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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

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Abstract

Effects of the antiparasitic drug, ivermectin, on the dung beetles, *Caccobius jessoensis* Harold, 1867 and the rare species, *Copris ochus* Motschulsky, 1860 and *Copris acutidens* Motschulsky, 1860 were studied in laboratory and field experiments in Hokkaido, Japan. Ivermectin was detected in dung from 1 to 21 or 28 days following treatment, with a peak on the first day after treatment in two pour-on administrations (500 μg kg^{-1}), although there were considerable differences between the two peaks. In *C. jessoensis*, brood balls constructed by the female were not reduced in the dung of treated cattle except for seven days after treatment in experiment 2. Also, there was no significant difference in the mean weight of brood balls between dung from treated and control cattle. However, the emergence rates were significantly reduced in dung 1–3 days after treatment. In the field study, brood balls constructed by *C. jessoensis* were more abundant in dung from treated cattle in experiment 1, but adult emergence was significantly reduced at one and seven days after treatments. Adult mortality of *C. ochus* Motschulsky at 90 days after the beginning of rearing was 11.1% in dung from control cattle with 22 brood balls constructed, whereas it was 84% in dung from treated cattle with no brood balls and/or ovipositioning. Also, in *C. acutidens* Motschulsky, adult mortality at 90 days after the beginning of rearing was 3.6% in dung from control cattle with 13 brood balls constructed, whereas it was 94.1% in dung from treated cattle with no brood balls or ovipositioning. The environmental risk in the use of ivermectin during breeding period of dung beetles in pasture is discussed.

Introduction

Ivermectin, a member of the avermectin family of drugs derived from an actinomycete isolated from soil in Japan, is a broad-spectrum parasiticide against nematode and arthropod parasites of domestic livestock (Miller *et al.*, 1981; Benz, 1985; Drummond, 1985). Regardless of the formulations, cattle do not metabolize ivermectin, so most of it is excreted in the faeces of treated cattle (Campbell *et al.*, 1983) and remains in the faeces to act against dung-breeding flies of economic importance. However, ivermectin residues in dung are not selective and kill non-target beneficial insects, which would otherwise accelerate degradation of the dung (Wall & Strong, 1987). The elimination of dung-decomposing insects, which help to return nutrients

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to the soil, may lead to important consequences in pasture ecosystems, as undegraded dung pats increase, fouling the grassland and reducing available grazing area.

Extensive works on the effects of this drug on dung-breeding insects have been done by Strong & Wall (1988, 1994), Roncalli (1989), Madsen *et al*. (1990), Fincher (1992), Sommer & Nielsen (1992), Sommer *et al*. (1992), Strong (1992, 1993), Lumaret *et al*. (1993), Floate (1998a) and Floate *et al*. (2002). A recent review was published by Floate *et al*. (2005).

Since 1996, with the spread of pour-on formulation, ivermectin has been used widely in Japan to control parasites in cattle. Iwasa *et al*. (2005a,b) reported on the effects of ivermectin on dung-breeding flies and beetles (*Liatongus minutus* Motschulsky, 1860) in Hokkaido, but further surveys involving other dung beetles are needed.

In Japan, especially, the number of dung beetles in pastures are rapidly decreasing and currently 43 species have been designated as 'rare' or 'endangered' species by the Ministry of Environment or Prefectural Governor (Tsukamoto, 2003).

The purpose of this study is to evaluate the effects of dung voided by ivermectin-treated cattle on the reproduction and survival of dung beetles, *Caccobius jessoensis* Harold, 1867, *Copris ochus* Motschulsky, 1860 and *Copris acutidens* Motschulsky, 1860; the latter are listed as 'rare' or 'endangered' species in Japan.

Materials and methods

Cattle and dung collection

The experiments were carried out at a pasture of the Obihiro University of Agriculture and Veterinary Medicine. In experiment 1, four Holsteins (dry cows, aged 43–60 months) were selected and ivermectin (Ivomec® Topical, Merial Limited) was topically applied on 30 May 2004 using pour-on formulation at the recommended dose of $500\,\mu g\,kg^{-1}$ of body weight. Four Holsteins (lactating cows, aged 40–72 months) were used as controls. In experiment 2, three Holsteins (dry cows, aged 44–60 months) and one Japanese black cow (breeding cow, aged 71 months) were selected, and ivermectin was applied in the same way on 10 July 2004. Three Holsteins (dry cows, aged 42–70 months) and one Japanese black cow (breeding cow, aged 67 months) were used as controls. The cows fed primarily on timothy and orchard grass in a pasture; however, in experiment 1, the treated-cows (dry) and control cows (lactating) were given 2 and 6 kg of commercial concentrate (corn and soybean grain) per cow per day, respectively. In experiment 2, both the treated and control cows were given 2 kg of commercial concentrate per cow per day. The cows were grazed separately in a pasture in experiment 1; but, in experiment 2, they grazed together in the pasture. None of the cows had been treated previously with anthelmintics or insecticides. Dung pats were collected immediately after defecation at 1, 3, 7, 14, 21, 28 and 35 days following treatment. In each collection, dung pats from four cows in each group were mixed and frozen at $-20°C$ until used.

Determination of ivermectin concentrations

The concentration of ivermectin in the faeces was determined using high-performance liquid chromatography (HPLC) following the method of Payne *et al*. (1995) with some modifications: on each sample day, only one faecal sample from a dung pat was analysed; the extraction was carried out with homogenization (PHYSCOTRON, Microtec Co., Ltd) at 10,000 rpm for 1 min (instead of sonication); and avermectin B1 was added as an internal standard.

The effects of ivermectin on reproduction and the emergence of Caccobius jessoensis *Harold*

Laboratory bioassays. *C. jessoensis* is a small but important dung-burying beetle, especially in Hokkaido, Japan. In the spring, male-female pairs cooperate to make dung brood balls beneath the dung, and eggs are laid inside the brood balls. Hatched larvae feed on the dung and grow to pupae in the summer. Parental adults do not remain for the purpose of brood-caring in the same dung pat. The new generation emerges in the autumn and overwinters as an adult. Adults tested were collected from the pasture of the Obihiro University of Agriculture and Veterinary Medicine. One 90 g faecal sample from both the treatment and control groups in experiments 1 and 2 was placed on volcanic ash-soil (15 cm in depth) in a container (12 cm diameter × 18 cm deep). Three male-female pairs were placed on the faeces in each container, which was covered with gauze and maintained at 22°C (16L-8D). Faeces was replaced with fresh samples once per week for 6–8 weeks; and, at that time, volcanic ash-soil from each container was sieved to collect any brood balls that were produced. These brood balls were placed in a plastic cup (8 cm diameter × 4 cm deep) containing andosol and were kept at 22°C (16L-8D) until adult emergence, 1–2 months later. Rearing tests for the treatment and control cattle were replicated 4–8 times for each dung collection date.

Field experiment. Twenty-four artificial dung pats (700 g, three pats each per collection date for the treated and control cows) for 1, 7, 14 and 21 days post-treatment were deposited each on a 17-cm layer of andosol in a plastic box (33 × 33 × 19 cm). Boxes were then placed in a field area adjacent to the pasture on 3 July, for experiment 1, and on 7 August, for experiment 2, for seven days for exposure to insect activity. One month after exposure, the brood balls constructed by the dung beetles were collected and examined. Brood balls obtained were kept at 22°C (16L-8D) until the adults emerged.

The effects of ivermectin on adult mortality and reproduction of Copris ochus *Motschulsky and* C. acutidens *Motschulsky*

C. ochus is a large dung-burying beetle, which is distributed throughout Japan, Korea and China. In spring and summer in Japan, male-female pairs of *C. ochus* feed on dung and cooperate in excavating an underground chamber, which is provisioned with a mass of dung; and adults remain a long time in the same dung pat for the purpose of brood-caring. This species may, over the course of the winter, become either adults or mature larvae (Bang *et al*., 2004). We tested adults collected in the Hidaka subprefecture of Hokkaido in July and August. One 100 g faecal sample (one-day dung after treatment in experiment 2) from each treatment and control group was placed on the volcanic ash-soil (30 cm deep) in a container (30-cm diameter × 40 cm deep). One male-female pair was placed on the faeces in each container, which was covered with gauze, and maintained at room temperature (22–28°C). In total, 12 pairs plus three females (when males were not available) and 11 pairs plus three females were tested in the control and treatment groups, respectively.

C. acutidens is a medium-sized dung-burying beetle, which is also distributed throughout Japan and adjacent countries in East Asia. In spring (June), overwintering male-female pairs also cooperated in the construction of a brood chamber that is provisioned with fresh dung for oviposition. Adults remain and spend time in the same dung pat to care for the brood-balls from spring to summer. In autumn (September), a new generation emerges as adults and hibernates over the course of the winter. Adults tested were collected in the Yamanashi Prefecture of Honshu in June and September. A 50 g faecal sample (one-day dung after treatment in experiment 2) from each treatment and control group was placed on volcanic ash-soil (15 cm deep) in a container (12-cm diameter × 18 cm deep). One male-female pair was placed on the faeces in each container, which was covered with gauze and maintained at the same room temperature as *C. ochus*. Faeces were replaced with fresh samples once per week for up to 90 days; and, at the same time, ash-soil from each container was examined to collect any brood balls produced. In total, 12 pairs plus four females and 15 pairs plus four females were tested in the control and treatment groups, respectively.

Data analysis

Differences of values between the control and treatment groups were analysed by the non-parametric Mann-Whitney *U* test. Fisher's PLSD comparisons yielded differences of values among the dates of dung collection.

Results

Determination of ivermectin concentration

Ivermectin residues attained maximal concentrations at 1 day after treatment in both administrations, but their concentrations were greatly different 1 and 3 days after treatment (fig. 1). Both concentration peaks declined to a similar level 7 days after treatment, and ivermectin was detected for up to 21 (experiment 1) or 28 days (experiment 2). No ivermectin was detected at 28 days (experiment 1) and 35 days (experiment 2) after treatments; the determination limit was 0.002 ppm. In the control cattle, no ivermectin was detected in experiment 1, but in experiment 2 some low concentrations were detected 1 day (0.036 ppm), 3 days (0.026 ppm) and 7 days (0.01 ppm) after treatment.

The effects of ivermectin on adult survival, brood ball production and emergence of C. jessoensis *Harold*

Laboratory bioassay. In experiments 1 and 2, there appeared to be no effects of ivermectin on adult mortality of *C. jessoensis* (data not shown). In experiment 1, the number

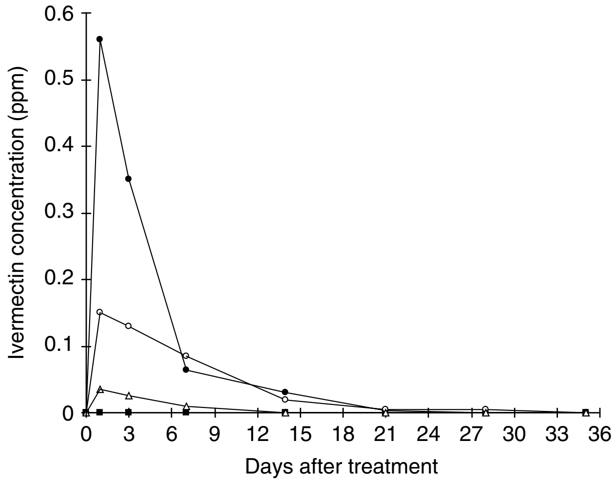

Fig. 1. Ivermectin concentration in dung (ppm of wet weight) after treatments (500 μg kg^{-1}) (–●–, treatment (exp.1); –○–, treatment (exp.2); –■–, control (exp.1); –△–, control (exp. 2)).

of brood balls constructed per female by *C. jessoensis* were significantly more abundant in ivermectin-treated cows 1 and 7 days after treatment (table 1). Conversely, numbers of brood balls were significantly lower 7 days after treatment in experiment 2. No significant difference in the numbers of brood balls was found on other dates between control and treated cows. There were also no differences between control and treated cows regarding the weight of brood balls in each experiment. However, adult emergence rates were severely reduced or null at 1 and 3 days ($P < 0.01$) after treatment in both experiments with 62.0–77.8% recovery at 7 days and nearly complete recovery at 14 days. Some emergences 3 days after treatment in experiment 2 may be attributed to the lower concentration of ivermectin excreted in dung for experiment 2 than experiment 1. The average number of F1 adult offspring per female was low 1 and 3 days after treatment in both experiments, in agreement with the emergence rates (table 1).

Field experiment. Data for brood balls collected from soil beneath the dung pats after one week exposure are shown in table 2. The brood balls of dung beetles, *Caccobius jessoensis* Harold and *Liatongus minutus* (Motsculsky), were obtained; however, as there were very few of the latter species, only data of *C. jessoensis* were shown. In experiment 1, the number of brood balls constructed by *C. jessoensis* was 184; these were more abundant on all collection dates from treated cattle than from those of the control cows. Adult emergence rates from the dung of the treated cattle were null at 1 day and 5.8% at 7 days after treatment; these results were significantly different from those of 14 and 21 days after treatment (32.6% and 23.1%, respectively). In experiment 2, brood balls amounted to only a small number in both control (9) and treatment (11) cows, and no significant difference was found between them. Adult emergence rates from the dung of treated cattle were significantly reduced at 1 and 7 days after treatment.

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Table 1. Numbers and weights of brood balls, emergence rates and F1 adult offspring/female in *Caccobius jessoensis* reared in dung from treated and control cattle in experiments 1 and 2.

Days after treatment	Experiment 1		Experiment 2	
	Control	Treatment	Control	Treatment
	No. of brood balls/female		No. of brood balls/female	
1	1.10 ± 0.67a (22)	3.27 ± 0.65a* (49)	1.38 ± 0.43a (29)	2.42 ± 0.78a (26)
3	1.46 ± 0.60a (19)	1.36 ± 1.04a (26)	1.10 ± 0.55a (23)	0.93 ± 0.23b (25)
7	1.82 ± 0.80a (31)	4.17 ± 1.36a* (50)	2.42 ± 0.79a (29)	0.79 ± 0.19b* (27)
14	1.56 ± 0.37a (28)	3.67 ± 0.66a (22)	1.56 ± 0.27a (64)	1.73 ± 0.27ab (33)
	Weight of brood ball (g)		Weight of brood ball (g)	
1	1.3 ± 0.1a	1.3 ± 0.1a	0.97 ± 0.05a	1.10 ± 0.04a
3	1.2 ± 0.1a	1.6 ± 0.1a	0.90 ± 0.04a	0.84 ± 0.05a
7	1.6 ± 0.2a	2.3 ± 0.4a	0.91 ± 0.03a	1.05 ± 0.06a
14	1.0 ± 0.05a	1.0 ± 0.05a	1.03 ± 0.03a	0.93 ± 0.05a
	Emergence rate (%)		Emergence rate (%)	
1	95.5 ± 0.05a	0a**	100 ± 0a	0a*
3	63.2 ± 0.18b	0a**	91.3 ± 0.08ab	24.0 ± 0.12a**
7	80.7 ± 0.09a	62.0 ± 0.12b	82.2 ± 0.18b	77.8 ± 0.14b
14	92.9 ± 0.06a	95.5 ± 0.06b	93.8 ± 0.02a	90.9 ± 0.04b
	F1 adult offspring/female		F1 adult offspring/female	
1	1.05	0	1.38	0
3	0.92	0	1.00	0.22
7	1.04	2.60	2.01	0.61
14	1.45	3.55	1.51	1.57

Values in parentheses are total numbers of brood balls.
*, ** Significantly different between control and treatment within the rows in each experiment (*$P < 0.05$, **$P < 0.01$; Mann-Whitney *U* test).
Values followed by the same letters within the columns are not significantly different (Fisher's PLSD; $P < 0.05$).

Table 2. Emergence rates and numbers of brood balls of *Caccobius jessoensis* recovered from soil beneath dung pats of control and treated cattle after seven days exposure in the field.

Days after treatment	Experiment 1		Experiment 2	
	Control	Treatment	Control	Treatment
	No. of brood balls ± SD/pat		No. of brood balls ± SD/pat	
1	1.33 ± 0.3 (4)	21.3 ± 1.9*(64)	1.67 ± 0.3 (5)	0.67 ± 0.7 (2)
7	0 (0)	11.3 ± 2.4*(34)	1.33 ± 0.3 (4)	3.0 ± 0.6 (9)
14	1.33 ± 0.3 (4)	14.3 ± 2.3*(43)	0 (0)	0 (0)
21	0 (0)	11.7 ± 0.9*(35)	0 (0)	0 (0)
	Emergence rate (%) ± SD		Emergence rate (%) ± SD	
1	50 ± 0.3	0a	60 ± 0.17	0*
7	–	5.8 ± 0.03a	100	44.4 ± 0.6*
14	50 ± 0.3	32.6 ± 0.4b	–	–
21	–	23.1 ± 1.5b	–	–

Values in parentheses are total numbers of brood balls.
* Significantly different between control and treatment within the rows in each experiment ($P < 0.05$; Mann-Whitney *U* test).
Values followed by the same letters within the collumns are not significantly different ($P < 0.05$; Mann-Whitney *U* test).

The effects of ivermectin on adult survival and brood ball production of Copris ochus *Motschulsky and* Copris acutidens *Motschulsky*

Table 3 shows mortality and brood ball numbers for adult *C. ochus* emerging from the dung of the control and treated cows. From the dung of treated cattle, 12% and 40% of the adults emerged from tunnels and died 30 and 60 days, respectively, after rearing began. Upon examination of the nests after 90 days, accumulated adult mortality was very high, reaching 84%; no brood balls were constructed in the nests. Alternatively, in the dung of the control cattle, there were no dead adults emerged from the tunnels at 30 and 60 days after the start of rearing, and accumulated adult mortality was only 11.1% after 90 days. Twelve pairs plus three females in the control dung produced 22 brood balls. For *C. acutidens*, in the dung of treated-cattle, 17.6% and 64.7% of the adults emerged from tunnels and died at 30 and 60 days, respectively, after the start of rearing (table 3). Adult mortality after 90 days was very high (94.1%) without brood

Table 3. Accumulated adult mortality (%) and numbers of brood balls of *Copris ochus* and *C. acutidens* reared in dung from control and treated cattle.

Species		No. of adults	Mortality (%) Days after beginning of rearing 30	60	90	No. of brood balls
C. ochus	Control	27 (♀15, ♂12)	0	0	11.1	22
	Treatment	25 (♀14, ♂11)	12†	40†	84*	0
C. acutidens	Control	28 (♀16, ♂12)	3.6†	3.6†	3.6	13
	Treatment	34 (♀19, ♂15)	17.6†	64.7†	94.1*	0

† Percentages are based on numbers of dead adults found on surface of soil in rearing cages.
* Significantly different between control and treatment in each experiment ($P < 0.05$; Mann-Whitney U test).

ball production. In dung from the control cattle, the accumulated adult mortality was 3.6% from 30 to 90 days after rearing had begun. Twelve pairs plus four females constructed 13 brood balls.

Discussion

Ivermectin by pour-on formulation was excreted in dung for at least 14 to 28 days after treatment with a peak at the first or third day post-treatment (Sommer *et al*., 1992; Iwasa *et al*., 2005a,b). The present results are similar to those reported in previous papers, but there was considerable difference of concentrations at 1 and 3 days after treatment in the two experiments. It is known that the concentration of ivermectin in faeces can be affected by the diet of cattle (Cook *et al*., 1996). Although the reason for the difference in concentrations between the two experiments is unclear, concentrations may have varied with diet or physiological differences of the cattle. Some low concentrations, at 1 day (0.036 ppm), 3 days (0.026 ppm) and 7 days (0.010 ppm) in the control group of experiment 2, are due to contamination in the dung collection or to self-contamination through mutual grooming of the two groups in the same pasture, as shown by Barber & Alvinerie (2003); these concentrations did not affect adult emergence. High concentrations of ivermectin 1 and 3 days after treatment reflect a severe reduction of adult emergence of *C. jessoensis* on the same dates (fig.1, table 1).

In *Onthophagus binodis* Thunberg, dung from cattle treated with abamectin by injection caused a reduction in the mean number of brood balls up to four weeks (Ridsdill-Smith, 1988) and 34 days (Dadour *et al*., 2000) after treatment. On the other hand, there were no significant differences in the mean numbers of brood balls produced by *Onthophagus gazella* (Fabricius) (Fincher, 1992; Yamashita *et al*., 2004) and *Euoniticellus intermedius* (Reiche) (Fincher, 1992) in dung from ivermectin-treated cattle for both injection and pour-on formulations. The present results of brood ball construction and weight of brood balls in experiments 1 and 2 show that brood ball construction in *C. jessoensis* is also not affected by pour-on treatment of the recommended dose of ivermectin. In experiment 1, a significant increase in numbers of brood balls at 1 and 7 days post-treatment may be influenced by differences of diets as mentioned below. In either case, it is probable that the effects of ivermectin on brood ball construction are considerably different between species.

It is known that some dung beetles are attracted to ivermectin-containing dung pats, but ivermectin alone is not attractive to dung beetles (Wardhaugh & Mahon, 1991; Holter *et al*., 1993; Floate, 1998b). In field experiment 1, brood balls found in the soil beneath dung pats after 7 days exposure were more abundant in the dung of treated cattle than in that of the control cattle (table 2). However, it is unclear whether *C. jessoensis* had a preference for dung from ivermectin-treated cattle over that of the control cattle, because there were some differences in the amount of concentrate diets (corn and soybean grain) given between treated (dry) and control (lactating) cattle in experiment 1. In experiment 2, in which there was no difference in the amount of concentrate diet between treated and control cattle, it is difficult to compare the effects of ivermectin residues on the attraction and colonization of dung beetles because of the small number of brood balls constructed. The small numbers of brood balls in experiment 2 (middle of July) may be due to the short breeding period of *C. jessoensis*. Floate *et al*. (2005) emphasized that factors such as animal diet or dung moisture are more important on the effects of ivermectin on the attraction and colonization of dung-dwelling insects. Further investigation is needed.

In *Onthophagus binodis*, the larval survival was significantly prevented up to one week (Ridsdill-Smith, 1988), and adult emergence was greatly reduced up to 3–6 days (Dadour *et al*., 2000) after treatment in dung from cattle treated with abamectin by injection. Fincher (1992) reported that the emergence of adult *Onthophagus gazella* and *Euoniticellus intermedius* in the dung from steers treated with ivermectin by injection was reduced for two and one weeks after treatment, respectively. Krüger & Scholtz (1997) noted that adult emergence of two dung beetles, *Euoniticellus intermedius* and *Onitis alexis* Klug, were reduced in the dung of steers treated with ivermectin by injection for 2–14 days and 2–7 days after treatment, respectively. In pour-on formulation, emergence of adults in dung from cattle treated with ivermectin was reduced for 1–2 weeks in *Euoniticellus intermedius* (Fincher, 1996), 2–3 weeks in *Onthophagus gazella* (Fincher, 1996) and two weeks in *Liatongus minutus* (Iwasa *et al*., 2005b). At present, however, there is no report on the effect of ivermectin on larvae of the genus *Caccobius*. The present results of laboratory and field experiments show that pour-on formulation of the recommended dose of ivermectin could greatly reduce adult emergence of *C. jessoensis* for at least 3–7 days, returning to normal 14 days after treatment. The effect of the pour-on formulation of ivermectin on *C. jessoensis*, which is a small species (5–8 mm body length), is short-term in comparison with those of medium-sized dung beetles, mentioned above. Further surveys would be needed to corroborate the relationships of body size of dung beetles and the effect of ivermectin (if any) on emergence reduction.

Ivermectin and abamectin residues in dung have no effect on adult mortality in sexually mature dung beetles *Onthophagus binodis* and *Euoniticellus intermedius* (Ridsdill-Smith, 1988; Fincher, 1992) and *Bubas bubalus* (Oliver) (Wardhaugh & Rodriguez-Menendez, 1988), whereas sexually immature adults of the dung beetle, *O. binodis*, exhibited some mortality in abamectin residues in dung (Houlding *et al*., 1991; Dadour *et al*., 2000). Wardhaugh & Rodriguez-Menendez (1988) showed that ivermectin residues in dung caused a high mortality of newly emerged adult *Copris hispanus* (Fabricius), although no mortality was found among sexually matured adults. This high mortality in newly-emerged adults might be due to an accumulation of a great amount of dung ingested. Actually, it is known that adult dung beetles generally emerge sexually immature and then go through a period of intense feeding (Halffter & Edmonds, 1982). Bang *et al*. (2004) showed that, in spring (June and July), adults of *C. ochus* were overwintering beetles and the emergence of a new generation commenced in late summer (August) in Korea and that both undergo a period of intensive feeding before oviposition; whereas, it is known that, in spring (June), adults of *C. acutidens* are also overwintering beetles and a new generation of beetles emerge in autumn (September). Adults of *C. ochus* and *C. acutidens* tested were collected in July–August, and June and September, respectively. It was indicated that ivermectin dung residues cause a high mortality in the field-collected beetles of two *Copris* species regardless of the season and generation. Asian *C. ochus* is a large dung beetle, similar to the European *C. hispanus*, whereas *C. acutidens* is a medium-sized species. All are brood-care species. From spring to summer in Japan, male-female pairs of these species eat dung and cooperate in excavating an underground chamber, which is provisioned with a mass of dung; adults remain in the same dung pat for brood-care for a long time. Ivermectin concentrations remain relatively stable in dung pats for at least 45 days in the field (Sommer *et al*., 1992). Therefore, adults increase their exposure to ivermectin residue in dung and are likely to be affected by it, as compared with non-brood-care species, which nest repeatedly and move from pad to pad.

It is well known that *C. ochus* is widely distributed in East Asia and has a high dung-decomposing activity; an average weight of their brood balls is about 40 g per ball (Bang *et al*., 2001). However, this species has rapidly decreased in pastures in the past ten years and is listed as a ‘rare’ or ‘endangered’ species in 25 prefectures of Japan (Hori, 2005). *C. acutidens* is also designated as a ‘rare’ species in four prefectures in Japan. These *Copris* species are exposed to a variety of threats, e.g. frequent renovation of grassland, change of dung composition due to an increase of concentrate diets and residues of veterinary parasiticides and drugs used in pastures. In Japan, ivermectin has been used in pastures throughout the country with the advent of pour-on formulation since 1996. Most public stock farms treated cattle with ivermectin 2–3 times from spring to autumn. Treatment with ivermectin in spring and summer coincides with the period of breeding activity of dung beetles. The results of the present study suggest that the decrease of *C. ochus* in Japanese pastures can be partly attributed to the contamination of dung with anthelmintic drugs such as ivermectin. McCracken (1993) noted that rare species of dung beetles could be put at risk by use of avermectins because they may not have recolonization potential. Use of harsh anthelmintics such as ivermectin, during the breeding season of dung-dwelling insects, could especially accelerate the reduction of the populations of rare dung beetles such as *Copris*. Krüger & Scholtz (1998) suggested that the use of ivermectin affected community structure through a reduction in species diversity in a large-scale field study. Many other common dung beetles, such as *C. jessoensis*, whose larvae are affected by ivermectin also, possibly, have long-term risks affecting the reduction of their populations in Japanese pastures.

Floate *et al*. (2002) compared four endectocide products for their effects on non-pest insects in cattle dung and showed that they were ranked in descending order of adverse effect: doramectin > ivermectin > eprinomectin > moxidectin. Choosing endectocide products, which least affect dung-decomposing insects, is important. Moreover, the effects of these products on dung-degradation should be assessed to avoid environmental hazards in pasture.

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