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Toxicological effect of ivermectin on the survival, reproduction, and feeding activity of four species of dung beetles (Coleoptera: Scarabaeidae and Geotrupidae) in Japan

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Abstract

We investigated the effects of the antiparasitic drug ivermectin on the dung beetles *Copris acutidens* Motschulsky, *Onthophagus bivertex* Heyden, *O. lenzii* Harold and *Phelotrupes auratus auratus* Motschulsky in Japan. Ivermectin was detected in cattle dung from 1 to 3 or 7 days post-treatment, with a peak at 3 days post-treatment in two pour-on administrations (500 μg kg^{-1}). In *C. acutidens*, adult survivals and numbers of brood balls were significantly reduced in dung collected at 3 and 7 days post-treatment, and adult emergence rates were significantly decreased in dung collected at 7 and 14 days post-treatment. Feeding activity of *C. acutidens* was inhibited in dung collected at 3 days post-treatment, but was not significantly different from that seen in control dung at 7 and 14 days post-treatment. In *O. bivertex* and *O. lenzii*, there were no effects of ivermectin on adult survival or feeding activities, but the numbers of brood balls of *O. bivertex* constructed in dung collected at 3 and 7 days post-treatment were significantly lower than observed with control dung. The adult emergence rates of *O. bivertex* and *O. lenzii* were significantly reduced in dung collected at 1 to 3 and 1 to 7 days post-treatment, respectively. In *P. auratus*, there were no effects of ivermectin on adult survival, oviposition, feeding activity, or larval survival (until the third instar) in dung at 3 days post-treatment. The environmental risks affecting the populations of dung beetles in Japan are discussed.

Keywords: Ivermectin, *Copris*, *Onthophagus*, *Phelotrupes*, Japan, ecotoxicity

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Introduction

Helminths parasites adversely affected domestic livestock through an effect on weight gain, milk yield, and reproductive ability (Steelman, 1976; Byford *et al*., 1992). To control these parasites, veterinary drugs with various treatment methods (subcutaneous injection, oral administration, or pour-on formulation) have been developed. In particular, macrocyclic lactones (MLs) such as ivermectin, are highly effective in eliminating intestinal nematodes and arthropod parasites such as blood-sucking flies, mites, and lice (Miller *et al*., 1981; Benz, 1985; Schröder, 1992; Iwasa *et al*., 2007; Adler *et al*., 2016). After MLs are applied to animals, most of the active ingredient is excreted into dung (Campbell *et al*., 1983), and it remains for a long time in dung (Wohde *et al*., 2016), where it can kill the larvae of dung-breeding insects (Drummond, 1985; Floate *et al*., 2001). However, residues of MLs in dung negatively affects non-target insects such as dung beetles and non-pest flies inhabiting dung pats in pastures (Floate *et al*., 2005), which may have long term ecological consequence.

Dung beetles play an important role in dung decomposition, and they provide important ecosystem services which include improvement of nutrient cycling and soil aeration, seed dispersal, and suppression of parasites of domestic animals in pastures (Bornemissza, 1970; Nichols *et al*., 2008;

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Manning *et al*., 2016). Reduction of dung beetles may result in important consequences in pastures. It has been reported that ivermectin residues in dung reduce the survival and reproduction of dung beetles (Ridsdill-Smith, 1988; Wardhaugh & Rodriguez-Menendez, 1988; Houlding *et al*., 1991; Fincher, 1992; Holter & Sommer, 1993; Lumaret *et al*., 1993, 2012; Sommer *et al*., 1993; Krüger & Scholtz, 1997; Dadour *et al*., 1999; Errouissi *et al*., 2001; Iwasa *et al*., 2005, 2007). Ivermectin residues in dung also suppressed feeding activity or dung removal of dung beetles (Wardhaugh & Rodriguez-Menendez, 1988; Dadour *et al*., 1999; Pérez-Cogollo *et al*., 2015; Manning *et al*., 2017). These adverse effects may be associated with the delay of dung decomposition (Wall & Strong, 1987; Madsen *et al*., 1990; Floate, 1998; Krüger and Scholtz, 1998*a*, *b*; Römbke *et al*., 2010) and reduction of diversity (Verdú *et al*., 2018) in pastures. For conservation of pasture ecology (pastureland ecosystems), a detailed evaluation of anthelmintic drugs such as ivermectin on dung beetles is an important issue.

In Japan, ivermectin has been widely used, with the pour-on formulation introduced in 1996. However, there have been only a few papers about the effects of ivermectin on dung beetles in Japan (Yamashita *et al*., 2004; Iwasa *et al*., 2005, 2007). It is therefore necessary to obtain further information about the effects on dung beetles. This study was carried out to assess the effect of pour-on formulation of ivermectin on the survival, reproduction, and feeding activity of four dung beetle species: *Copris acutidens* Motschulsky, *Onthophagus bivertex* Heyden, *O. lenzii* Harold and *Phelotrupes auratus auratus* Motschulsky, which are important dung-decomposing species in Japan.

Materials and methods

Application of ivermectin and dung collection

The experiments were performed in a pasture of Yachiyo Farm southwest of Obihiro City, Hokkaido, Japan. In experiment 1, ten Holstein cattle (aged 10–30 months) were selected, and ivermectin (Ivomec Topical; Merial, Japan) was applied by pour-on formulation with the recommended dose of 500 $\mu g\ kg^{-1}$ of body weight on 15 June 2015. In experiment 2, ten Holsteins were selected, and ivermectin was applied on 24 June 2016. Ten untreated Holsteins were used as a control. All cattle were pastured in Yachiyo Farm and mainly fed with green grass composed of Kentucky bluegrass, timothy, and Ladino clover.

Dung pats were collected immediately after defecation in the morning from treated and control groups on the day preceding treatment and at 1, 3, 7, 14, and 21 days after treatments. In each collection, a total of about 10 kg of dung from ten cows in each group was thoroughly homogenized and stored at −20°C until used.

Analysis of ivermectin concentration

The concentration of ivermectin in cattle dung was analyzed using high-performance liquid chromatography (HPLC) following the method of Payne *et al*. (1995) with some modifications. Two fecal samples (each 5 g) from dung collected from treatment and control groups at each date were analyzed, and the extraction was carried out with homogenizer (Physcotron, Microtec Co., Ltd) at 10,000 rpm for 1 min (instead of sonication), and the concentration was calculated from a calibration curve of a standard solution. The concentrations detection limit was determined to be 0.05 ppm.

The effect of ivermectin on the survival and reproduction of four species of dung beetles

Three species of Scarabaeidae (*C. acutidens*, *O. bivertex*, and *O. lenzii*) and one species of Geotrupidae (*P. auratus*) were selected as important dung beetles which are distributed throughout Japan (Hayakawa, 1981; Kawai *et al*., 2005; Imura, 2007).

C. acutidens *Motschulsky*

C. acutidens is a medium-sized (9.7–16.0 mm) dung beetle which depends on dung of cattle, horse, and deer. Adults of *C. acutidens* were collected in Hokkaido in June 2015. A 70 g sample of dung collected from control and treated (3, 7, and 14 days after treatment in experiment 1) cattle was placed on andosol (commercial black soil for horticulture) (15 cm depth) in a plastic container (12 cm diameter, 18 cm depth) which was covered with gauze, and one male–female pair was placed on the dung surface. The rearing test was performed for 90 days; this period corresponds to the breeding season (June–August) of *C. acutidens* in Japan. Thereafter, dung samples were replaced with fresh ones every week. After every 30 days following the onset of rearing, the soil in the container was sieved to collect the brood balls (one egg laid per one brood ball). The brood balls collected were placed in a plastic cup (5 cm diameter, 3 cm depth) filled with andosol to record adult emergence rate. Rearing tests were replicated ten times for each dung from treatments and control.

O. bivertex *Heyden and* O. lenzii *Harold*

O. bivertex is small/medium sized dung beetle (5.2–6.8 mm), which is distributed in open and flat land. Adults of *O. bivertex* were collected in Hokkaido in June 2016. A 50 g sample of dung collected at 1, 3, and 7 days after treatment and control in experiment 2 was placed on andosol (15 cm depth) in a plastic container (12 cm diameter, 18 cm depth), and three male–female pairs were placed on the dung surface. Dung samples were replaced with fresh dung, and the soil in the container was sieved to collect the brood balls once per week for 56 days. Rearing tests were replicated five times for each dung treatment and control.

O. lenzii is medium-sized dung beetle (body size is 7.1–9.2 mm), which is distributed throughout Japan. Adults were collected in Tochigi prefecture in July 2016. A 50 g dung sample collected at 1, 3, 7, and 14 days after treatment and control in experiment 2 was used for rearing tests. The rearing method was the same as *O. bivertex*, and rearing tests were replicated five times for each treatment and control.

P. auratus auratus *Motschulsky*

P. a. auratus is a large dung beetle (body size: 12.4–22.0 mm) and it is widely distributed from flatland to highland. Adults were collected in the pasture of Yachiyo Farm, located in southwest of Obihiro City, Hokkaido in August 2015. A 200 g dung sample collected at 3 days after treatment and control in experiment 1 was placed on andosol (30 cm depth) in a plastic container (30 cm diameter, 40 cm depth), and two male–female pairs were placed on the dung surface. Dung samples were replaced with fresh dung once per week for 90 days; this period corresponds to their breeding season (August–October) in Hokkaido, Japan. The andosol in the container was sieved to collect the brood mass (one egg laid per

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one brood mass) every 30 days. At this time, we confirmed one egg laid per one brood mass. Rearing tests were replicated five times for each dung treatment and control group. The rearing tests on adults and larvae of *C. acutidens*, *O. bivertex*, *O. lenzii*, and *P. a. auratus* were conducted at a constant temperature of 22°C (light regime 16 L:8 D).

The effect of ivermectin on the feeding activity of four species of dung beetles

The dung samples placed in rearing containers were inspected when they were replaced with new ones. They were described following the level of feeding 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 the surface of dung; 2 = two or more feeding traces on the surface of dung; 3 = most of the feces was degraded, but surface parts of dung was left; 4 = all of the feces was degraded into fiber or carried to a burrow).

Statistical analysis

The adult mortality of four species of dung beetle was analyzed using the log-rank test for differences between control and treatments. Then, for *C. acutidens*, *O. bivertex*, and *O. lenzii*, Bonferroni's method was used to apply a multiple testing adjustment of *P*-values for each comparison between control and treatment groups. The significance level which was required by the Bonferroni correction was set at 0.008 (=0.05/6: *C. acutidens* and *O. bivertex*) or 0.005 (=0.05/10: *O. lenzii*).

Generalized linear mixed models (GLMMs) with log-link function were used to analyze the numbers of brood balls or masses (Poisson error) and the adult emergence rates or larval survival (binomial error). The numbers of brood balls (masses) and adult emergence rates (larvae survival) were response variables, the type of feces (each treatment and control) were explanatory variables, and replicates were random effects.

The feeding activity levels for each treatment and control were evaluated using GLMMs with Poisson error distribution and log-link function. The estimated feeding levels (0–4) were response variables, type of feces were explanatory variables, and replicates were random effects. The significance level for multiple comparisons was adjusted by Tukey's method. All analyses were performed using R 3.2.4 software (R Development Core Team, 2016).

Results

Concentration of ivermectin residues in cattle dung

Ivermectin residues in dung attained maximal concentration at 3 days after treatment (fig. 1), followed by a marked decline by 7 days in both experiments. In experiment 1, ivermectin was not detected at 7, 14, and 21 days after treatment (<0.05 ppm). In experiment 2, ivermectin in dung at 7 days was about one-third of maximal concentration, and no ivermectin was detected at 14 and 21 days (<0.05 ppm) after treatment. No ivermectin was detected in dung from all dates in both control cattle.

The effect of ivermectin on survival, reproduction, and feeding activity of C. acutidens

In dung collected from control cattle, all of adults survived until 90 days after the beginning of rearing (table 1). In dung collected at 3 days post-treatment, however, accumulated adult mortality were significantly higher than the control at 30, 60, and 90 days after the beginning of rearing, and with 40% of maximal mortality at 90 days (table 1; 30 days: $\chi^2 = 7.7$, $P < 0.008$; 60 days: $\chi^2 = 11.6$, $P < 0.008$; 90 days: $\chi^2 = 13.7$, $P < 0.008$). Similarly, the adult mortality in dung collected at 7 days post-treatment were significantly high at 60 and 90 days after the beginning of rearing (table 1; 60 days: $\chi^2 = 5.8$, $P < 0.008$; 90 days: $\chi^2 = 7.9$, $P < 0.008$). In dung collected at 14 days post-treatment, there was no significant difference in adult mortality between control and treatment. There were 5.3 ± 1.2 brood balls in dung collected from control cattle, but there was no brood ball in dung at 3 days post-treatment. In dung collected at 7 days post-treatment, the numbers of brood balls were significantly low (table 1; $Z = -2.6$, $P < 0.05$), returning to levels of the control at 14 days post-treatment. The adult emergence rate in dung from control cattle was 96.1%, whereas those in dung collected at 7 and 14 days post-treatment were significantly decreased (table 1; $Z = -5.7$, $P < 0.001$; $Z = -2.9$, $P < 0.01$, respectively).

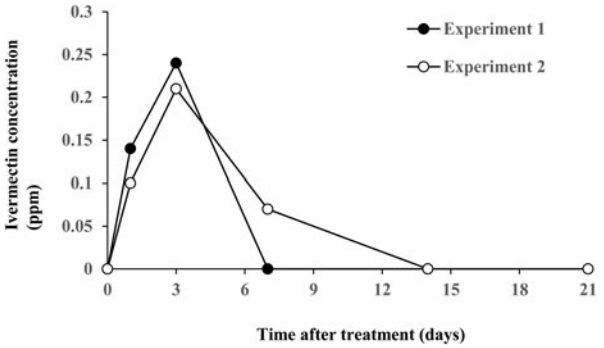

Fig. 1. Ivermectin concentrations in dung (ppm of wet weight) after treatments (pour-on treatment: 500 μg kg^{-1}) in experiments 1 and 2.

In dung collected at 7 and 14 days post-treatment, there were no significant differences in feeding activity levels between at 7 and 14 days post-treatment and control (fig. 2). In dung collected at 3 days post-treatment, however, the feeding activity was significantly reduced compared to control (fig. 2; $Z = -4.4$, $P < 0.001$).

The effect of ivermectin on survival, reproduction, and feeding activity of O. bivertex

Adult mortality of *O. bivertex* in all treatments and control ranged from 53.3 to 63.4%, and there were no significant differences between each treatment and control (table 2). The numbers of brood balls increased significantly in dung collected at 1 day post-treatment compared to control (table 2; $Z = 3.2$, $P < 0.05$). In contrast, females in dung collected at 3 and 7 days post-treatment constructed significantly fewer brood balls than the control (table 2; $Z = -4.1$, $P < 0.001$; $Z = -7.2$, $P < 0.001$, respectively). Adult emergence rates in dung collected at 1 and 3 days post-treatment were significantly lower than control (table 2; $Z = -2.96$, $P < 0.05$; $Z = -3.8$, $P < 0.001$, respectively), but an equivalent level to the control was restored at 7 days post-treatment.

There were no significant differences in the feeding activities of *O. bivertex* between all treatments and control (fig. 3),

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Table 1. Accumulated adult mortality, number of brood balls, and adult emergence rate of *C. acutidens* in dung from control and treatment groups in experiment 1.

Days after treatment	Adult mortality (%) Days after the beginning of rearing 30	60	90	No. of brood ball/replicate (mean ± SD)	Adult emergence rate (%) (mean ± SD)
Control	0[a]	0[a]	0[a]	5.3 ± 1.2[a] (53)	96.1 ± 8.7[a]
Day 3	25.0 ± 26.4[b]	35.0 ± 24.2[b]	40.0 ± 21.0[b]	0	0
Day 7	5.0 ± 15.8[a]	20.0 ± 25.8[c]	25.0 ± 26.4[c]	2.9 ± 2.4[b] (29)	8.3 ± 23.6[b]
Day 14	5.0 ± 15.8[a]	5.0 ± 15.8[a]	5.0 ± 15.8[a]	3.7 ± 2.6[a] (37)	68.3 ± 23.5[c]

Each value is a mean of ten replicates involving one pair of male and female.
Values in parentheses are total numbers of brood balls.
In each column, the means not associated with the same letter differ among control and treatments in experiment 1 (adult mortalities: log-rank test followed by Bonferroni method $P < 0.0083$, numbers of brood balls and adult emergence rate: GLMM followed by Tukey's multiple comparisons $P < 0.05$).

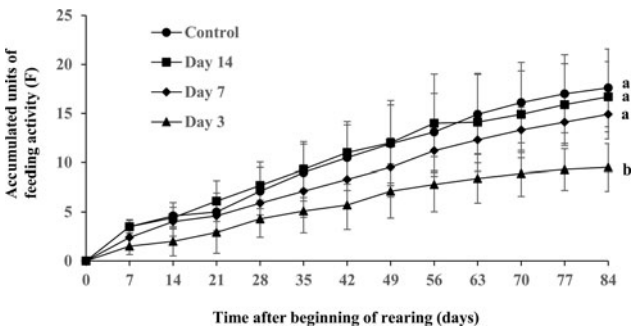

Fig. 2. Accumulated feeding activity (F) of *C. acutidens* in dung from control and treatments in experiment 1. Different letters show significant difference among control and treatments (GLMM followed by Tukey's multiple comparisons: $P < 0.05$).

Table 2. Accumulated adult mortality, number of brood balls, and new adult emergence rate of *O. bivertex* in dung from control and treatment groups in experiment 2.

Day after treatment	Adult mortality (%)	No. of brood balls/replicate (mean ± SD)	Adult emergence rate (%) (mean ± SD)
Control	53.3 ± 13.9[a]	47.2 ± 7.8[a] (236)	39.8 ± 9.8[a]
Day 1	63.4 ± 7.5[a]	62.2 ± 35.7[b] (311)	25.5 ± 12.7[bc]
Day 3	56.7 ± 14.9[a]	19.8 ± 9.1[c] (99)	20.4 ± 13.5[b]
Day 7	60.0 ± 22.4[a]	30.8 ± 13.3[d] (154)	35.3 ± 15.7[ac]

Each value is a mean of five replicates involving three pairs of male and female.
Value in parentheses is the total number of brood balls.
In each column, the means not associated with the same letter differ among control and treatments in experiment 2 (adult mortalities: log-rank test followed by Bonferroni method $P < 0.0083$, numbers of brood balls and adult emergence rate: GLMM followed by Tukey's multiple comparisons $P < 0.05$).

but those in dung collected at 1 and 7 days post-treatment differed significantly (fig. 3; $Z = -2.67$, $P < 0.05$).

The effect of ivermectin on survival, reproduction, and feeding activity of O. lenzii

There were no significant differences in adult mortality of *O. lenzii* between each treatment (1, 3, 7, and 14 days post-treatment) and control (table 3). Numbers of brood balls in dung collected at 3, 7, and 14 days post-treatment were not significantly different from those of the control, but those in dung collected at 1 day post-treatment were significantly more abundant (table 3; $Z = 3.63$, $P < 0.05$). In control dung, adult emergence rate was 72.9%, but those in dung from 1, 3, and 7 days post-treatment were statistically smaller than that of control dung (table 3; $Z = -9.12$, $P < 0.001$; $Z = -7.06$, $P < 0.001$; $Z = -5.26$, $P < 0.001$, respectively), returning to control level at 14 days post-treatment.

There were no significant differences in the feeding activities of *O. lenzii* between all treatments (3, 7, and 14 days post-treatment) and control (fig. 4).

The effect of ivermectin on survival, reproduction, and feeding activity of P. a. auratus

Adult mortalities of *P. auratus* were 5% and zero in dung collected at 3 days post-treatment and control, respectively, showing no significant difference between them (table 4). Females constructed some brood masses (control: 15, treatment: 13 masses), and there was no significant difference between treatment and control. The larvae in brood masses developed to the third instar, but all of them died before pupation in dung from both treatment and control cattle. No significant difference in the feeding activities of *P. a. auratus* was found between control and treatment (fig. 5).

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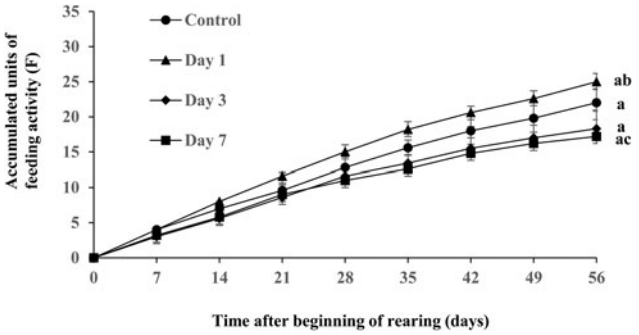

Fig. 3. Accumulated feeding activity (F) of *O. bivertex* in dung from control and treatments in experiment 2. Different letters show a significant difference between treatments (GLMM followed by Tukey's multiple comparisons: $P < 0.05$).

Table 3. Accumulated adult mortality, number of brood balls, and new adult emergence rate of *O. lenzii* in dung from control and treatment groups in experiment 2.

Day after treatment	Adult mortality (%)	No. of brood balls/replicate (mean ± SD)	Adult emergence rate (%) (mean ± SD)
Control	56.7 ± 25.3[a]	24.4 ± 16.4[a] (122)	72.9 ± 15.5[a]
Day 1	63.3 ± 13.9[a]	37.2 ± 15.4[b] (186)	7.6 ± 10.3[bc]
Day 3	60.0 ± 36.5[a]	19.8 ± 8.8[a] (93)	0.7 ± 1.6[c]
Day 7	76.7 ± 14.9[a]	18.6 ± 10.4[a] (99)	11.6 ± 14.8[b]
Day 14	60 ± 14.9[a]	21.4 ± 4.7[a] (107)	56.8 ± 11.5[a]

Each value is a mean of five replicates involving three pairs of male and female.
Value in parentheses is the total number of brood balls.
In each column, the means not associated with the same letter differ among control and treatments in experiment 2 (adult mortalities: log-rank test followed by Bonferroni method $P < 0.005$, numbers of brood balls and adult emergence rate: GLMM followed by Tukey's multiple comparisons $P < 0.05$).

Discussion

Ivermectin was excreted in dung for 2–4 weeks with peak concentration at 1 or 3 days post-treatment by pour-on formulation (Sommer & Nielsen, 1992; Herd *et al*., 1996; Dadour *et al*., 2000; Iwasa *et al*., 2005, 2007). The present results are similar to the previous studies, but there was a considerable difference in concentration at 7 days after treatment between the two trials: no detectable residues in experiment 1 and 0.07 ppm in experiment 2. The detection limit concentration of ivermectin in our study was 0.05 ppm, but a reduction of adult emergence rates of *C. acutidens* in dung collected at 7 and 14 days after treatment in experiment 1 suggests that an undetectable concentration of ivermectin may have been excreted in dung for at least 14 days after treatment.

Previous studies show that the adult survival of dung beetles of genera *Aphodius*, *Caccobius*, *Euoniticellus*, *Liatongus*, and *Onthophagus* are not affected by ivermectin residues in dung (Ridsdill-Smith, 1988; Fincher, 1992; Lumaret *et al*., 1993; Yamashita *et al*., 2004; Iwasa *et al*., 2005, 2007; Pérez-Cogollo *et al*., 2017). Similarly, in our results, there were no effects of ivermectin on adult survivals of *O. bivertex*, *O. lenzii*, and *P. auratus*. In contrast, ivermectin residues in cattle dung reduced the survival of newly emerged adults of *Copris hispanus* Linnaeus (Wardhaugh & Rodriguez-Menendez, 1988) and mature adults of *Copris ochus* Motschulsky and *C. acutidens* (Iwasa *et al*., 2007). In the present study, the mortality of mature adults of *C. acutidens* increased in dung collected at 3 and 7 days post-treatment, although dung collected at those dates had no effect on adult survival of *O. bivertex* and *O. lenzii*. It is suggested that adults of *Copris*, which are large dung beetles, have a higher drug sensitivity against ivermectin than those of other genera in Scarabaeidae. Ortiz *et al*. (2017) reported a new method for the quantitative determination of ivermectin in bodies of dung beetles which ingested the dung containing ivermectin. It is expected that the relationship between the body sizes of dung beetles and the amount of intake of ivermectin residues in contaminated dung will be investigated using the new analysis method.

It has been suggested that there are no effects of ivermectin present in cattle dung on the production of brood balls of small dung beetles belonging to the genera *Caccobius*, *Liatongus* (Iwasa *et al*., 2005, 2007), and *Euoniticellus* (Fincher, 1992). In the genus *Onthophagus* which comprises small to medium-sized dung beetles, no reduction of brood balls of *Digitonthophagus gazella* Fabricius (body length: 9–13 mm) was observed in dung from cattle treated by injection and pour-on formulation of ivermectin (Fincher, 1992; Sommer & Nielsen, 1992; Yamashita *et al*., 2004). In *O. binodis* Thunberg (body length: 10–13 mm), however, the numbers of brood balls were reduced for 4 weeks in dung from abamectin-treated cattle by injection (Ridsdill-Smith, 1988). In addition, Pérez-Cogollo *et al*. (2017) found that brood mass production by *Onthophagus landolti* Harold (body length: 5.0–5.5 mm) was almost completely suppressed in dung collected from ivermectin-treated cattle (ivermectin 1 and 3.15%) at 3, 6, and 14 days post-treatment. In the present study, the number of brood balls of *O. bivertex* decreased in dung collected at 3 and 7 days after treatment from ivermectin-treated cattle, but there was no adverse effect on those of *O. lenzii* although this species belongs to the same genus. Thus, it is suggested that the effects of ivermectin on the production of brood balls vary among the species even within the same genus (*Onthophagus*) regardless of the body size of adults.

Iwasa *et al*. (2007) reported that adults of *C. ochus* and *C. acutidens* produced no brood balls in dung collected at 1 day after ivermectin treatment, and Wardhaugh & Rodriguez-Menendez (1988) observed aberrant ovarian development of *Bubas bubalus* Oliver and reduction of brood balls of *C. hispanus* in dung from ivermectin-treated cattle. Correspondingly, our results show that the reproduction of *C. acutidens* was completely inhibited in dung collected at 3 days post-

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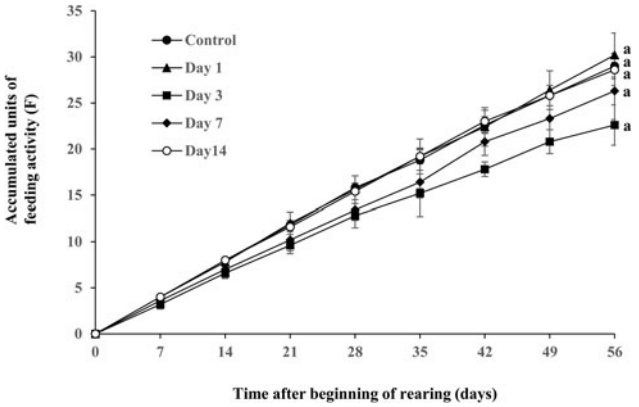

Fig. 4. Accumulated feeding activity (F) of *O. lenzii* in dung from control and treatments in experiment 2. Same letters show no significant difference between treatments (GLMM followed by Tukey's multiple comparisons: $P < 0.05$).

Table 4. Accumulated adult mortality, number of brood masses, and survival of third instar larvae rate of *P. a. auratus* in dung from control and ivermectin-treated cattle in experiment 1.

Day after treatment	Adult mortality (%) Days after the beginning of rearing 30	60	90	No. of brood masses/replicate (mean ± SD)	Survival of third instar larvae (%) (mean ± SD)
Control	0	0	5.0	3.0 ± 2.0 (15)[a]	70.0 ± 24.5[a]
Day 3	0	5.0	5.0	2.6 ± 1.1 (13)[a]	70.0 ± 29.8[a]

Each value is mean of five replicates involving two pairs of male and female.
Value in parentheses is the total number of brood masses.
In each column, the means not associated with the same letter differ among control and treatment in experiment 1 (adult mortalities: log-rank test followed by Bonferroni method $P < 0.05$, numbers of brood masses and survival of larvae: GLMM followed by Tukey's multiple comparisons $P < 0.05$).

treatment and was significantly reduced until 7 days post-treatment. Therefore, it is conceivable that the reproduction of the relativity large dung beetles belonging to the genus *Copris* are affected by ivermectin residues compared to other genera.

Adult emergence rates of *D. gazella* in dung from cattle treated with ivermectin by injection were reduced for 1–2 weeks after treatment (Fincher, 1992; Sommer & Nielsen, 1992). Ridsdill-Smith (1988) reported that larval survival of *O. binodis* was reduced in dung at 1–4 weeks after treatment with avermectin B1 by injection. In the present study, reduction periods of adult emergence rates differed between *O. bivertex* (1, 3 days post-treatment) and *O. lenzii* (1, 3, and 7 days post-treatment). Iwasa *et al*. (2007) suggested that the effective period of pour-on formulation of ivermectin on adult emergence rates of *C. jessoensis* (small beetle: 5–8 mm body length) was shorter (3–7 days after treatment) than for medium-sized dung beetles such as species of the genus *Onthophagus*. This present result agrees with Iwasa *et al*. (2007), suggesting that the effects of ivermectin on larval survival (adult emergence) are associated with the body size of dung beetles among the related genera and species.

Wardhaugh & Rodriguez-Menendez (1988) showed that the larval survival of *C. hispanus* was reduced in dung collected at 1 and 8 days after ivermectin-treatment by injection, and equivalent levels to the control were restored in dung collected at 16 days post-treatment. Our results revealed that the reduction of adult emergence rates of *C. acutidens* occurred in dung collected at 3, 7, and 14 days after treatment by the pour-on method of ivermectin. Although the concentrations of ivermectin in dung collected at 7 and 14 days post-treatment were less than the detection limit, adult emergence rates of *C. acutidens* decreased by 8.3% (7 days post-treatment) and 68.3% (14 days post-treatment) compared to control (table 1). Thus, it seems likely that the larvae of *C. acutidens* are sensitive to the undetectable concentration of ivermectin in dung.

Dung removal by *O. landolti* was decreased in dung spiked with ivermectin at 0.1, 1.0, and 10.0 mg kg^{-1} (Pérez-Cogollo *et al*., 2015). Wardhaugh & Rodriguez-Menendez (1988) reported that the feeding activity of *C. hispanus* was restrained in cattle dung treated with ivermectin by injection for 1–8 days post-treatment. Dung dispersal by *Onthophagus taurus* Schreber was suppressed in dung at 7 and 10 days after ivermectin-treatment by injection (Dadour *et al*., 1999). The present results show that the feeding activity of *C. acutidens* was suppressed in dung at 3 days post-treatment, same as the species mentioned above, although there were no adverse effects on those of *O. bivertex*, *O. lenzii*, and *P. auratus*. Martínez *et al*. (2016) reported that ivermectin residues in dung caused the cessation of yolk synthesis in oocytes and reduction of body fat reserves of *Euoniticellus intermedius* Reiche because activities of feeding and oviposition were inhibited. Ivermectin suppresses the neurotransmission produced by γ-aminobutyric acid and glutamate in invertebrates, resulting in somatic paralysis and death (Keane & Avery, 2003; Geary & Moreno, 2012). Indeed, muscle contraction of *Scarabaeus cicatricosus* Lucas decreased in dung containing ivermectin, resulting in a reduction of the locomotor capacity and foraging success (Verdú *et al*., 2015). It is assumed that the remarkable reduction of adult survival and brood ball construction of *C. acutidens* in dung at 3 days post-treatment in our study can also be attributed to the antifeedant and paralysis affected by ivermectin.

Regarding the effects of ivermectin on Geotrupidae species, Lumaret (1996) showed that ivermectin residues in horse dung have no effect on adult survival of *Anoplotrupes stercorosus* Hartmann. The results presented here show that

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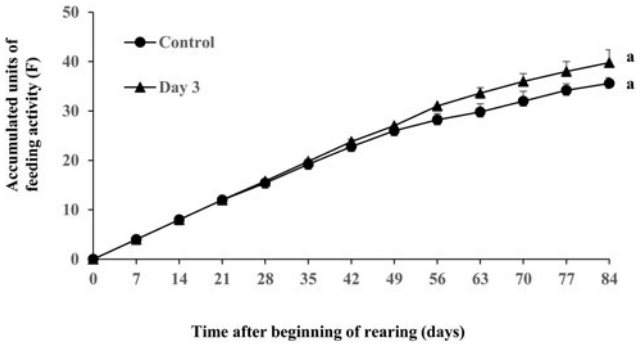

Fig. 5. Accumulated feeding activity (F) of *P. a. auratus* per replicates in dung from control and ivermectin-treated cattle in experiment 1. Same letters show no significant difference between treatments (GLMM followed by Tukey's multiple comparisons: $P < 0.05$).

there were no effects of ivermectin on adult survival and brood mass construction of *P. a. auratus*, although the adults fed a large amount of dung residual in the maximum concentration of ivermectin (3 days after treatment). Geotrupid adult beetles have mandibulate type of mouth parts, and their food habit is likely to differ from absorption type of scarabid beetles. Actually, adults of *Thorectes lusitanicus* Jekel (Geotrupidae) feed on oak acorn, semi-dry and dry dung (Verdú *et al.*, 2010), showing that they feed on nondecomposed plant material. Hence, it is assumed that the lower effects of ivermectin on adults of *P. auratus* than on other large species such as *Copris* (Scarabaeidae: filter-feeder) are also associated with a difference of feeding habits. Elucidation of the tolerance mechanism in dung beetles to ivermectin is needed in relation to their feeding habits in both adults and larvae.

Evaluations for the effects of ivermectin on dung beetles which have been studied all over the world are focused on common species which are small to medium sized (Floate *et al.*, 2005), and there are few investigations on rare species facing extinction. McCracken (1993) suggested that the rare species of dung beetles in the genera of *Copris* and *Onthophagus* are put at risk by use of avermectin owing to low recolonization potential. Moreover, the decrease of *C. ochus* and *C. acutidens*, which are listed as rare or endangered species in many prefectures of Japan, can be partly attributed to the residues of ivermectin in dung (Iwasa *et al.*, 2007). *O. bivertex* was also designated as an endangered species in some prefectures of Japan.

From our results that brood ball constructions and larval survival of *C. acutidens* and *O. bivertex* were inhibited by ivermectin residues, it is suggested that these two species have a higher drug sensitivity against ivermectin than common species such as *O. lenzii* and *P. auratus*. In particular, dung beetles of *Copris* construct fewer brood balls (2–10) per female than those of other dung beetles such as *Onthophagus*, and they stay in their nests for a long period to take care of their offspring in the breeding season. Frequent use of anthelmintics such as ivermectin in the breeding season could possibly cause a serious impact on the population of *Copris* species. Moreover, it is known that paracoprid species (such as *Copris* and *Onthophagus*) have an ability to bury a large amount of dung per day (Bang *et al.*, 2005). Further investigation is necessary to clarify the association between inhibition of activities of dung beetles (feeding and reproduction) by ivermectin and dung degradation, involving the body size of dung beetles.

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References

Adler, N., Bachmann, J., Blanckenhorn, W.U., Floate, K.D., Jensen, J. & Römbke, J. (2016) Effects of ivermectin application on the diversity and function of dung and soil fauna: regulatory and scientific background information. *Environmental Toxicology and Chemistry* **35**, 1914–1923.

Bang, H.S., Lee, J.H., Kwon, O.S., Na, Y.E., Jang, Y.S. & Kim, W. H. (2005) Effects of paracoprid dung beetles (Coleoptera: Scarabaeidae) on the growth of pasture herbage and on the underlying soil. *Applied Soil Ecology* **29**, 165–171.

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.

Byford, R.L., Craig, M.E. & Crosby, B.L. (1992) A review of ectoparacites and their effect on cattle production. *Journal of Animal Science* **70**, 597–602.

Campbell, W.C., Fisher, M.H., Stapley, E.O., Albers-Schönberg, G. & Jacob, T.A. (1983) Ivermectin: a potent new antiparasitic agent. *Science* **221**, 823–828.

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.

Dadour, I.R., Cook, D. & Hennessy, D. (2000) Reproduction and survival of the dung beetle *Onthophagus binodis* (Coleoptera: Scarabaeidae) exposed to abamectin and doramectin residues in cattle dung. *Environmental Entomology* **29**, 1116–1122.

https://doi.org/10.1017/S0007485319000385 Published online by Cambridge University Press

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. (1998) Off-target effects of ivermectin on insects and on degradation in southern Alberta, Canada. *Bulletin of Entomological Research* **88**, 25–35.

Floate, K.D., Spooner, R.W. & Colwell, D.D. (2001) Larvicidal activity of endectocides against pest flies in the dung of treated cattle. *Medical and Veterinary Entomology* **15**, 117–120.

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.

Geary, T.G. & Moreno, Y. (2012) Macrocyclic lactone anthelmintics: spectrum of activity and mechanism of action. *Current Pharmaceutical Biotechnology* **13**, 866–872.

Hayakawa, H. (1981) A pasture-cleaning operation by means of dung beetles. *The Insectarium* **18**, 14–23.

Herd, R.P., Sams, R.A. & Ashcraft, S.M. (1996) Persistence of ivermectin in plasma and faeces following treatment of cows with ivermectin sustained-release, pour-on or injectable formulations. *International Journal for Parasitology* **26**, 1087–1093.

Holter, P. & Sommer, C. (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.

Houlding, B., Ridsdill-Smith, T.J. & Bailey, W.J. (1991) Injectable abamectin causes a delay in scarabaeine dung beetle egg-laying in cattle dung. *Australian Veterinary Journal* **68**, 185–186.

Imura, O. (2007) Diversity and functions of dung beetles in pasture. *Japanese Society of Grassland Science* **53**, 47–51.

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.

Kawai, S., Hori, S., Kawahara, M. & Inagaki, M. (2005) Atlas of Japanese Scarabaeidae Vol. 1 coprophagous group. p. 189 *in* the Japanese society of Scarabaeoideans (*Ed*.) Roppon-Ashi Entomological Books, Tokyo.

Keane, J. & Avery, L. (2003) Mechanosensory inputs influence *Caenorhabditis elegans* pharyngeal activity via ivermectin sensitivity genes. *Genetics* **164**, 153–162.

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, Ecosystems, & Environment* **61**, 123–131.

Krüger, K. & Scholtz, C.H. (1998*a*) Changes in the structure of dung insect communities after ivermectin usage in a grassland ecosystem. I. Impact of ivermectin under drought conditions. *Acta Oecologica* **19**, 425–438.

Krüger, K. & Scholtz, C.H. (1998*b*) Changes in the structure of dung insect communities after ivermectin usage in a grassland ecosystem. II. Impact of ivermectin under high rainfall. *Acta Oecologica* **19**, 439–451.

Lumaret, J.P. (1996). Comparative effects of moxidectin 2% equine gel and ivermectin 1.87% equine gel on dung non-target fauna when used at the recommended dose in horse. American Cyanamid Company, Princeton, NJ. Report No. GASD. Cyanamid Websters Pty Ltd, Baulkham Hills, NSW (unpublished).

Lumaret, J.P., Galante, E., Lumbreras, 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., Floate, K., Römbke, J. & Wardhaugh, K (2012) A review on the toxicity and non-target effects of macrocyclic lactones in terrestrial and aquatic environment. *Current Pharmaceutical Biotechnology* **13**, 1004–1060.

Madsen, M., Nielsen, B.O., Holter, P., Pedersen, O.C., Jespersen, J.B., Jensen, K.M.V., Nansen, P. & Grønvold, J. (1990) Treating cattle with ivermectin: effects on the fauna and decomposition of dung pats. *Journal of Applied Ecology* **27**, 1–15.

Manning, P., Slade, E.M., Beynon, S.A. & Lewis, O.T. (2016) Functionally rich dung beetle assemblages are required to provide multiple ecosystem services. *Agriculture, Ecosystems & Environment* **218**, 87–94.

Manning, P., Slade, E.M., Beynon, S.A. & Lewis, O.T. (2017) Effect of dung beetle species richness and chemical perturbation on multiple ecosystem functions. *Ecological Entomology* **42**, 577–586.

Martínez, M.I., Lumaret, J.P., Ortiz Zayas, R. & Kadiri, N. (2016) The effects of sublethal and lethal doses of ivermectin on the reproductive physiology and larval development of the dung beetle *Euoniticellus intermedius* (Coleoptera: Scarabaeidae). *The Canadian Entomologist* **149**, 461–472.

McCraken, D.I. (1993) The potential for avermectins to affect wildlife. *Veterinary Parasitology* **48**, 273–280.

Miller, J.A., Kunz, S.E., Oehler, D.D. & Miller, R.W. (1981) Larvicidal activity of Merck 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., Amezquita, S. & Favila, M.E. & Scarabaeinae Research Network (2008) Ecological functions and ecosystem services provided by Scarabaeinae dung beetle. *Biological Conservation* **141**, 1461–1474.

Ortiz, A.J., Cortez, V., Azzouz, A. & Verdú, J.R. (2017) Isolation and determination of ivermectin in post-mortem and in vivo tissues of dung beetles using a continuous solid phase extraction method followed by LC-ESI+-MS/MS. *PLoS One* **12**, e0172202. doi: 10.1371/journal.pone.0172202.

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.

Pérez-Cogollo, L.C., Rodríguez-Vivas, R.I., Reyes-Novelo, E., Delfín-González, H. & Muñoz-Rodríguez, D. (2017) Survival and reproduction of *Onthophagus landolti*

https://doi.org/10.1017/S0007485319000385 Published online by Cambridge University Press

(Coleoptera: Scarabaeidae) exposed to ivermectin residues in cattle dung. *Bulletin of Entomological Research* **107**, 118–125.

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.

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.

Römbke, J., Coors, A., Fernández, Á.A., Förster, B., Fernández, C., Jensen, J., Lumaret, J.P., Cots, M.Á.P. & Liebig, M. (2010) Effects of the parasiticide ivermectin on the structure and function of dung and soil invertebrate communities in the field (Madrid, Spain). *Applied Soil Ecology* **45**, 284–292.

Schröder, J. (1992) Chemical control of ticks on cattle. pp. 175–184 *in* Fivaz, B., Petnoy, T. & Horak, I. (*Eds*), *Tick Vector Biology*. Berlin, Heidelberg, Springer.

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., Grønvold, J., Holter, P., Madsen, M. & Nansen, P. (1993) Dung burial activity and development of ivermectin exposed *Diastellopalpus quinquedens* in a field experiment. *Entomologia Experimentalis et Applicata* **66**, 83–89.

Steelman, C.D. (1976) Effects of external and internal arthropod parasites on domestic livestock production. *Annual Review of Entomology* **21**, 155–178.

Verdú, J.R., Casas, J.L., Lobo, J.M. & Numa, C. (2010) Dung beetles eat acorn to increase their ovarian development and thermal tolerance. *PLoS One* **5**, e10114. https://doi.org/10.1371/journal.pone.0010114.

Verdú, J.R., Cortez, V., Ortiz, A.J., González-Rodríguez, E., Martinez-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**, 13912. https://doi.org/10.1038/srep13912.

Verdú, J.R., Lobo, J.M., Sánchez-Piñero, F., Gallego, B., Numa, C., Lumaret, J.P., Cortez, V., Ortiz, A.J., Tonelli, M., García-Teba, J.P., Rey, A., Rodríguez, A. & Durán, J. (2018) Ivermectin residues disrupt dung beetle diversity, soil properties and ecosystem functioning: an interdisciplinary field study. *Science of the Total Environment* **618**, 219–228.

Wall, R. & Strong, L. (1987) Environmental consequence of treating cattle with the antiparasitic drug ivermectin. *Nature* **327**, 418–421.

Wardhaugh, K.G. & Rodriguez-Menendez, 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 beetles, *Copris hispanus* L., *Bubas bubalus* (Oliver) and *Onitis belial* F. *Journal of Applied Entomology* **106**, 381–389.

Wohde, M., Blanckenhorn, W.U., Floate, K.D., Lahr, J., Lumaret, J.-P., Römbke, J., Scheffczyk, A., Tixier, T. & Düring, R.A. (2016). Analysis and dissipation of the antiparasitic agent ivermectin in cattle dung under different field conditions. *Environmental Toxicology and Chemistry* **35**, 1924–1933.

Yamashita, N., Yoshida, N., Watanabe, A. & Mikami, A. (2004) Effects of the anthelmintics on development of the dung degrading insects. *Tohoku Agricultural Research* **57**, 119–120 (in Japanese).

https://doi.org/10.1017/S0007485319000385 Published online by Cambridge University Press