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The negative effects of the residues of ivermectin in cattle dung using a sustained-release bolus on *Aphodius constans* (Duft.) (Coleoptera: Aphodiidae)

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Abstract – This paper reports the findings of two trials into the effects of the treatment of cattle with ivermectin slow-release (SR) bolus on the larval development of the dung beetle *Aphodius constans* Duft. Rectal faecal samples were collected prior to treatment and every 3 and 2 weeks in a first and second trial, respectively, and up to 156 days post-administration of the SR bolus. Faecal ivermectin concentration reached a peak at 63 days post-treatment (1427 $ng \cdot g^{-1}$) and ivermectin was detected up to 147 days post-treatment in the first trial (7.2 $ng \cdot g^{-1}$). First stage larvae of *A. constans* were reared with control or contaminated dung and adult beetles were counted after emergence. In the first trial, the comparison of pairwise samples showed that ivermectin prevented the development of larval *A. constans* until day 105, while at day 135 the rate of emergence was still significantly lower than the corresponding series of control ($p < 0.05$). In the second trial, the difference between control and treated series remained significant until 143 days post-treatment, with no emergence until 128 days post-administration of SR bolus to cattle. These results show the negative effect of ivermectin on the development of larval *A. constans*, even at a low concentration (38.4 $ng \cdot g^{-1}$). The administration of ivermectin sustained-release bolus to cattle was highly effective in killing dung beetle larvae for approximately 143 days after treatment. The results were similar when dung was obtained from a single animal kept alone, or from a blending of faecal pats obtained from a group of animals kept in field conditions during the whole trial period.

ivermectin / sustained-release (SR) bolus / cattle / dung beetle / rate of emergence / environmental risk

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Résumé – Effets négatifs des résidus d'ivermectine délivrés par un bolus à diffusion lente sur la survie du coprophage *Aphodius constans* (Duft.) (Coleoptera: Aphodiidae). À la suite du traitement de bovins par un bolus à diffusion lente d'ivermectine (12 $mg \cdot j^{-1}$), deux expérimentations indépendantes ont été conduites pour en étudier les conséquences sur le développement larvaire du coléoptère coprophage *Aphodius constans* Duft. Des échantillons de bouse ont été prélevés avant le traitement puis régulièrement, respectivement chaque 3 et 2 semaines pour la première et la seconde expérimentation, ceci jusqu'à 156 jours après l'administration du bolus aux animaux. Les bouses utilisées ont été congelées puis stockées plusieurs mois avant leur utilisation. La concentration fécale d'ivermectine atteint un pic 63 jours après le traitement (1427 $ng \cdot g^{-1}$) et l'ivermectine était encore détectable dans la bouse après 147 jours (7.2 $ng \cdot g^{-1}$). Des larves d'*A. constans* ont été nourries avec de la bouse (plusieurs séries, correspondant chacune à une date après traitement) et les imagos obtenus à l'issue du développement larvaire ont été décomptés à l'émergence. Dans la première expérience (animal isolé), la mortalité des larves a été totale jusqu'à 105 jours après le traitement, tandis que 135 jours après l'administration du bolus la bouse contenait encore suffisamment d'ivermectine pour que le taux d'émergence soit significativement inférieur à celui du témoin ($p < 0.05$). Dans la seconde expérience (bouses homogénéisées provenant de plusieurs animaux traités), les différences entre séries bolus et séries témoins restaient significatives jusqu'à 143 jours après le traitement, avec une émergence nulle jusqu'à 128 jours après le traitement. Ces résultats démontrent l'effet négatif de l'ivermectine sur le développement larvaire d'*Aphodius constans*, même à faible concentration (38.4 $ng \cdot g^{-1}$). On peut considérer que l'administration d'un bolus à diffusion lente d'ivermectine a un effet négatif sur les larves de coprophages jusqu'à 143 jours après le traitement. Ces résultats sont similaires si la bouse provient d'animaux isolés ou en mélange.

ivermectine / bolus à diffusion lente / bouse / coléoptère coprophage / taux d'émergence / risques environnementaux

1. INTRODUCTION

In grazed ecosystems, the recycling of organic materials of animal origin is a complex linkage of slow processes in which microorganisms and the edaphic fauna intervene at the soil surface as well as in the upper layers of the soil, where climatic conditions influence the activity of dung-breeding insects (Scarabaeidae, Hydrophilidae, Staphylinidae, larvae of Diptera) [11]. When beetles utilise dung pats, they dig small tunnels which weaken the pats and, at the same time, beetles inoculate the heart of pats with microorganisms as they carry spores of telluric fungi and microorganisms on their integument. Consequently, the presence of beetles stimulates microbial activity [2, 12]. Under such conditions, pats progressively become soil annexes, with the network of tunnels making the colonisation of pats by edaphic mesofauna easier.

It has been shown [11] that the activity of dung beetles in dung pats during the first month after deposition is essential for their breakdown. The artificial exclusion of all insects (both flies and beetles) from fresh cattle dung pats for only one month after the deposition of pats considerably lowers the rate of decay. Pats covered with wire gauze excluding beetles for one month took 1.7 to 2.2 times longer to decay than pats freely exposed to insects [11]. The exclusion of all insects for only one month shows the potential risks for pastures if dung beetles were eliminated by broad-spectrum systemic parasiticides administered to livestock for the control of helminths.

Ivermectin has a high potency at low concentrations against nematodes and arthropods, in particular against dung beetles and flies [8, 18, 20, 21], and it has been demonstrated that dung pats containing ivermectin are degraded significantly slower than pats containing no ivermectin [3, 13, 20, 21]. Although some authors [3, 15] have previously recommended that cattle breeders treat their cattle with subcutaneous injection of ivermectin when there is negligible dung beetle activity, the spread of the use of ivermectin slow-release bolus invalidates this recommendation, as ivermectin can be detected both in plasma and faeces up to 160 days post-administration of a sustained-release (SR) bolus to calves [1]. This 5-month period is so long that environmental risks become evident, as already expressed [8].

This paper presents the findings of an investigation into the effects of the treatment of cattle with ivermectin slow-release bolus on the larval development of the dung beetle *Aphodius constans* Duft. (Coleoptera: Aphodiidae). This is a very common species, widespread and distributed all over temperate and Mediterranean Europe. The adult beetles are active from November to May and oviposit into large and compact cattle dung pats. This species constitutes a good model for bioassays as it is very easy to rear in laboratory and the entire egg, larval and pupal development of *A. constans* takes place in dung pats. Typically, larvae spend all their life in contact with dung and they feed their way inside [10].

2. MATERIALS AND METHODS

2.1. Bolus

According to the manufacturer, the Ivomec® SR bolus device (Merial, Lyon, France) delivers ivermectin at a dose rate of 12 mg·day^{-1} over 135 days. It contains 1.72 g of ivermectin and is designed for cattle weighing between 150 and 450 kg.

2.2. Animals

Two trials were carried out independently.

1) Two clinically healthy Charolais steers, weighing approximately 350 kg each, were used in the first trial. One animal was treated with an intraruminal bolus; the second animal remained untreated (control). Animals were reared indoors and kept in separate boxes during the whole experimental period in the INRA experimental station of Nouzilly (France). The trial was conducted between April and September 1998.

2) Fifty 1.5-year-old Aubrac heifers, weighing 320 kg each on average, were used in the second trial. Twenty-six animals were treated with an intraruminal bolus; the other animals remained untreated (control). Animals were kept in field conditions during the whole experimental period in a farm close to Montpellier (France). No additional feed was supplied except hay and fresh water, provided via automatic drinkers. During the whole trial period, the two groups grazed in separated fields. The trial was conducted between March and August 1999.

2.3. Drug administration

The administration of the ivermectin SR bolus was given via a gun provided by the manufacturer and was designed to deliver the specific bolus under investigation. The animals were observed closely during the first 24 hours post-treatment to ensure that the bolus was not regurgitated.

2.4. Faecal sample collection

In the first trial, rectal faecal samples were collected prior to treatment and at 21, 43, 63, 85, 105, 135 and 147 days after administration of the SR bolus. The faeces samples were frozen in plastic bags at –20 °C until they were to be used to feed beetles. In the second experimental trial, rectal faecal samples were collected prior to treatment and at 4, 14, 28, 42, 56, 70, 84, 98, 112, 128, 143 and 156 days after the treatment. The dung collected from all animals of the second trial (treated and control animals, separately) was mixed thoroughly before being frozen.

2.5. Analytical methods

Ivermectin residues in the dung collected in the first trial were analysed by high performance liquid chromatography (HPLC) with automated solid phase extraction and fluorescence detection by the method previously described for ivermectin [1].

2.6. Feeding *Aphodius constans* larvae on cattle dung

First stage larvae were obtained in the field (experimental farm close to Montpellier, Southern France) from adult beetles feeding on ivermectin-free cattle dung. Two independent assays were carried out with this beetle species in the laboratory. (1) The dung from the first trial was used to produce ten replicates for days 21, 43 and 63 after treatment and 15 replicates for days 85, 105 and 135. Each replicate consisted of a plastic container (6 cm in diameter and 10 cm in height) filled with dung. (2) In the second trial, the dung from cattle grazed on pasture was sorted into 5-7 replicates as the control and into 15 replicates as the treated sample in days 4, 14, 28, 42, 56, 70, 84, 98, 112, 128, 143 and 156 after treatment. The two assays were conducted between April and June 2000, using dung previously frozen.

Ten first stage larvae obtained from the field (untreated cattle) were settled in each container at the bottom of small holes made into the dung. Containers were closed by a gauze lid and were kept under laboratory conditions (room temperature ranging between 21 and 23 °C) until the emergence of the beetles.

2.7. Statistical analysis

Adult beetles were counted after emergence and the average rates of emergence were compared (control and treated samples). We used the average rates of emergence as if the number of larvae in each container was the same (10 larvae per container), and the number of replicates was different between the control and the treated sample. The non-parametric Mann-Whitney test was used for a comparison of the samples (treated and control) taken pairwise [16] because the rate of emergence of adults for each date did not follow a normal distribution. All analyses were undertaken using the Statgraphics® Plus version 2 statistical package.

3. RESULTS

Faecal ivermectin reached a peak at 63 days after treatment (1427 $ng \cdot g^{-1}$). Ivermectin was detected up to 147 days after treatment in the first trial (7.2 $ng \cdot g^{-1}$), with 38.4 $ng \cdot g^{-1}$ at day 135 after treatment. Ivermectin was not detected in the control steer. The newly hatched *A. constans* beetles began to emerge 6 weeks after the larvae were settled in containers and continued two weeks later. Newly emerged beetles were counted three times as from the first emergence of beetles and cumulative emergence was taken into account in the calculation. In the control steer, the rate of emergence of beetles ranged from 39 to 55% (Tab. I). In the treated steer, the rate of emergence of beetles ranged from 0 to 32.7%, with no emergence until 105 days after administration of sustained-release bolus to the steer. The comparison of the whole samples taken pairwise showed that ivermectin stopped the larval development of *A. constans* until day 105, while in D135 the rate of emergence was still significantly lower than the corresponding series of control ($p < 0.05$).

In the control dung in the second trial, the rate of emergence of beetles ranged from 32 to 58.6% (Tab. II). In treated cattle, the rate of emergence of beetles ranged from 0 to 35.3%, with no emergence until 128 days after administration of sustained-release bolus. The difference between the two groups (control and treated) was significant

Table I. Mean percentage *Aphodius constans* emergence (± SD) from dung removed from untreated and ivermectin-treated steer (trial one). Ten larvae were reared in dung collected from 21 up to 135 days after administration of sustained-release bolus to steer (treatment) and from untreated steer (control).

Time after treatment (days)	*Control*		*Treatment*		*Statistical significance*
	N° of replicates	*% emergence (± SD)*	*N° of replicates*	*% emergence (± SD)*	
21	10	40 (± 25)	10	0	$P < 0.001$
43	10	39 (± 24)	10	0	$P < 0.001$
63	10	55 (± 22)	10	0	$P < 0.001$
85	15	40.7 (± 29)	15	0	$P < 0.001$
105	15	41.3 (± 26)	15	0	$P < 0.001$
135	15	54 (± 29)	15	32.7 (± 14.4)	$P < 0.05$

Table II. Mean percentage *Aphodius constans* emergence (± SD) from dung removed from untreated and treated ivermectin in cattle grazed on pasture (trial two). Ten larvae were reared in dung collected from 4 up to 156 days after administration of sustained release bolus to cattle (treatment) and from untreated cattle.

Time after treatment (days)	*Control*		*Treatment*		*Statistical significance*
	N° of replicates	*% emergence (± SD)*	*N° of replicates*	*% emergence (± SD)*	
4	5	38.0 (± 30.3)	15	0	$P < 0.001$
14	5	42.0 (± 21.7)	15	0	$P < 0.001$
28	5	32.0 (± 13.0)	15	0	$P < 0.001$
42	5	46.0 (± 32.1)	15	0	$P < 0.001$
56	5	54.0 (± 18.2)	15	0	$P < 0.001$
70	5	56.0 (± 27.9)	15	0	$P < 0.001$
84	7	50.0 (± 30.0)	15	0	$P < 0.001$
98	7	35.7 (± 29.4)	15	0	$P < 0.001$
112	7	35.7 (± 22.3)	15	0	$P < 0.001$
128	7	52.9 (± 24.3)	15	0	$P < 0.001$
143	7	58.6 (± 26.7)	15	24.7 (± 27.0)	$P < 0.05$
156	7	57.1 (± 26.9)	15	35.3 (± 26.4)	n.s.

n.s.: not significant

($p < 0.001$). The difference between control and treated groups remained significant until 143 days post-treatment ($p < 0.05$). Differences were not significant between control and treated dung at day 156 after treatment.

4. DISCUSSION

The evaluation of ivermectin in the dung of a single animal was only made to verify if the concentrations obtained in trial 1 were in accordance with those previously documented by Alvinerie et al. [1] using the same analytical methods. The persistence of ivermectin in dung corresponded both in concentration and time with the results described in Alvinerie et al. [1]. Our results show the negative effect of ivermectin on the larval development of *Aphodius constans*, even at low concentration (38.4 $ng \cdot g^{-1}$). The administration of ivermectin sustained-release bolus to cattle was highly effective in killing dung beetle larvae for approximately 143 days after treatment. The results were similar when dung was obtained from a single animal kept alone, or from a blending of pats obtained from a group of animals kept in field conditions during the whole trial period. *Aphodius* larvae constitute a good predictive model for what should appear in the future, as the pats deposited on grazed pastures persist as hot spots of ivermectin. Sommer et al. [17] demonstrated that the toxicity is aggravated by the aggregation of many insects from the pasture ecosystem in pats.

The persistence of ivermectin in faeces for more than 4.5 months after administration of an ivermectin slow-release bolus has already been demonstrated [1]. In the latter study, the authors showed that faecal ivermectin concentration gradually increased to reach a peak concentration of 4.1 $\mu g \cdot g^{-1}$ at 4 days post-administration, and that the level dropped to a steady-state concentration of 1.18 $\mu g \cdot g^{-1}$ which was maintained up until 120 days after treatment. Herd et al. [9] reported a lower plateau concentration (0.5 $\mu g \cdot g^{-1}$) that lasted up until 49 days after treatment. In the present experiment, the concentration ranged between 0.7 and 1.4 $\mu g \cdot g^{-1}$ between days 4 and 105 after treatment, with no emergence of beetles during this period. By day 135 after administration, with a concentration at 0.038 $\mu g \cdot g^{-1}$, the rate of emergence of beetles was still significantly lower than in the control dung ($p < 0.05$).

Wall and Strong [21] first drew attention to the powerful insecticidal effect of avermectins on non-target species of the dung-breeding community. Ivermectin residues in cattle dung particularly affect Cyclorrhaphan Diptera [20]. The larval mortality of *Musca domestica* [13] and *Musca vetustissima* [15, 22, 23] is significantly higher than in controls more than one month after a subcutaneous injection of ivermectin to cattle. It is the same for other Diptera such as *Neomyia cornicina* [6, 7, 24] or *Scatophaga stercoraria* [19] whose larvae were unable to survive in dung collected up to one month after the cattle had been treated with ivermectin. In the same way, ivermectin has well-documented effects on a variety of beetles associated with dung and has been shown to have a wide range of sublethal effects in addition to its acute toxicity [17, 18]. The fertility and the adult emergence rate decreased, whereas the mortality of newly hatched adults and the mortality of larvae could be important. This was demonstrated for several dung beetle species such as *Onthophagus*, *Euoniticellus*, *Copris*, *Onitis* and *Aphodius* genera [4, 13, 14, 17, 22, 24].

In conclusion, this study has for the first time linked the patterns of faecal excretion of ivermectin administered from a sustained-release bolus over the full period of delivery with the larval mortality of a widespread dung beetle species. This suggests that the faecal excretion of high ivermectin concentrations for prolonged periods after bolus administration to cattle may represent a threat to the ecosystem, as a reduction of insect activity was associated with slower dung pat degradation [5, 11, 21, 22].

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