Effect of ivermectin on the survival and fecundity
of *Euoniticellus intermedius* (Coleoptera: Scarabaeidae)

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Abstract: The State of Veracruz in Mexico is one of the main cattle producers, and uses several veterinary products for disease and parasite control. For parasite control, ivermectin is one of the most frequently used substances. Nevertheless, even though previous research conducted in other countries has found that this product has negative effects on beneficial coprophagous fauna, no studies have described its effects on coprophagous insects at a local scale in Veracruz, Mexico. This study evaluated *Euoniticellus intermedius* survival, fecundity, fertility and preimaginal development under laboratory conditions when ivermectin was added to cattle dung at three different concentrations. The design included two controls (spiked dung), and the following product concentrations: 0.01, 1.0 and 100ppm, which were homogenized with wet cattle dung. 20 female-male *E. intermedius* couples between five and 15 days old were used and kept at 27°C, 70% RH, and 12h light for 10 days. The survival of all specimens, the fertility of 20 females and the gonadal maturity of 17 males were verified. The larval development in 162 pieces of brood-mass was examined, and a total of 974 larvae developed and reached adulthood. The highest ivermectin concentration was toxic at 1.0ppm dose, the survival of adults was reduced to almost the half, and at 100ppm, total mortality was observed. The effects on specimen reproductive systems showed that the ovary was not affected, that the testicle size increased, and that the fecundity and weight of brood-masses were reduced. Pre-imaginal development increased 0.5 times at 0.01ppm concentration, and the width of the cephalic capsule in third instar larvae diminished. The prolonging of development time may cause a phase lag in the field activity cycle, this lag may reduce the number of *E. intermedius* individuals and the efficiency of the environmental services that they provide. Rev. Biol. Trop. 60 (1): 333-345. Epub 2012 March 01.

Key words: dung beetles, toxicity, brood-mass, pre-imaginal development.

Dung beetles perform an important ecological activity within cattle pastureland (Nichols *et al*. 2008), because they bury cattle dung to nest and feed on, and in this way, they prevent the proliferation of flies and some cattle parasites while returning nutrients to the soil (Edwards & Aschenborn 1987, Chirico *et al*. 2003).

The coprophagous beetle fauna is highly diverse in Mexican cattle pasturelands (Halffter *et al*. 1992, Martín-Piera & Lobo 1993). In Veracruz and in other 14 Mexican states, the non-native species include *Euoniticellus intermedius* (Reiche) and *Digitonthophagus gazella* (Fabricius), which, because of their great adaptation capacity, have dispersed from Texas into mexican territory (Montes de Oca & Halffter 1998). These exotic species, originating in Africa, were introduced to the USA in 1972 with the purpose of controlling flies

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and excess cattle dung (Fincher *et al.* 1983, Fincher 1986).

Veracruz is the principal cattle producing state in Mexico, with a population of almost 4.5 million (Gobierno de Veracruz 2009), 93% of which are regularly rid of parasites (INEGI 2007). Parasiticides may alter common cattle pasture fauna (Martínez *et al.* 2000, Martínez & Lumaret 2006). One of the main problems is caused by veterinary medicines such as Ivermectin, a macrocyclic lactone often used as parasiticide for cattle (Boxall *et al.* 2004).

The residues of Ivermectin are released in dung, from where they contaminate the environment and affect other innocuous soil fauna like dung beetles (Lumaret & Errouissi 2002, Floate *et al.* 2005). The presence of ivermectin in dung may change the attraction of beetles toward the dung, which may alter the feeding and reproduction of the beetles (Holter *et al.* 1993, Krüger *et al.* 1998 a, Krüger *et al.* 1998 b, Errouissi & Lumaret 2010). Furthermore, the maturation process of the adults may be retarded (Sommer *et al.* 1993, Krüger & Scholtz 1997, Wardhaugh *et al.* 2001). Consequently, the biological cycles and the services fulfilled by dung beetles within grazing land may change, thus affecting the productivity of this agroecosystem (Fincher 1981, Davis *et al.* 2004, Losey & Vaughan 2006).

Euoniticellus intermedius is one of the most abundant dung beetle species in the tropical pastureland in Veracruz (Montes de Oca & Halffter 1998, Carolina Flota pers. comm.). This beetle is a diurnal, digger species, preferring open pastureland and cattle dung, especially dung deposited between 24h and 36h earlier. For reproduction, the females dig galleries in the soil beneath the dung. In every gallery, various brood-masses are made, each of which is formed of dung, buried, compacted, and hollowed out by the female for depositing an egg into, which afterward is covered with more dung. The time of larval development varies according to temperature, but at 27.8°C, the new adult emerges after 34.5 days. The females lay their first egg after four days, and on average, they deposit 2.3 eggs daily (Blume 1984).

Ivermectin toxicity tests have been made on *E. intermedius* using its residues in the dung of animals treated subcutaneously at a dose of 0.2mg/kg (Krüger & Scholtz 1997). The toxicity of ivermectin and deltamethrin has also been detected in *E. fulvus* (Wardhaugh *et al.* 1998, Wardhaugh *et al.* 2001). In addition to its toxicity, it has been observed that under field conditions, the presence of ivermectin may also change the diversity of dung insect communities (Krüger & Scholtz 1998 a, Krüger & Scholtz 1998 b).

Toxicity studies on veterinary substances like ivermectin, and their effects on the environment and non-target fauna has been developed for years, mainly in the USA and the European Union (Boxall *et al.* 2004, VICH 2004, Knacker *et al.* 2005), but little research has been done in Mexico; therefore, it was necessary to study these effects to document the consequences of using these substances on dung beetle fauna.

The objective of the present study was to evaluate the effect of ivermectin on the survival and fecundity of *E. intermedius* adults and on survival and development from the egg to adulthood.

MATERIALS AND METHODS

Although recently standardized tests have been developed to evaluate the toxicity of veterinary substances like ivermectin (Lumaret *et al.* 2007, Römbke *et al.* 2007, Römbke *et al.* 2009, OECD 2009), these tests have not yet been carried out in Mexico; thus, we chose to employ a laboratory test proposed by Wardhaugh (2002). This test is based on using a non-treated control and treatments with various dilutions of the substance to be tested, which was diluted with acetone to obtain the concentration needed (spiked dung). Because a solvent substance was utilized, another control was established for this substance (solvent control). The treatment took 10 days with three changes of fresh dung for renewing the dose that was to be tested. For the application of this protocol, a considerable number of *E. intermedius* adults

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of known age are required, beginning with the breeding of individuals collected in the field.

Species and site of collection: To establish a breed of *Euoniticellus intermedius,* adult individuals were collected at the "San Roman" ranch located in La Laguna in the municipality of Medellin de Bravo, Veracruz, Mexico (18°58'19.37'' N - 96°04'51.43'' W, 37masl). Four collections were made over four days between June-July 2009. In every collection, at least 30 dung pats, distributed on the pasture, were revised manually. Dung pats were one or two days old and were naturally deposited by cattle. 132 adults were obtained and taken to the insectary of the Instituto de Ecología A.C. in Xalapa.

Rearing under laboratory conditions: 43 vertical terraria were prepared, consisting of rectangular, transparent plastic boxes of 11×16×5cm with small holes in the top wall for air interchange. In each terrarium, 700g of humid, sifted soil, two females, and one male were placed in the box with 50g of fresh cattle dung for feed. The terraria were kept in an insectary under controlled environmental conditions of 27°C±2°C, 70% relative humidity and a photoperiod of 14h of light.

Each terrarium was revised weekly to change the food and identify the initiation date of the brood-mass, which appeared adhered to the terrarium walls. 20 days after establishment of the terraria, a plastic trap can of approximately 50mL was placed at its top, filled with soil to half its volume, and the other half was filled with fresh dung (Blume & Aga 1975) to remove the parents and leave the brood-mass alone in the terrarium. Subsequently, from 30 days after establishment of the terraria, they were examined every three days to obtain the recently emerged adults that had entered the trap. These newly emerged individuals were placed in different terraria according to their sex and age. After every revision of the traps, the dung supply was renewed. This procedure was repeated until there were no more emergences.

Bioassays: To establish the experimental treatments, we followed the protocol proposed by Wardhaugh (2002) for DOTTS (Dung Organism Toxicity Testing Standardization Group) with modifications.

A commercial ivermectin solution at 1% was used ("Iverfull" injectable solution of 1% ivermectin, Aranda Laboratories, SAGAR-PA Reg.Q-0449-170). Starting from this concentration, three experimental solutions were prepared, besides the two controls. Each solution was prepared in 50mL of acetone to get concentrations of 0.01, 1.0 and 100ppm of ivermectin. Acetone allows for the dilution of the commercial ivermectin solution because of its oily nature, and its volatility prevents the adding of more water to the dung.

Each ivermectin solution was thoroughly mixed for at least five minutes to ensure even distribution in 5kg of wet dung from cows not recently treated with a parasiticide. Final concentrations of ivermectin were 0.01, 1.0, and 100mg/kg of wet dung (low, medium and high concentration, respectively). Two controls, CS and CF, were established, the first with 5kg of dung + 50mL of acetone, whereas the second control of 5kg dung remained free of substances. Dung with ivermectin and the two controls were left exposed to the air at least 30min to eliminate the solvent. After venting, the dung pats were divided into subsamples of 1kg, hermetically sealed and frozen until their use.

The experimental units consisted of terraria made of rectangular, transparent plastic boxes of 7×14×3.5cm placed in a vertical position and with ventilation holes at the top part filled with 400g of humid, sifted soil from the collection site, free of agricultural chemicals. A pair of *E. intermedius* (female and male chosen at random) was installed in each terrarium, with ages varying from 5-15 days.

The treatments with ivermectin separately received 50g of dung at different ivermectin concentrations. Those of the CS control received 50g of dung with the solvent, and those of the CF control received 50g dung free of substances. 20 terraria per treatment were utilized.

The terraria of each of the five treatments were examined every three days to remove the non-utilized dung and to place 50g of fresh,

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previously defrosted dung in the box corresponding to every treatment. After the 10 days of observation, all the boxes were opened; the surviving adults were counted as well as the number and weight of the elaborated brood-mass.

Three to seven adults of the surviving individuals per treatment were dissected to extract their reproductive systems (according to Martínez's [2002] techniques) to observe and compare their gonadal maturity and their accessory glands.

The data collected included the weight and number of brood-masses, the fecundity of the females, the mortality of the offspring, and the development time from egg to adult. Fecundity was defined as the number of brood-masses produced by the females during the first 10 days period. To check the viability of the brood-masses after opening all the terraria, a sample of 10-50 brood-masses obtained, from each treatment was randomly taken. The selected brood-mass samples were opened to define the stage of development they had reached: egg or larva of the first, second, or third instar, as well as the size of the cephalic capsules of the larvae, according to the method by Hernandez-Martinez & Martinez (2003).

To determine the time of development, the remaining brood-masses of every treatment were put in independent terraria until the emergence of adults. Emerged individuals were checked every three days. The development time to the adult stage was determined for each treatment. The emerged adults were grouped according to age and sex and fed with dung free of any veterinary medicine. The longevity of beetles was compared according to the initial larval treatments.

The results were analyzed with the SAS program (SAS Institute Inc. 2004). The survival of adults was evaluated with a logistic regression model (LOGISTIC procedure), which utilizes the chi-squared (χ^2) statistic to compare the effect by treatment and sex.

Variations of the ovary in surviving females were determined by the number of oocytes present per ovariole, and by the size of the basal oocyte, by means of a general linear model (GLM procedure) analysis. The number of oocytes was analyzed following the linear Log model with Poisson's distribution (GENMOD procedure).

The presence of bodies of resorption in the ovary was compared by means of the logistic regression model, using the chi-squared (χ^2) statistic. The variations of testis volume and of accessory gland reservoirs of the surviving males were examined with an analysis of variance by way of unequal repetitions (GLM procedure). A fecundity analysis was conducted by the number and mean weight of the brood-mass elaborated in treatments.

The number of brood-masses was analyzed according to a linear Log model with Poisson's distribution (GENMOD procedure). Brood-mass weight was compared using analysis of variance through unequal repetitions (GLM procedure). The viability of brood-masses at 10 days was studied based on a two-way contingency table of the observed and expected frequencies for each larval stage and for each treatment. The association between ivermectin treatment and the stage was established with the chi-squared (χ^2) statistic at a confidence level of 95%. The variation of cephalic capsule size per larval stage among treatments was examined with an analysis of variance by way of unequal repetitions (GLM procedure).

In all the analyses, a confidence interval of 95% was maintained, and cases of significant difference ($p < 0.05$) were subsequently compared by contrasts. The time from hatching to the adult stage of the new generation was compared by survival curves (LIFETEST procedure) according to the frequency of daily emergence and the period required for their development in each treatment. Curves were compared by a log-rank test with a significance level of $p < 0.05$; the same test was applied to compare the survival of the new adults (Zar 1999). For better interpretation of the results, the survival data were presented in a graph as its complement; in other words, the accumulated values of adult emergence were depicted.

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RESULTS

Ivermectin treatments significantly affected the survival of adults exposed for 10 days (Table 1) (χ^2=8.98, d.f.=3, p<0.05) without significant difference with respect to the sex of adults (χ^2=0.11, d.f.=1, p=0.739). The survival of adults in the solvent control (CS), and in the control (CF) was 70 and 67.5%, respectively, without significant difference (χ^2=0.058, d.f.=1, p=0.809).

The survival of adults fed with dung containing the lower concentration of ivermectin (IL) was 62.5%, and did not significantly differ from that of the control groups (χ^2=0.469, d.f.=1, p=0.493). At the medium concentration of ivermectin (IM), the survival of adults was 40%, significantly different from the survival of adults at low concentration (χ^2=3.98, d.f.=1, p=0.046), and adults of the control groups (χ^2=8.78, p=0.003). The highest lethal effect was observed among adults exposed to dung containing the highest concentration of ivermectin (IH) (all adults died in a short time).

The females surveyed after treatment with ivermectin and those of the control groups did not show a significant difference in the number of oocytes present per ovariole ($F_{3.16}$=1.54, p=0.24), either by the size of basal oocytes ($F_{3.16}$=1.14, p=0.36) or by the presence of an extra-ovarian resorption body (χ^2=5.43, d.f=3, p=0.14) (Table 2).

In males, however, significant differences in the follicle size of the testicles were observed ($F_{3.30}$=302, p=0.045) (Table 2). No significant difference was obtained in their volume between the two control groups ($F_{1.30}$=1.02, p=0.32) or between the two groups exposed to ivermectin ($F_{1.30}$=0.27, p=0.609). However, controls and treated groups differed significantly from each other ($F_{1.30}$=7.3, p=0.011). As for the size of accessory glands, reservoirs of the male reproductive system did not show considerable difference between controls and treated groups ($F_{3.20}$=0.63, p=0.6).

Female fecundity depended on treatments ($F_{4.67}$=14.65, p<0.001) (Table 3). The amount of brood-masses produced by females of the

TABLE 1

Characteristics of the survival rates of *E. intermedius* fed for 10 days on cattle dung with three different ivermectin doses and in two control groups

Treatments	Ivermectin in dung (ppm)	Acetone in dung (ml/kg)	Total number of surviving adults	Ratio of surviving females/males
CF- Control free of substances	0	0	28	16/12
CS- Control solvent	0	10	27	14/13
IL- Ivermectin low dose	0.01	10	25	12/13
IM- Ivermectin medium dose	1.0	10	16	7/9
IH- Ivermectin high dose	100	10	0	-

TABLE 2

Variations in the gonads of adult *E. intermedius* after treatment application

Treatments	Females				Males		
	Examined females	Number of oocytes per ovariole [1]	Length of basal oocyte (mm)[1]	Females with body of resorption	Examined males	Volume of testis follicle (mm³)[1]	Volume of accessory gland reservoirs (mm³)[1]
CF	4	9.75 ± 1.5	1.25 ± 0.34	2	4	4.46 ± 1.73	3.22 ± 2.11
CS	7	7.71 ± 2.28	1.70 ± 0.56	7	5	3.83 ± 0.45	3.51 ± 1.95
IL	6	9.5 ± 1.37	1.47 ± 0.19	4	4	5.56 ± 1.60	4.89 ± 2.09
IM	3	8.66 ± 1.52	1.85 ± 0.84	1	4	5.21 ± 1.33	2.72 ± 2.85

[1]. Means ± standard deviation.

two controls did not present significant differences ($F_{1.67}=1.35$, p=0.24). Similarly, no significant difference was obtained between the controls and females of the IL treatment ($F_{1.67}=0.31$, p=0.58), contrary to the significant differences obtained by IM treatment compared with controls ($F_{1.67}=34.45$, p<0.001) and with IH treatment ($F_{1.67}=21.81$, p<0.001), respectively. The fecundity of the females with IH was lower than that found in the other treatments, both with controls ($F_{1.67}=26.6$, p<0.001) and with IL ($F_{1.67}=23.16$, p<0.001) or IM treatment ($F_{1.67}=6.7$, p=0.01).

Brood-mass mean weight also showed a significant difference among treatments ($F_{3.62}=9.74$, p<0.001) (Table 3). No significant difference was observed between CF and CS ($F_{1.62}=4.07$, p=0.048) or between the CF and IL treatments ($F_{1.62}=1.95$, p=0.167). The difference was significant between the CF and IM treatments ($F_{1.62}=10.64$, p<0.01); similarly, the difference between the CS and IL treatments ($F_{1.62}=11.65$, p<0.01) as well

as the CS and IM treatments ($F_{1.62}=27.2$, p<0.001) was significant. Finally, there was no significant difference between the IL and IM treatments ($F_{1.62}=3.65$, p=0.06).

Ten days after initiating the treatment, within brood-masses, variations were obtained among the different pre-imaginal stages according to the treatments ($\chi^2=44.9$, d.f.=9, p<0.001) (Table 4). In the control groups and in the IL treatment, the larval mortality was low, and the proportions of the first, second and third instars were similar. In the IM treatment, the larval viability was low (four larvae out of ten hatched and attained only the first instar). In the IH treatment, only two brood-masses were obtained, but no eggs hatched.

In the cephalic capsules of the larvae of the first instar, there were no significant differences, nor were there significant differences in length ($F_{3,9}=1.29$, p=0.334) or in width ($F_{3,9}=2.20$, p=0.157) (Table 5). In the second instar, there were no significant differences for length ($F_{2,43}=0.147$, p=0.862) or

TABLE 3
Variation in the fecundity of *E. intermedius* after treatment application

Treatments	Total production of brood-masses per treatment	Brood-masses per female		
		min-max	Number[1]	Mean weight (g) [1]
CF	357	1 - 30	17.8 ± 8.8	2.53 ± 0.33
CS	361	5 - 28	18.0 ± 9.7	2.36 ± 0.29
IL	334	1 - 28	16.7 ± 9.6	2.29 ± 0.17
IM	82	1 - 9	4.1 ± 2.9	2.05 ± 0.27
IH	2	0 - 1	0.1 ± 0.3	-

[1]. Means ± standard deviation.

TABLE 4
Viability and frequency per pre-imaginal stage observed in brood-masses produced by *E. intermedius* after 10 days of treatment application

Treatment	Observed brood-masses	Individual in development	Pre-imaginal stage			
			Embryo	First instar	Second instar	Third instar
CF	50	46	0	3	20	23
CS	50	41	3	3	8	27
IL	50	43	0	5	18	20
IM	10	4	2	2	0	0

width ($F_{2,43}$=0.314, p=0.732). The third instar did not show significant differences in length ($F_{2,67}$=2.59, p=0.082); it did, however, show significant differences in width ($F_{2,67}$=7.55, p<0.001). The larvae of this instar, having received the IL treatment, presented the least width of cephalic capsules.

In IL treatment, the emergence of adults reached 65.5% compared with the control groups, which had 70% emergence (Table 6). The difference between the two controls and the treated groups was significant (χ^2=48, d.f.=2, p<0.001), unlike the difference in sexual proportion (χ^2=0.95, d.f.=2, p=0.62). The differences were significant between the controls and the IL treatment as well in the development time from egg to adult and in the survival rate of individuals (χ^2=388, d.f.=2, p<0.001) (Fig. 1).

Within the control groups, low but significant differences were observed in the survival curves of adults (χ^2=10.45, d.f.=1, p<0.05)

even though development times were similar. However, in the IL treatment, the mean development time into an adult of the new generation was 1.1 to 1.6 times longer than that observed in the control groups.

The survival rates of adults that emerged from controls and the IL treatment were similar (χ^2=0.63, d.f.=2, p=0.72). The generation obtained from larvae of the IL treatment had a life span of 18.13 days (DE=5.55, n=123); this value was similar to those obtained for the chemical-free control group (18.12 days, DE=5.52, n=208), and the solvent control group (17.6 days, DE=5.82, n=202). The state of sexual maturity of the emerged adults was not defined, but in some cases, the females made brood-masses whose viabilities were not verified.

DISCUSSION

The low concentration (0.01ppm) of ivermectin was not lethal for adults nor did it

TABLE 5
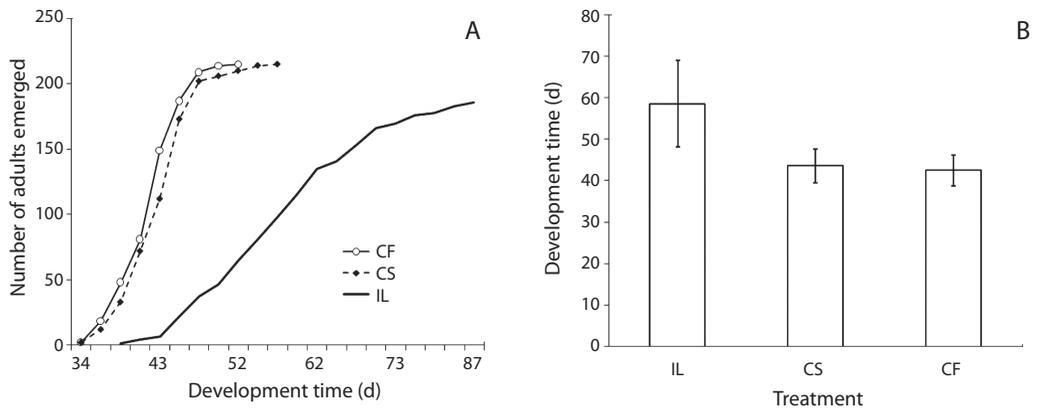Variation in the cephalic capsule sizes of *E. intermedius* larvae after treatment application

Treatments	Size of the cephalic capsule (mm)					
	First instar		Second instar		Third instar	
	Width [1]	Length [1]	Width [1]	Length [1]	Width [1]	Length [1]
CF	1.34 ± 0.05	0.79 ± 0.05	1.77 ± 0.05	1.16 ± 0.08	2.21 ± 0.05	1.50 ± 0.09
CS	1.24 ± 0.06	0.88 ± 0.08	1.75 ± 0.05	1.14 ± 0.08	2.19 ± 0.06	1.47 ± 0.08
IL	1.30 ± 0.07	0.82 ± 0.08	1.75 ± 0.06	1.14 ± 0.08	2.13 ± 0.07	1.44 ± 0.08
IM	1.39 ± 0.06	0.91 ± 0.09	-	-	-	-

[1] Means ± standard deviation.

TABLE 6
Emergence and time of development to adulthood of the new generation of *E. intermedius*, after treatment application

Treatments	Observed brood-masses	Emerged Females/Males	Development time to adulthood (d)	
			Min-max	Mean [1]
CF	307	105/110	34 - 52	42.46 ± 3.64
CS	311	115/100	34 - 57	43.56 ± 4.05
IL	284	94/92	38 - 87	58.54 ± 10.42
IM	72	0	-	-

[1]. Means ± standard deviation.

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Fig. 1. (A) Accumulated frequency of emerged *E. intermedius* adults and (B) variation of development time to adultness after treatment with 0.01ppm of ivermectin (IL) and control solvent (CS), and substance -free control (CF), (mean ± SD).

affect their fertility and fecundity. The larvae survived and emerged, but their time of development increased. The medium concentration (1.0ppm) was lethal for more than half of the adults and reduced their fecundity. None of the larvae emerged. Finally, the high concentration (100ppm) was lethal for all adults, and oviposition was not performed.

The effects of reduction in survival and fecundity in *E. intermedius* treated with ivermectin-spiked dung were observed in the same species, but using another methodology (Krüger & Scholtz 1997). This effect was also observed in *Copris hispanus, Digitonthophagus gazella, Euoniticellus fulvus, Aphodius ater* and *A. rufipes* that had been fed on the dung of animals recently treated with 0.2mg/kg of injectable ivermectin (Wardhaugh & Rodriguez-Menendez 1988, Sommer *et al.* 1993, Wardhaugh *et al.* 1993, O'Hea *et al.* 2010). On the contrary, *Caccobius jessoensis* and *Liatongus minutus* increase their fecundity in response to the action of ivermectin (Iwasa *et al.* 2005, Iwasa *et al.* 2007).

In the third instar larvae of *E. intermedius*, the concentration of 0.01ppm of ivermectin reduced cephalic capsule width, which could be related to a reduced feeding capacity. In *D. gazella* larvae exposed to ivermectin in the dung of animals after treatment, anatomical alterations in head structures were observed and were related with a reduced feeding capacity (Sommer *et al.* 1993). These anatomical changes in the head may have occurred in *E. intermedius* but were not studied.

The ivermectin at 0.01ppm concentration was only sublethal for *E. intermedius* larvae, but it increased their development time by 50%, these results show that the larvae represent the most unstable phase of the life cycle. Krüger and Scholtz (1997) perceived that in this same species, the development took two and a half times longer in specimens exposed to dung samples from day one and 14 after the treatment of cattle with ivermectin; but these authors did not determine the exact concentration utilized for each day of treatment, and therefore, comparisons cannot be made.

Several studies have demonstrated the ecotoxicity effect of ivermectin on coprophagous fauna (Wardhaugh & Rodriguez-Menendez 1988, Wardhaugh *et al.* 1993, Wardhaugh *et al.* 2001, Lumaret & Errouissi, 2002, Floate *et al.* 2005). In some of these cases (Errouissi *et al.* 2001, Hempel *et al.* 2006, Lumaret *et al.* 2007), we even know the effective concentration levels (EC), that may cause an adverse response or the concentration considered lethal

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(LC) for the species. More analyses with higher concentrations between 0.01 and 0.1ppm of ivermectin in dung are needed to establish the effective or lethal concentrations for larvae and adults of *E. intermedius*.

Recently, Römbke *et al*. (2010) showed that the determined ivermectin concentration in dung, obtained between days two to seven after treatment, varied from 0.05 to 0.11mg/kg of fresh weight (approximately 80% moisture in the dung); the equivalent values varied between 0.31 to 0.81mg/kg dung dry weight. This variation between the ivermectin concentration in fresh and dry dung had an increment of six to seven times; therefore, the authors recommended that care be taken in interpreting the results when dealing with ivermectin concentrations in samples of dung fresh or dry weight. In this study, though utilizing the method of dung spiked with ivermectin, the concentration of 0.01ppm was much lower than the minimum concentration in fresh dung determined by Römbke *et al*. (2010). The concentration of 1.0ppm was close to the maximum of 1mg/kg ivermectin in dung dry weight determined by Sommer *et al*. (1992) and Römbke *et al*. (2010). The concentration of 100ppm was beyond that found in the dung of subcutaneously or topically treated animals.

According to the preliminary results obtained from *E. intermedius*, the most adequate concentration interval of ivermectin must vary from 0.01 to 0.1ppm in fresh dung. More analyses with concentrations between 0.01 and 1.0ppm are necessary to establish the effective or lethal concentrations for *E. intermedius* larvae. In addition, more studies with other parasiticides and other species of dung beetles should be performed to determine their effects.

The change in development time of beetles such as *E. intermedius*, in addition to the time lag at the moment of emergence of the new adults in the field, may alter the abundance and diversity of dung beetle fauna common in cattle pastures (Krüger & Scholtz 1998a, Krüger & Scholtz 1998b, O'Hea *et al*. 2010, Römbke *et al*. 2010). The changes in abundance of an ecologically important species on cattle pastureland could also affect the productivity and health of grasslands, as observed in other countries (Edwards & Aschernborn 1987, Doube *et al*. 1988, Hutton & Giller 2003, Davis *et al*. 2004, Louzada & Carvalho-Silva 2009).

In *E. intermedius*, the impact on larval development is most important. Some theoretical models predicting the impact of ivermectin according to the frequency and dose of application indicate that the highest negative effect on dung beetle communities becomes evident when applications to cattle are frequent and coincide with the emergence of new generations. According to these models, the continuous and long-term use of ivermectin reduces the population of dung beetles (Sherrat *et al*. 1998, Wardhaugh *et al*. 1998, Wardhaugh *et al*. 2001).

Because of the aforementioned results, the knowledge of the biology of dung beetles is fundamental, but environmental health in cattle raising zones is also important. In Rancho "San Román" (La Laguna, Veracruz), ivermectin is subcutaneously applied to the cattle at a concentration of 0.2mg/kg twice a year (Florencio Portillo R., pers. com.). However, on other farms in the State of Veracruz, ivermectin application is known to vary from 2-4 times a year (Martínez & Cruz 2009); therefore, its presence in dung can be quite variable throughout the year.

Ivermectin has been utilized by the cattle farmers of Veracruz for more than 20 years (Martínez & Cruz 2009) without any regulation on its consumption and application; therefore, the prognostication of ecotoxicological effects on coprophagous fauna is of serious concern.

The State of Veracruz, as one of the main cattle producers in the country (Gobierno de Veracruz 2009), consumes large amounts of ivermectin, which could also be the case in other states of Mexico (Martinez & Cruz 2009). In Mexico, few studies reflect the problem of contamination originating from veterinary substances, parasiticides among them (Martínez *et al*. 2000, Lumaret & Martínez 2005, Martínez & Lumaret 2006, Martínez & Cruz 2009). This paper is the first in this

country dealing with the effect of ivermectin. It is necessary to carry out more toxicity tests of this parasiticide in other species and to study the abundance and diversity of dung beetle species in cattle raising areas.

It is recommended that cattle farmers apply parasiticide on dates subsequent to the emergence of the new generations. Likewise, the life cycle of the most common parasites in the cattle drove and their levels of parasitization should be known before applying any parasiticide. These practices would reduce the parasiticide impact on other beneficial fauna in cattle pasturelands and the associated economic losses to cattle farmers (Fincher 1981, Losey & Vaughan 2006).

Likewise, in Mexico, the use and sale of this type of substance should be regulated as in other countries, such as those of the European Union, Japan, and the U.S.A. (VICH 2004, Knacker *et al*. 2005, OECD 2009).

RESUMEN

El estado de Veracruz en México, es uno de los principales productores de ganado vacuno en México, asimismo utiliza diversas medicinas veterinarias para el control de enfermedades y parásitos. La ivermectina es una de las substancias más utilizadas para el control de parásitos. Sin embargo, se sabe por estudios hechos en otros países, que esta substancia tiene efectos negativos sobre la fauna coprófaga benéfica como los escarabajos del estiércol, pero no se han estudiado sus efectos sobre la fauna coprófaga de Veracruz o de México. Este estudio se realizó en condiciones de laboratorio, en donde se utilizó el estiércol vacuno a tres diferentes concentraciones de ivermectina para determinar su efecto sobre la supervivencia, fecundidad, fertilidad y desarrollo preimaginal de *Euoniticellus intermedius*. Por lo tanto, las tres concentraciones que se emplearon fueron: 0.01, 1.0 y 100ppm de ivermectina homogeneizada en estiércol vacuno fresco y dos testigos. Además, se utilizaron 20 parejas hembra-macho por tratamiento, entre cinco y 15 días de edad y mantenidos por 10 días a 27°C, 70% HR y 12hr luz. Se determinó la supervivencia de todos, la fertilidad en 20 hembras y el estado de madurez gonádica en 17 machos. Se determinó el desarrollo preimaginal en 162 masas-nido y 974 se dejaron continuar el desarrollo hasta la emergencia de los adultos. La ivermectina es tóxica a mayor concentración. La supervivencia de adultos se redujo casi a la mitad a dosis de 1.0ppm y fue nula a 100ppm. El ovario no fue afectado. Los testículos incrementaron de tamaño. La fecundidad y el peso de las masas-nido se redujeron. El desarrollo preimaginal se incrementó 0.5 veces a concentración 0.01ppm y las larvas del tercer estadio redujeron el ancho de la cápsula cefálica. El alargamiento del tiempo de desarrollo puede causar desfase de su ciclo de actividad en campo, lo que podría reducir su número y la eficiencia de los servicios ambientales que proporcionan.

Palabras clave: escarabajos coprófagos, toxicidad, masas-nido, desarrollo preimaginal.

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