing tests were replicated five times for each collection date of dung from treated and control cattle.

The effect of eprinomectin on survival and reproduction of C. ochus

C. ochus is the largest dung-burying beetle in Japan; it is distributed in Japan, Korea, and China. A 100 g sample of dung collected at 3 days after treatment in experiment 1 and control, and collected at 7 and 14 days after treatment in experiment 2 and control, were placed on andosol (30 cm depth) in a plastic container (30 cm diameter × 40 cm depth). One male–female pair was placed on dung in each container, and the rims of containers were wrapped in gauze. Feces were replaced with fresh samples once per week for 90 days; this period corresponds to the breeding season (July–September) of *C. ochus* in Japan. At that time, andosol from each container was sieved to collect the brood balls produced by the beetles every 30 days. The balls collected were placed in a plastic cup (12 cm diameter × 6 cm depth) with andosol and until adult emergence. Rearing tests of adults and larvae were replicated ten times for each collection date of dung from treated and control cattle at 22°C with a light regime of 16L:8D.

The effects of eprinomectin on feeding activity of O. lenzii and C. ochus

Each dung sample in the rearing container was inspected when it was replaced with fresh dung, and the level of feeding (F) was assessed on an arbitrary scale of 0–4 based on Wardhaugh & Rodriguez-Menendez (1988) with some modifications (i.e., 0 = no feeding; 1 = one feeding trace on surface of dung; 2 = feeding trace of two or more; 3 = most feces was eaten, but surface part of dung was left; 4 = all of the feces was decomposed into fiber or carried to a burrow).

Statistical analysis

The log-rank test was used to test for differences in the adult mortality between controls and treatments. Then, a multiple testing adjustment of *P*-values was applied using the Bonferroni method for each comparison between the groups. The Bonferroni correction required setting the significance level at 0.0083 (=0.05/6; *O. lenzii* in experiment 1) or 0.0167 (=0.05/3; *C. ochus* in experiment 2).

The generalized linear mixed models (GLMMs) with Poisson error and log-link function were used to test the numbers of brood balls and feeding levels, and the GLMMs with binomial error and log-link function were used to test the adult emergence rates. The numbers of brood balls and adult emergence rates were response variables, days of dung post-treatment were explanatory variables, and replicates were random effects. In the feeding activity, feeding levels on each date were the response variables; days after the

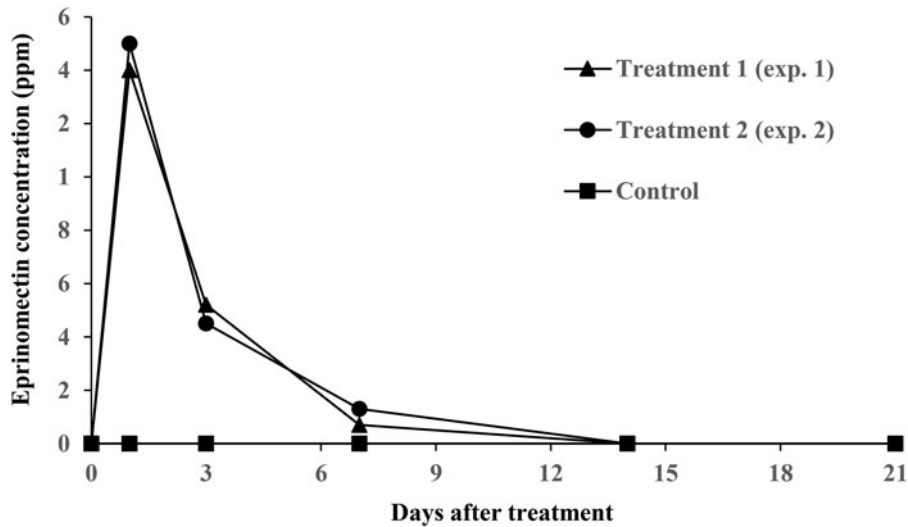


Fig. 1. Eprinomectin concentrations in dung (ppm of wet weight) after treatment ($500 \mu\text{g kg}^{-1}$) in experiments 1 and 2. No eprinomectin was detected in both controls (experiments 1 and 2).

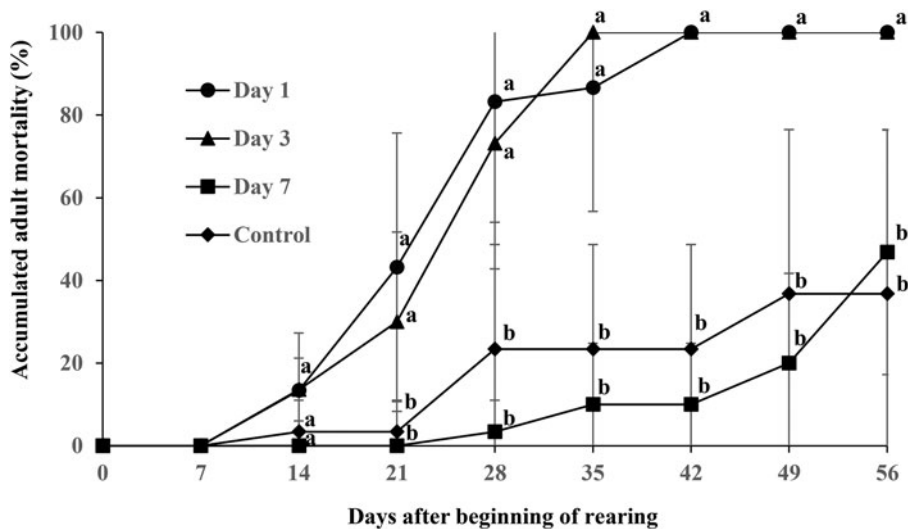


Fig. 2. Accumulated adult mortality of *O. lenzii* in dung from control and eprinomectin treated cattle in experiment 1. Different letters indicate significant differences ($P < 0.0083$) among control and treatments (log-rank test followed by the Bonferroni correction).

beginning of rearing and type of dung were explanatory variables, and replicates were random effects. Significance levels for comparisons on these tests were adjusted by Tukey's method. All tests were performed using R 3.2.4. Software (R Development Core Team 2016).

Results

Determination of eprinomectin concentration

Eprinomectin in cattle dung attained maximal concentration at 1 day after treatment, followed by marked declines at 3 and 7 days, and it was not detected at 14 days or later in

experiments 1 and 2 (fig. 1). No eprinomectin was detected in control dung of both control experiments.

The effects of eprinomectin on adult survival, brood ball production, emergence, and feeding activity of *O. lenzii*

Adult mortalities of *O. lenzii* in dung at 7 days post-treatment were not significantly different from those in control dung from 14 to 56 days after the beginning of rearing (fig. 2). However, in dung at 1 and 3 days post-treatment, adult mortalities significantly increased from 21 to 56 days, and attained 100% at 42 and 35 days after the start of rearing, respectively (fig. 2; $\chi^2 = 39.8$, $P < 0.0083$; $\chi^2 = 33.7$, $P < 0.0083$). The numbers

Table 1. Number of brood balls constructed and adult emergence rates of *O. lenzii* in dung from control and eprinomectin-treated cattle in experiment 1.

	Days after treatment	No. of brood balls/female (mean ± SD)	Adult emergence rate (%) (mean ± SD)
Control	–	17.0 ± 5.5 ^a (85)	41.9 ± 22.5 ^a
Treatment	1	0	0
	3	0.4 ± 0.9 ^b (2)	0 ^b
	7	18.8 ± 5.7 ^a (94)	4.5 ± 6.4 ^b

Each value is a mean of five replicates involving three pairs of each parent.

Values in parentheses are total numbers of brood balls.

In each column, means not associated with the same letter differ at $P < 0.05$ among control and treatments (GLMM followed by Tukey's multiple comparisons).

of brood balls per female were 17.0 in dung from control cattle, but no brood balls were constructed in dung at 1-day post-treatment. In dung at 3 days post-treatment, the numbers of brood balls were significantly reduced (table 1; $Z = -5.2$, $P < 0.001$), and an equivalent level to the control was restored at 7 days post-treatment. The adult emergence rate was 41.9% in dung from control cattle, but that in dung at 3 and 7 days post-treatment was significantly reduced (table 1; $Z = -5.04$, $P < 0.001$).

Fig. 3 shows the estimates of accumulated feeding activity of *O. lenzii* per dung pat from control and treated cattle. There were no significant differences in feeding rates at 7 days post-treatment. However, in dung from 1 and 3 days post-treatment, feeding rates were significantly suppressed compared with control ($Z = -4.7$, $P < 0.001$; $Z = -3.1$, $P < 0.05$, respectively).

The effects of eprinomectin on adult survival, brood ball production, emergence, and feeding activity of C. ochus

Table 2 shows the adult mortality, numbers of brood balls constructed, and adult emergence rate of *C. ochus* at 3, 7, and 14 days post-treatment in dung from control and treated cattle in experiments 1 and 2. In dung from control cattle in experiments 1 and 2, accumulated adult mortalities of *C. ochus* were 10 and 20%, respectively, until 90 days after the beginning of rearing. However, in dung from 3 days post-treatment (experiment 1), accumulated adult mortalities were 90% at 60 days and 100% at 90 days after the beginning of rearing, showing a marked difference with controls (60 days: $\chi^2 = 18.1$, $P < 0.0083$; 90 days: $\chi^2 = 30.2$, $P < 0.0083$, respectively). Meanwhile, normal adult mortalities, which were equivalent to the level of controls, were restored at 7 and 14 days post-treatment (experiment 2) at all dates after the beginning of rearing. The numbers of brood balls constructed per female in control dung were 4.3 (experiment 1) and 4.4 (experiment 2), but there were no brood balls in dung at 3 days (experiment 1) post-treatment. The numbers of brood balls in dung at 7 days post-treatment were significantly reduced (experiment 2; $Z = -2.7$, $P < 0.05$), and an equivalent level to the control was restored at 14 days post-treatment. In dung from control cattle, adult emergence rates were 100% (experiment 1) and 71.6% (experiment 2). However, there was no oviposition in dung at 3 days post-treatment, and all offspring died at an early stage of development (egg or first instar of larva) in dung at 7 and 14 days post-treatment (data not shown).

Fig. 4 shows the estimates of accumulated feeding activity of *C. ochus* per dung pat from control and treated cattle (experiments 1 and 2). The feeding levels of *C. ochus* in dung at 3 days post-treatment were significantly suppressed from 7 to 49 days after the beginning of rearing between control and treatment (experiment 1; $Z = -7.6$, $P < 0.001$). However, there were no differences in the feeding levels at 7 and 14 days post-treatment between control and treatment ($Z = 0.3$, $P = 0.95$; $Z = -1.3$, $P = 0.35$, respectively).

Discussion

In previously published studies eprinomectin pour-on formulation was found to cause dung contamination for 1–29 days post-treatment with a peak at 2 or 3 days post-treatment (Lumaret *et al.*, 2005; Iwasa & Sugitani, 2014). Present results which showed a peak at 1 day post-treatment are different from those of above literatures. Moreover, the maximum detectable level of eprinomectin in this study was 3–5 times higher than those of preceding studies (Lumaret *et al.*, 2005; Iwasa & Sugitani, 2014). Cook *et al.* (1996) and Iwasa *et al.* (2007, 2008) reported that concentrations of ivermectin and moxidectin in dung can be affected by the diet of cattle. In the present study, the site and diet were the same as in a preceding study (Iwasa & Sugitani, 2014). Sommer *et al.* (1992) suggested that ivermectin concentrations in cattle dung increased by the metabolization of organic matter in field exposed pats. It seems likely that the difference of concentrations of eprinomectin residues in dung was attributed to other factors such as contents of organic matter and moisture in dung which were affected by weather and the condition of cattle (age, body weight, diarrhea). It is, therefore, necessary to clarify the relation between the quality of dung and eprinomectin residues.

Other works have shown that dung from cattle treated with ivermectin had no effect on the survival of adult beetles which were small-to-medium sizes, such as *Caccobius*, *Euoniticellus*, *Liatongus*, and *Onthophagus* (Fincher, 1992; Lumaret *et al.*, 1993; Iwasa *et al.*, 2005, 2007). In large dung beetles, however, ivermectin residues in dung caused a high mortality for newly emerged adults of *Copris hispanus* Linnaeus, *Onitis belial* Fabricius (Wardhaugh & Rodriguez-Menendez, 1988), and matured adults of *Copris acutidens* Motschulsky and *C. ochus* (Iwasa *et al.*, 2007). In dung from cattle treated with eprinomectin, there was also no effect on survival of matured adults of *Caccobius jessoensis* Harold and *Liatongus minutus* Motschulsky (Iwasa & Sugitani, 2014). Wardhaugh *et al.* (2001) reported that dung from eprinomectin-treated cattle had no effect on matured adult survival of *Onthophagus taurus* Schreber, but mortality of newly emerged adults increased in dung at 3 days post-treatment. The present results for adult mortalities of two species (figs 1 and 2 and table 2) show that high concentrations (0.45–1.5 ppm) of eprinomectin after treatment at the recommended pour-on dose cause a high adult mortality in *O. lenzii* and *C. ochus*. We suggest that the effect of eprinomectin on adult survival varies by species even in the same genus (*Onthophagus*). Furthermore, dung beetles belonging to the genus *Copris* may have a higher drug sensitivity to eprinomectin than other genera same as ivermectin.

It has been reported that there were no effects of ivermectin on production of brood balls of *Euoniticellus intermedius* Reiche (Fincher, 1992), *Onthophagus gazella* Fabricius (Fincher, 1992; Sommer & Nielsen, 1992), *Diastellopalpus quinquegens* Bates (Sommer *et al.*, 1993), *C. jessoensis*, and *L. minutus* (Iwasa

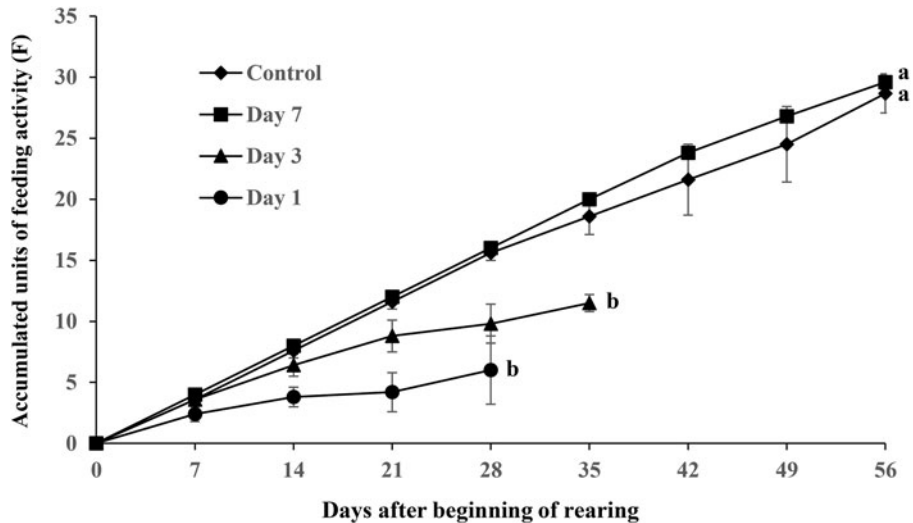


Fig. 3. Accumulated feeding activity (F) of *O. lenzii* in dung from control and eprinomectin treated cattle in experiment 1. Different letters indicate significant differences ($P < 0.05$) among control and treatments (GLMM followed by Tukey's multiple comparisons).

Table 2. Accumulated adult mortality, number of brood balls constructed, and adult emergence rates of *C. ochus* in dung from control and eprinomectin-treated cattle in experiments 1 and 2.

	Days after treatment	Adult mortality (%)			No. of brood balls/female (mean \pm SD)	Adult emergence rate (%)
		Days after the beginning of rearing				
		30	60	90		
Experiment 1	Control	10.0 \pm 21.1	10.0 \pm 21.1	10.0 \pm 21.1	4.3 \pm 1.1(43)	100
	3	30.0 \pm 34.9	90.0 \pm 31.6*	100*	0	0
Experiment 2	Control	10.0 \pm 21.1 ^a	15.0 \pm 24.2 ^a	20.0 \pm 25.8 ^a	4.4 \pm 2.8 ^a (44)	71.6 \pm 24.9
	7	15.0 \pm 24.2 ^a	20.0 \pm 25.8 ^a	25.0 \pm 26.4 ^a	2.2 \pm 2.6 ^b (22)	0
	14	15.0 \pm 24.2 ^a	15.0 \pm 24.2 ^a	20.0 \pm 25.8 ^a	3.8 \pm 1.8 ^a (38)	0

Each value is a mean of ten replicates involving one pair of parents each.

Values in parentheses are total numbers of brood balls.

*Significantly different vs. control in experiment 1 (log-rank test: $P < 0.05$).

In each column, means not associated with the same letter differ among control and treatments in experiment 2 (adult mortalities: log-rank test followed by the Bonferroni method, $P < 0.0167$, numbers of brood balls: GLMM followed by Tukey's multiple comparisons, $P < 0.05$).

et al., 2007). However, the numbers of brood balls of *C. hispanus* were reduced in dung from ivermectin-treated cattle at 3 days post-treatment (Wardhaugh & Rodriguez-Menendez, 1988), and those of *Onthophagus landolti* Harold decreased at concentrations of 0.1, 1.0, and 10.0 ppm in dung spiked with ivermectin (Pérez-Cogollo *et al.*, 2015). Moreover, there were no brood balls and/or oviposition of *C. acutidens* and *C. ochus* in dung from ivermectin-treated cattle at 1 day after treatment (Iwasa *et al.*, 2007). In dung from eprinomectin-treated cattle, there was no effect on the number of brood balls of *C. jessoensis* and *L. minutus* (Iwasa & Sugitani, 2014). In newly emerged adults of *O. taurus*, however, brood balls in dung from eprinomectin-treated cattle decreased at 3 days post-treatment (Wardhaugh *et al.*, 2001). The present results suggest that a high concentration of eprinomectin residues in dung could cause a significant reduction in the numbers of brood balls of *O. lenzii* and *C. ochus*, and its effect may persist longer in *C. ochus*.

In most cases, larval survivals or adult emergence rates of dung beetles (*Aphodius*, *Caccobius*, *Copris*, *Diastelopalpus*,

Digitonthophagus, *Euoniticellus*, *Liatongus*, and *Onthophagus*) decreased in dung from ivermectin-treated cattle (Wardhaugh & Rodriguez-Menendez, 1988; Fincher, 1992; Lumaret *et al.*, 1993; Sommer *et al.*, 1993; Krüger & Scholtz, 1997; Errouissi *et al.*, 2001; Wardhaugh *et al.*, 2001; Iwasa *et al.*, 2007). However, there are few reports on the effects of eprinomectin on larvae of dung beetles. Wardhaugh *et al.* (2001) showed that eprinomectin residues in dung at 3–7 days post-treatment reduced the larval survival of *O. taurus*. Adult emergence rates of *C. jessoensis* and *L. minutus* were reduced in dung from eprinomectin-treated cattle at 1–3 days post-treatment (Iwasa & Sugitani, 2014). The present results show that adult emergence rates of *O. lenzii* and *C. ochus* were reduced at 1, 3, 7 days and 3, 7, 14 days post-treatment, respectively (tables 1 and 2). The period during which eprinomectin-treated cattle produce dung toxic to larvae of *O. lenzii* is consistent with that of *O. taurus*, and both periods are longer than those for *C. jessoensis* and *L. minutus*. Iwasa *et al.* (2007) suggested that the effect of ivermectin on the larval survival of small dung beetles such as *C. jessoensis* is short-term in comparison with

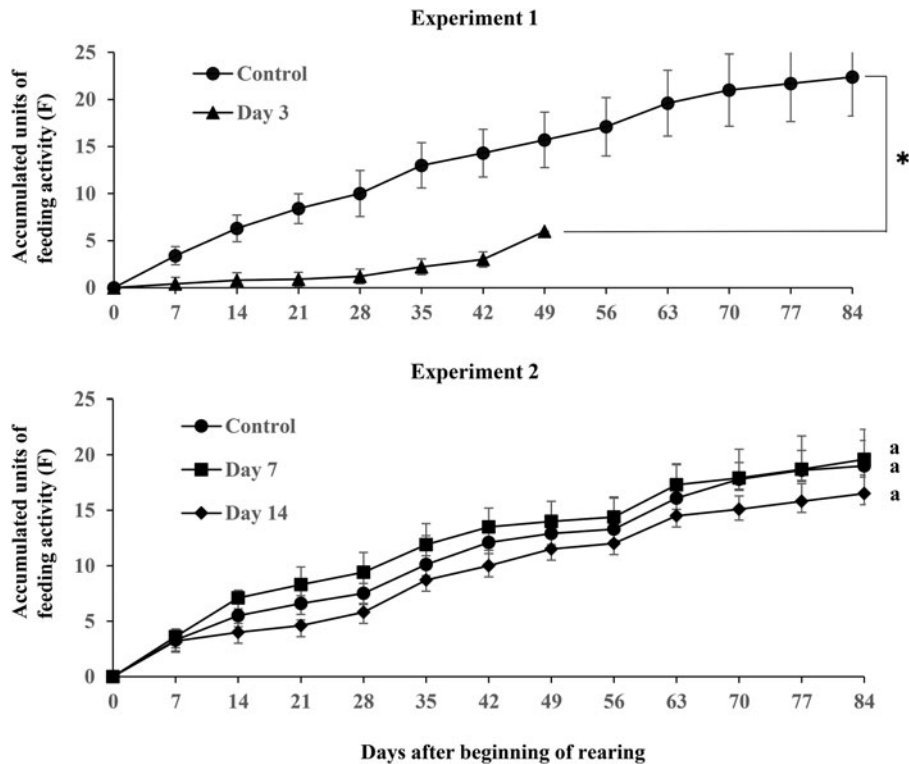


Fig. 4. Accumulated feeding activity (F) of *C. ochus* in dung from control and eprinomectin treated cattle in experiments 1 and 2. *Significantly different vs. control in experiment 1 (GLMM: $P < 0.05$). Values followed by same letters within each day after the beginning of rearing are not significantly different in experiment 2 (GLMM followed by Tukey's multiple comparisons: $P < 0.05$).

medium-sized dung beetles. The same phenomenon may be present in dung from cattle treated with eprinomectin.

As shown in the present results, all offspring of *C. ochus* died at an early stage (egg or first instar of larva) in dung from 14 days post-treatment (table 2), although eprinomectin was undetected in it (fig. 1). Doherty *et al.* (1994) reported that the adult emergence rates of *O. gazella* were severely reduced at a very low concentration (0.008 ppm) in dung directly spiked with abamectin. It is possible that dung from 14-days post-treatment with undetectable residues of eprinomectin (<0.05 ppm) could cause a high toxicity against the early stage of *C. ochus*. *Copris* species are listed as 'rare' or 'endangered' species in many prefectures of Japan (Hori, 2005). Iwasa *et al.* (2007) suggested that the decrease of *C. ochus* in Japan is partly attributed to the contamination of dung with anthelmintic drugs such as ivermectin. For evaluation of ecotoxicity of drugs used in pastures, attention should be paid to impact on the population of dung beetles such as *Copris* species, which show a high drug sensitivity.

Wardhaugh & Rodriguez-Menendez (1988) showed that the feeding activity of *C. hispanus* was greatly suppressed at 1–8 days after treatment by ivermectin injection. In their field experiment, Dadour *et al.* (1999) reported that dung dispersal by *O. taurus* decreased at 7 and 10 days after treatment in dung from cattle treated with ivermectin by injection. Dung removal by *O. landolti* was inhibited in dung which was spiked with ivermectin at 0.1, 1.0 and 10.0 ppm (Pérez-Cogollo *et al.*, 2015). The present results indicate that dung from cattle treated by pour-on formulation at the recommended dose of

eprinomectin causes a high concentration of eprinomectin residues, associated with the suppression of feeding of *O. lenzii* and *C. ochus*. This concentration occurred in dung produced at 3 days post-treatment when adult survival was also affected (fig. 1 and table 2).

It is known that avermectin acts on both glutamate-gated and GABA-gated chloride channels in nerve and muscle cells of invertebrates, resulting in somatic paralysis and death (Cully *et al.*, 1994; Arena *et al.*, 1995). Verdú *et al.* (2015) reported that ivermectin in dung decreased the olfactory and locomotor capacities of *Scarabaeus cicatricosus* Lucas, and it caused a significant negative effect on foraging success. Eprinomectin may have an effect on the feeding activity of dung beetles in the same mode of action as ivermectin, causing reduction of adult survival.

Our results show a significant effect of eprinomectin on survival, reproduction, and feeding activity of two species of dung beetles which have an important function in dung dispersal and decomposition in Japan. Further study is necessary to clarify the effect of eprinomectin on other dung beetles inhabiting dung pats and to evaluate the impact of this drug on dung degradation in the pasture.

Acknowledgements

We wish to thank Drs T. Oshida and N. Kumano of Obihiro University of Agriculture and Veterinary Medicine, and Dr T. Nakamura of Hirosaki University for their valuable suggestions. We are also grateful to Mr. S. Yamanaka and K. Mihara

of Obihiro Yachiyo Farm, Mr. K. Hasegawa of Ôno Farm, Mr. T. Arai of Ôtawara city office, and Mr. T. Nakamura of Nakamura Farm in Mukawa town for their kind help and arrangements during the survey in pastures. Our thanks are also due to Assistant Professor G. A. Hill of Obihiro University of Agriculture and Veterinary Medicine for checking the English in this manuscript.

References

- Arena, J. P., Lui, K. K., Paress, P. S., Frazier, E. G., Cully, D. F., Mrozik, H. & Schaeffer, J. M. (1995) The mechanism of action of avermectin in *Caenorhabditis elegans*: correlation between activation of glutamate-sensitive chloride current, membrane binding, and biological activity. *Journal of Parasitology* **81**, 286–294.
- Benz, G. W. (1985) Animal health applications of ivermectin. *Southwestern Entomologist* **7**, 2–10.
- Bornemissza, G. E. (1970) Insectary studies on the control of dung breeding flies by the activity of the dung beetle, *Onthophagus gazella* F. (Coleoptera: Scarabaeinae). *Australian Journal of Entomology* **9**, 31–41.
- Cook, D. F., Dadour, I. R. & Ali, D. N. (1996) Effect of diet on the excretion profile of ivermectin in cattle faeces. *International Journal of Parasitology* **26**, 291–295.
- Cully, D. F., Vassilatis, D. K., Liu, K. K., Paress, P. S., Van-Der-Ploeg, L. H. T., Schaeffer, J. M. & Arena, J. P. (1994) Cloning of an avermectin-sensitive glutamate chloride channel from *Caenorhabditis elegans*. *Nature* **371**, 707–711.
- Dadour, I. R., Cook, D. F. & Neesam, C. (1999) Dispersal of dung containing ivermectin in the field by *Onthophagus taurus* (Coleoptera: Scarabaeidae). *Bulletin of Entomological Research* **89**, 119–123.
- Doherty, W. M., Stewart, N. P., Cobb, R. M. & Keiran, P. J. (1994) In-vitro comparison of the larvicidal activity of moxidectin and abamectin against *Onthophagus gazella* (F.) (Coleoptera: Scarabaeidae) and *Haematobia irritans exigua* De Meijere (Diptera: Muscidae). *Australian Journal of Entomology* **33**, 71–74.
- Drummond, R. O. (1985) Effectiveness of ivermectin for control of arthropod pests of livestock. *Southwestern Entomologist* **7**, 34–42.
- Errouissi, F., Alvinerie, M., Galtier, P., Kerboeuf, D. & Lumaret, J. P. (2001) The negative effects of the residues of ivermectin in cattle dung using a sustained-release bolus on *Aphodius constans* (Duft.) (Coleoptera: Aphodiidae). *Veterinary Research* **32**, 421–427.
- Fincher, G. T. (1992) Injectable ivermectin for cattle: effects on some dung-inhabiting insects. *Environmental Entomology* **21**, 871–876.
- Floate, K. D., Colwell, D. D. & Fox, A. S. (2002) Reduction of non-pest insects in dung of cattle treated with endectocides: a comparison of four products. *Bulletin of Entomological Research* **92**, 471–481.
- Floate, K. D., Wardhaugh, K. G., Boxall, A. B. A. & Scherratt, T. N. (2005) Fecal residues of veterinary parasiticides: nontarget effects in the pasture environment. *Annual Review of Entomology* **50**, 153–179.
- Gokbulut, C., Naturali, S., Rufrano, D., Anastasio, A., Yalinkilinc, H. S. & Veneziano, V. (2012) Plasma disposition and milk excretion of eprinomectin following pour-on administration in lactating donkeys. *Journal of Veterinary Pharmacology and Therapeutics* **36**, 302–305.
- Holter, P., Sommer, C., Grønbold, J. & Madsen, M. (1993) Effects of ivermectin treatment on the attraction of dung beetles (Coleoptera: Scarabaeidae and Hydrophilidae) to cow pats. *Bulletin of Entomological Research* **83**, 53–58.
- Hori, S. (2005) On inhabiting situation of the Japanese species of the scarabaeid genus *Copris* (Coleoptera, Scarabaeidae). *Konchu to Shizen [Insects and Nature]* **40**, 6–9 (in Japanese).
- Houlding, B., Ridsdill-Smith, T. J. & Bailey, W. J. (1991) Injectable abamectin causes a delay in scarabaeinae dung beetle egg-laying in cattle dung. *Australian Veterinary Journal* **68**, 185–186.
- Iwasa, M. & Sugitani, M. (2014) Effects of the veterinary anti-parasitic drug eprinomectin on dung beetles (Coleoptera: Scarabaeidae), the non-pest fly *Neomyia cornicina* and pest fly *Haematobia irritans* (Diptera: Muscidae) in Japan. *Applied Entomology and Zoology* **49**, 591–597.
- Iwasa, M., Nakamura, T., Fukaki, K. & Yamashita, N. (2005) Nontarget effects of ivermectin on coprophagous insects in Japan. *Environmental Entomology* **34**, 1485–1492.
- Iwasa, M., Maruo, T., Ueda, M. & Yamashita, N. (2007) Adverse effects of ivermectin on the dung beetles, *Caccobius jessoensis* Harold, and rare species, *Copris ochus* Motschulsky and *Copris acutidens* Motschulsky (Coleoptera: Scarabaeidae), in Japan. *Bulletin of Entomological Research* **97**, 619–625.
- Iwasa, M., Suzuki, N. & Maruyama, M. (2008) Effects of moxidectin on coprophagous insects in cattle dung pats in Japan. *Applied Entomology and Zoology* **43**, 271–280.
- Krüger, K. & Scholtz, C. H. (1997) Lethal and sublethal effects of ivermectin on the dung-breeding beetles *Euoniticellus intermedius* (Reiche) and *Onitis alexis* Klug (Coleoptera, Scarabaeidae). *Agriculture, Ecosystem and Environment* **61**, 123–131.
- Lumaret, J. P., Galante, E., Lumberas, C., Mena, J., Bertrand, M., Bernal, J. L., Cooper, J. F., Kadiri, N. & Crowe, D. (1993) Field effects of ivermectin residues on dung beetles. *Journal of Applied Ecology* **30**, 428–436.
- Lumaret, J. P., Errouissi, F., Galtier, P. & Alvinerie, M. (2005) Pour-on formulation of eprinomectin for cattle: fecal elimination profile and effects on the development of the dung-inhabiting Diptera *Neomyia cornicina* (L.) (Muscidae). *Environmental Toxicology and Chemistry* **24**, 797–801.
- Madsen, M., Nielsen, B. O., Holter, P., Pedersen, O. C., Jespersen, J. B., Vagn Jensen, K.-M., Nansen, P. & Grønbold, J. (1990) Treating cattle with ivermectin: effects on the fauna and decomposition of dung pats. *Journal of Applied Ecology* **27**, 1–15.
- Mason, W. A., Pomroy, W. E., Lawrence, K. E. & Schot, I. (2012) The effect of repeated, four weekly eprinomectin treatment on milk production in pasture-based, seasonally-calving dairy cattle. *Veterinary Parasitology* **189**, 250–259.
- Miller, J. A., Kunz, S. E., Oehler, D. D. & Miller, R. W. (1981) Larvicidal activity of Merk MK-933, an avermectin against the horn fly, stable fly, and house fly. *Journal of Economic Entomology* **74**, 608–611.
- Nichols, E., Spector, S., Louzada, J., Larsen, T., Amezcua, S. & Favila, M. E. (2008) Ecological functions and ecosystem services provided by Scarabaeinae dung beetle. *Biological Conservation* **141**, 1461–1474.
- Payne, L. D., Hicks, M. B. & Wehner, T. A. (1995) Determination of abamectin and/or ivermectin in cattle feces at low parts per billion levels using HPLC with fluorescence detection. *Journal of Agricultural and Food Chemistry* **43**, 1233–1237.
- Pérez-Cogollo, L. C., Rodríguez-Vivas, R. I., Delfín-González, H., Reyes-Novelo, E. & Ojeda-Chi, M. M. (2015) Lethal and sublethal effects of ivermectin on *Onthophagus landolti* (Coleoptera: Scarabaeidae). *Environmental Entomology* **44**, 1634–1640.

- R Development Core Team** (2016) R a Language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available online at <https://www.r-project.org>
- Reist, M., Forbes, A. B., Bonfanti, M., Beretta, W. & Pfister, K.** (2011) Effect of eprinomectin treatment on milk yield and quality in dairy cows in South Tyrol, Italy. *Veterinary Record* **168**, 484–487.
- Ridsdill-Smith, T. J.** (1988) Survival and reproduction of *Musca vetustissima* Walker (Diptera: Muscidae) and a scarabaeine dung beetle in dung of cattle treated with avermectin B1. *Journal of the Australian Entomological Society* **27**, 175–178.
- Schoop, W. L., Demontigny, P., Fink, D. W., Williams, J. B., Egerton, J. R., Mrozik, H., Fisher, M. H., Skelly, B. J. & Turner, M. J.** (1996a) Efficacy in sheep and pharmacokinetics in cattle that led to the selection of eprinomectin as a topical endectocide for cattle. *International Journal of Parasitology* **26**, 1227–1235.
- Schoop, W. L., Egerton, J. R., Eary, C. H., Haines, H. W., Michael, B. F., Mrozik, H., Eskola, P., Fisher, M. H., Slayton, L., Ostlind, D. A., Skelly, B. J., Fulton, R. K., Barth, D., Costa, S., Gregory, L. M., Campbell, W. C., Seward, R. L. & Turner, M. J.** (1996b) Eprinomectin: a novel avermectin for use as a topical endectocide for cattle. *International Journal of Parasitology* **26**, 1237–1242.
- Sommer, C. & Nielsen, B. O.** (1992) Larvae of the dung beetle *Onthophagus gazella* F. (Col., Scarabaeidae) exposed to lethal and sublethal ivermectin concentrations. *Journal of Applied Entomology* **114**, 502–509.
- Sommer, C., Steffansen, B., Overgaard Nielsen, B., Grønvold, J., Vagn Jensen, K.-M., Brøchner Jespersen, J., Springborg, J. & Nansen, P.** (1992) Ivermectin excreted in cattle dung after subcutaneous injection or pour-on treatment: concentrations and impact on dung fauna. *Bulletin of Entomological Research* **82**: 257–264.
- Sommer, C., Grønvold, J., Holter, P., Madsen, M. & Nansen, P.** (1993) Dung burial activity and development of ivermectin exposed *Diastellopalpus quinquevittatus* in a field experiment. *Entomologia Experimentalis et Applicata* **66**, 83–89.
- Verdú, J. R., Cortez, V., Ortiz, A. J., González-Rodríguez, E., Martínez-Pinna, J., Lumaret, J.-P., Lobo, J. M., Numa, C. & Sánchez-Piñero, F.** (2015) Low doses of ivermectin cause sensory and locomotor disorders in dung beetles. *Scientific Reports* **5**, 1–10. <https://doi.org/10.1038/srep13912>.
- Wall, R. & Strong, L.** (1987) Environmental consequence of treating cattle with the antiparasitic drug ivermectin. *Nature* **327**, 418–421.
- Wardhaugh, K. G. & Rodríguez-Menéndez, H.** (1988) The effects of the antiparasitic drug, ivermectin, on the development and survival of the dung-breeding fly, *Orthellia cornicina* (F.) and the scarabaeine dung beetle, *Copris hispanus* L., *Bubas bubalus* (Oliver) and *Onitis belial* F. *Journal of Applied Entomology* **106**, 381–389.
- Wardhaugh, K. G., Longstaff, B. C. & Morton, R.** (2001) A comparison of the development and survival of the dung beetles, *Onthophagus taurus* (Schreb.) when fed on the faeces of cattle treated with pour-on formulations of eprinomectin or moxidectin. *Veterinary Parasitology* **99**, 155–168.
- Williams, J. C., Stuedemann, J. A., Bairden, K., Kerboeuf, D., Ciordia, H., Hubert, J., Broussard, S. D., Plue, R. E., Alva-Valdes, R., Baggott, D. G., Pinkall, N. & Eagleson, J. S.** (1997) Efficacy of a pour-on formulation of eprinomectin (MK-397) against nematode parasites of cattle, with emphasis on inhibited early fourth-stage larvae of *Ostertagia* spp. *American Journal of Veterinary Research* **58**, 379–383.