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Effects of ivermectin on target and non-target dung-breeding flies (Diptera) in cattle dung pats

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Abstract: The effects of antiparasitic drug, ivermectin, on the dung-breeding flies were studied in laboratory and field experiments in Hokkaido, Japan. In two pour-on treatments (500 μg/kg), ivermectin was excreted in dung for up to 14 and 21 days after treatment, with peaks at 3 days and 1 day, respectively. Dung from treated cattle prevented the larval development of *Haematobia irritans* and *Neomyia cornicina* up to 14 days and reduced the larval and pupal survival rates at 21 days after treatment. In the field experiment using emergence traps, the numbers of emerged flies from dung pats were 3,655 (18 families) in control dung and 2,234 (16 families) in treated dung. Especially, *Musca bezzii, Ravinia striata, Sepsis duplicata, Sepsis thoracica, Sepsis cynipsea*, Sphaeroceridea, Empididae, Sciaridae and Chironomidae were severely reduced in treated dung. Reduction of cyclorrhaphaous flies similarly occurred at 1, 3 and 7 days after treatment, which coincides with high concentrations of ivermectin. Conversely, Ceratopogonidae and Phoridae increased in treated dung, regardless of days (1, 3 and 7) after treatment. Dry weights of flies emerged from dung pats of treated cattle in the field experiment were only 5.8% of those from dung of control cattle, indicating that dung-decomposing activity by fly larvae was supressed in dung of treated cattle.

Key words: ivermectin, pour-on, *Haematobia irritans*, *Neomyia cornicina*, non-target species, dung-breeding flies

INTRODUCTION

Ivermectin is a broad-spectrum parasiticide against nematode and arthropod parasites of domestic livestock. This drug is used in many parts of the world since its introduction in the early 1980s, because it is effective and convenient to use by various formulations. Whatever the formulations, ivermectin is excreted in cattle feces or dung pats (Campbell et al., 1983), where it retains insecticidal activity: this has a novel role of control of pest flies inhabiting dung. However, ivermectin residues in dung have a potential impact on non-target organisms and cause undesirable environmental consequences by interfer-

ing with dung-breeding insects involved in the degradation of dung on pasture (Wall and Strong, 1987).

It is well known that pest flies are only a few in dipterous fly fauna of cattle dung pats in pasture; a large majority is non-pest (Hammer, 1941; Laurence, 1954; Papp, 1970). The effects of ivermectin on these non-target dipterous flies in dung pats have been investigated by Wardhaugh and Rodriguez-Menendez (1988), Madsen et al. (1990), Sommer et al. (1992), Strong and Wall (1994), Floate (1998), Floate et al. (2001).

In Japan, ivermectin has been used on cattle throughout the country, especially with the spread of pour-on formulation.

Until now, however, no attempt has been made to evaluate the effect of this drug on dung-breeding flies in Japan.

The purpose of this study is to determine the effects of ivermectin residues in dung voided by treated cattle on target and non-target dung-breeding flies in Hokkaido, Japan.

Materials and Methods

Cattle and dung collection

The experiments were conducted at the pasture of Obihiro University of Agriculture and Veterinary Medicine. Five Holstein cows (ages 31–53 months) were selected and ivermectin (Ivomec® Topical; Merial Limited) was topically treated to them by pour-on formulation at the recommended dose 500 μg/kg body weight. Two treatments were done on 25 May and 22 June in 2002, and at the same time each 5 untreated cows (ages 45–52 months) were also selected and used as controls. None of the cows had been previously treated with anthelmintics or insecticides. Dung pats were collected immediately after defecation at 1, 3, 7, 14, 21, 28 and 35 days after treatments. In each collection, dung pats from 5 cows were mixed and frozen at −20℃ until used.

Determination of ivermevtin concentrations

Determination of ivermectin concentration in feces was performed by high-performance liquid chromatography (HPLC) following the method of Payne et al. (1995) with some modifications: the extraction was carried out by homogenizer (PHYSCOTRON, Microtec Co., Ltd.) at 10,000 rpm for 1 min instead of sonication, and avermectin B1 was added as internal standard.

Laboratory bioassays: Effects of ivermectin on pupation and emergence of Haematobia irritans *(Linnaeus) and* Neomyia cornicina *(Fabricius)*

The horn fly, *H. irritans* is a target species which is a blood-sucking pest of cattle. *N. cornicina* was chosen as non-target beneficial species which is commonly found in dung pats as coprophagous in Hokkaido. Eggs of *N. cornicina* were collected from the surface of fresh dung pats in pasture, while eggs of *H. irritans* were obtained by allowing gravid females to oviposit in test tubes (11 mm diameter × 75 mm length). Thirty to 40 eggs in both species were inoculated onto 30g of dung in plastic cups (8 cm diameter × 4 cm depth) doubled in large cups (12 cm diameter × 5.5 cm depth) with sawdust for pupation site. These cups were maintained at 25℃ (16L-8D) and the number of pupae was counted. The pupae obtained were transferred to another plastic cup (8 cm diameter × 4 cm depth), for adult emergence. Experiments were replicated 3 times in *H. irritans* and 5 times in *N. cornicina*.

Field experiment: Effects of ivermectin on fly emergence

Eighteen artificial dung pats (1 kg, three pats each per collection date for treated and control) of 1, 3, 7 days after treatment were each placed on a 17-cm layer of black soil in a plastic box (33 × 33 × 19 cm). Boxes with these dung pats were then placed in a field area adjacent to a pasture for exposure to insect activity for 7 days.

After exposure, these boxes were brought to grassland in the campus of the University and covered with emergence traps attached with cups for collection of emerged flies. Traps were checked everyday, and flies emerged from dung were collected, counted and determined into the species or families. These specimens were dried in the laboratory more than two months and their dry weights were measured by an electric chemical balance (sartorius BJ1500). These experiments were performed two times in mid-June and late July, and a total of 36 dung pats was observed.

Results

Determination of ivermectin concentrations

In two treatments, ivermectin residues in dung attained maximal concentrations at 3 and 1 days after treatments, respectively, and both peaks were followed by a decline with below half concentrations at 7 days after treatment (Fig. 1). Then, ivermectin was detected in dung up to 14 (Exp. 1) and 21 days (Exp. 2) after treatment, and not detected at 28 and 35 days after treatment; the determination limit was 0.002 ppm.

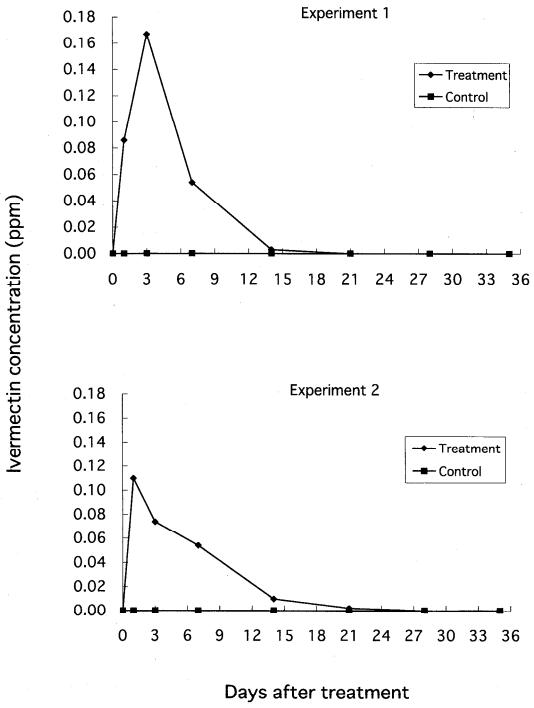

Fig. 1. Ivermectin concentrations in dung (ppm of wet weight) after pour-on treatments (500 μg/kg).

Effects of ivermectin on pupation and adult emergence of H. irritans *and* N. cornicina

Pupation rates of *H. irritans* in dung from control cattle was from 64.4 to 100% at 1–35 days after treatment (Table 1), whereas they were zero in dung from treated cattle at 1 to 14 days, 17.9% at 21 days, and 84.8 and 79.3% at 28 and 35 days after treatment, respectively. Emergence rates also showed the same tendency as the pupation rates.

Pupation rates of *N. cornicina* in dung from control cattle was from 86.9 to 99.3% at 1 to 35 days after treatment (Table 2). On the other hand, they were zero in dung from treated cattle at 1 to 14 days, about half (49.8%) at 21 days, and 95.9 and 97.2% at 28 and 35 days after treatment, respectively. Emergence rates also showed the same tendency as that of pupation rates.

Effects of ivermectin on fly emergence in field

In Experiments 1 and 2, a total of 5,889 flies comprising 19 families emerged from 36 dung pats (18 each control and treated), of which 3,655 flies (18 families) were from dung of control cattle and 2,234 flies (16 families) from dung of treated cattle (Table 3). The species identified into species were 1 species in Anthomyiidae, 4 species in Muscidae, 1 species in Sarcophagidae and 5 species in Sepsidae. Other families which include plural species were indicated as spp. The predominant families in total numbers were Ceratopogonidae (mainly *Forcipomyia* spp.) (1,666), followed by Sciaridae (1,381), Sphaeroceridae (1,230) and Phoridae (473).

Large reduction in the numbers of flies from dung of treated cattle occurred in Muscidae (*Musca bezzii* Patton et Cragg),

Table 1. Larval and pupal survival of *Haematobia irritans* in dung from cattle treated with ivermectin and in control dung.

Days after treatment	Pupation rate (%)±SD			Emergence rate (%)±SD		
	Control		Treatment	Control		Treatment
1	66.7±47.1	*	0	46.13±3.2	*	0
3	64.4±11.3	*	0	48.6±7.0	*	0
7	100	*	0	74.8±7.8	*	0
14	96.0±2.9	*	0	68.1±16.3	*	0
21	96.1±4.2	*	17.9±25.3	57.6±16.6	*	1.2±1.7
28	71.9±12.7	n.s.	84.8±16.8	53.6±13.6	n.s.	68.8±4.0
35	84.0±2.6	n.s.	79.3±7.1	54.4±14.6	n.s.	61.2±7.2

Each value is a mean of 3 replicates each inoculated 40 eggs.
*: Significantly different between control and treatment ($P<0.01$: Mann–Whitney U test).
n.s.: Not significant between control and treatment.

Table 2. Larval and pupal survival of *Neomyia cornicina* in dung from cattle treated with ivermectin and in control dung.

Days after treatment	Pupation rate (%)±SD			Emergence rate (%)±SD		
	Control		Treatment	Control		Treatment
1	86.9±13.3	*	0	72.9±17.2	*	0
3	99.3±1.4	*	0	93.3±3.0	*	0
7	89.6±7.5	*	0	86.1±9.0	*	0
14	91.8±1.5	*	0	87.5±5.3	*	0
21	96.8±4.0	*	49.8±14.5	91.5±6.2	*	34.1±17.5
28	98.6±2.8	n.s.	95.9±3.3	93.2±6.1	n.s.	93.1±5.8
35	94.6±5.0	n.s.	97.2±2.7	82.5±3.8	n.s.	92.5±1.3

Each value is a mean of 5 replicates each inoculated 30 eggs.
*: Significantly different between control and treatment ($P<0.01$: Mann–Whitney U test).
n.s.: Not significant between control and treatment.

Sarcophagidae (*Ravinia striata* (Fabricius)), Sepsidae (*Sepsis duplicata* Haliday, *S. thoracica* (R.-D.), *S. cynipsea* Linnaeus), Sphaeroceridae and Empididae in Cyclorrhapha, and Sciaridae and Chironomiidae in Nematocera. Conversely, emergence of Ceratopogonidae prominently increased in dung of treated cattle. The numbers of Phoridae were also more rather abundant in dung from treated cattle than in dung from control cattle.

In Sepsidae, *S. latiforceps* Duda emerged from dung pats of treated cattle in almost the same numbers as those of control cattle, although no flies emerged from dung of treated cattle in other 4 species.

The numbers of represented Cyclorrhapha species and families (*M. bezzii*, *R. striata*, Sepsidae, Sphaeroceridae, Empididae and Phoridae), and Nematocera families (Ceratopogonidae, Sciaridae, Chironomidae) emerged from dung of control and treated cattle at 1, 3 and 7 days after treatments are shown in Figs 2. In Cyclorrhapha (*M. bezzii*, *R. striata*, Sepsidae, Sphaeroceridae, Empididae), reduction in the numbers similarly occurred at 1, 3 and 7 days after treatment, indicating that ivermectin concentrations in these days are enough to affect their larval development. In Phoridae and Ceratopogonidae, emerged flies were more abundant at all days (1, 3 and 7) after treatment, bearing no relation to ivermectin concentrations.

Total dry weight of flies emerged in Experiments 1 and 2 was 1,915.9 mg in dung

Table 3. Numbers of flies emerged from dung pats (1 kg) of control and treated cattle by exposure for 7 days.

Families and species	Dung pats		Total
	Control	Treatment	
Anthomyiidae			
Paregle cinerella	1	4	5
Muscidae			
Musca bezzii	38	0	38
Hydrotaea abipuncta	1	1	2
Hebecnema umbratica	1	0	1
Coenosia sp.	3	4	7
Sarcophagidae			
Ravinia striata	142	0	142
Sepsidae			
Sepsis latiforceps	45	39	84
Sepsis duplicata	44	0	44
Sepsis thoracica	99	0	99
Sepsis cynipsea	47	0	47
Saltella sphondylii	1	0	1
Sphaeroceridae spp.	1,210	20	1,230
Chloropidae spp.	5	3	8
Drosophilidae spp.	13	7	20
Ephydridae spp.	1	3	4
Dolichopodidae spp.	3	6	9
Platystomatidae spp.	3	1	4
Empididae spp.	165	38	203
Phoridae spp.	207	266	473
Stratiomyidae spp.	0	2	2
Tipulidae (Limoniinae spp.)	20	0	20
Ceratopogonidae spp.	261	1,405	1,666
Chironomidae spp.	326	49	375
Mycetophilidae spp.	7	3	10
Sciaridae spp.	998	383	1,381
Psychodidae spp.	14	0	14
Total	3,655	2,234	5,889

Eighteen dung pats were observed in control and treatment, respectively.

from control cattle and 112.8 mg in dung from treated cattle (Table 4): the ratio was 17 : 1. In dung of control cattle, the proportion of large-sized Cyclorrhapha (*M. bezzii* and *R. striata*) was 78% in total weights.

Discussion

Sommer et al. (1992) reported that ivermectin by pour-on formulation was rapidly excreted in dung up to 14 days with a peak at the 1st day after treatment. Peak residue levels of avermectins including ivermectin administrated by subcutaneous injection are typically excreted at 3–6 days after injection (Sommer et al., 1992; Lumaret et al., 1993). The current results are similar to those of Sommer et al. (1992) and exhibit that ivermectin is excreted in dung at least up to 14 days after treatment, which coincides with the periods of 100% larval mortality of *H. irritans* and *N. cornicina*. Large reduction of pupation rates in two species at 21 days after treatment (Exp. 1) when no ivermectin was detected may be due to undetectable residues or metabolites of drug.

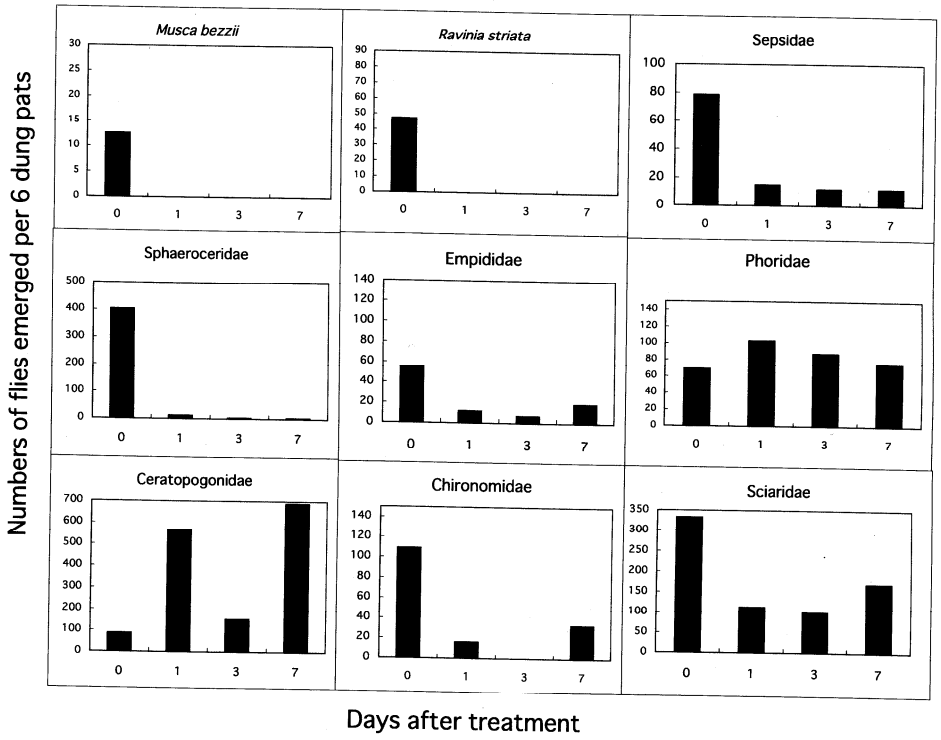

Fig. 2. Numbers of represented Cyclorrhapha and Nematocera flies emerged per 6 dung pats at 1, 3 and 7 days after treatments (0=Control).

Table 4. Dry weights of main coprophagous flies emerged from dung pats of control and treated cattle, respectively.

Families and species	Dry weights (mg)	
	Control	Treatment
Muscidae		
Musca bezzii	400.9	0
Sarcophagidae		
Ravinia striata	1,097.7	0
Sepsidae		
Sepsis latiforceps	18.0	15.6
Sepsis duplicata	6.2	0
Sepsis thoracica	39.6	0
Sepsis cynipsea	18.8	0
Saltella sphondylii	0.5	0
Sphaeroceridae spp.	84.7	1.4
Sciaridae spp.	249.5	95.8
Total	1,915.9	112.8

Eighteen dung pats were observed in control and treatment, respectively.

It has been reported that ivermectin residues in dung could effectively reduce the dipterous flies of economic importance, such as *Haematobia irritans*, *Stomoxys calcitrans* and *M. domestica* (Ridsdill-Smith, 1988; Fincher, 1992, 1996; Doherty et al., 1994; Wardhaugh et al., 1996; Floate et al., 2001). Fincher (1992) reported that emergence of the horn fly, *H. irritans* was reduced for 8 weeks when reared on dung from cattle treated with ivermectin by injection. In pour-on formulation, emergence of *H. irritans* was reduced for 5–6 weeks (Fincher, 1996) and 4–8 weeks (Floate et al., 2001), respectively. The period which affected survival of *H. irritans* in the present study is somewhat shorter than that in the previous reports. This may be attributed to the differences of breeds and diets of cattle. Uzuka et al. (1999) reported that no adult horn fly was on cattle body for 5 weeks after treatment of ivermectin by pour-on formulation in Obihiro, Hokkaido. It is considered that

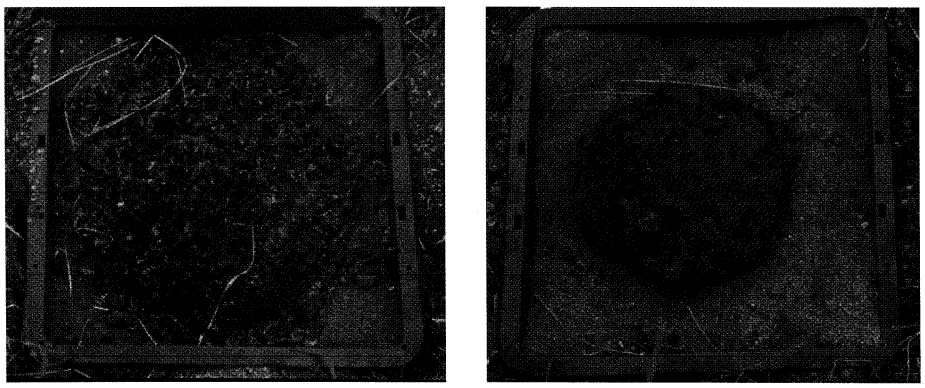

Fig. 3. Appearance of dung pats of 1 day after treatment (right) and control dung pat (left) (both 7 days old after exposure).

ivermectin by pour-on formulation contributes to reduction of horn fly population on cattle body during a certain period.

It has been reported that ivermectin inhibits larval development of non-target dung-breeding flies (Wall and Strong, 1987; Madsen et al., 1990; Strong, 1992; Floate, 1998). Mortality of larvae of *N. cornicina* in dung of injection formulation of ivermectin was 100% up to 32 days after treatment (Wardhaugh and Rodriguez-Menendez, 1988) and 97% up to 2 weeks after pour-on formulation (Sommer et al., 1992). The present results confirmed that pour-on formulation of ivermectin suppressively affects the larvae of non-target coprophagous flies, *N. cornicina* at least up to 21 days after treatment.

It is well known that Cyclorrhapha larva were affected in dung of ivermectin-treated cattle, whereas Nematocera larvae remained unaffected (Schmidt, 1983; Madsen et al., 1990; Sommer et al., 1992). The present results are similar to the previous reports. Especially, the larvae of *M. bezzii*, *R. striata*, *S. duplicata*, *S. thoracica*, *S. cynipsea*, Sphaeroceridae, Empididae, Chironomidae and Sciaridae in dung appeared to be severely reduced by ivermectin treatment; these are coprophagous, having a role as decomposer. It is concluded that ivermectin residues in dung by pour-on treatment extensively suppress development of non-target beneficial coprophagous fly larvae in dung pats.

According to Madsen et al. (1990), difference of sensitivity between Nematocera and Cyclorrhapha may be ascribed to differences in phenology, development and feeding habits. In the present study, however, the rather increased emergence of Ceratopogonidae (mainly genus *Forcipomyia* spp.) of Nematocera and Phoridae of Cyclorrhapha from dung of ivermectin-treated cattle is still a riddle; further investigation is necessary in consideration of the possibility of their attractions to ivermectin residues or metabolites in dung.

In the species level there has been no report on sensitivity of small-sized acalypterate flies of Cyclorrhapha against ivermectin up to the present. In Sepsidae, there was emergence of *S. latiforceps* from dung of treated cattle likewise that of control dung, nevertheless, there was no emergence from dung of treated cattle in other 4 sepsid species, suggesting that there are differences in sensitivity among the species within this family.

Papp (1970) investigated the dry weight values of flies developing in cattle dung pats and suggested that fly larvae have an important role in the disappearance of cattle dung pats in pasture. Dry weights of flies emerged from dung of treated cattle were only 1/17 (5.8%) of that from dung of control cattle, indicating that de-

composing activity by larvae was suppressed in dung pats of treated cattle. This activity of fly larvae in part is revealed in the appearance of dung pats; that is, typical control dung pat has spread out and its surface is soft and crumbled, whereas that of treated cattle has not spread out and its surface is crusty and hard (Fig. 3). Especially, dry weights of *M. bezzii* and *R. striata* occupied 78% in total weights; these large-sized Cyclorrhapha flies may be an important decomposer in dung pats. Further work would be needed to determine the effects of ivermectin on dung-breeding insects in relation to their roles in degradation of dung pats in pasture.

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