



**HAL**  
open science

## Licking behaviour induces partial anthelmintic efficacy of ivermectin pour-on formulation in untreated cattle.

Alain Bousquet-mélou, Philippe Jacquet, Hervé Hoste, Julien Clément,  
Jean-Paul Bergeaud, Michel Alvinerie, Pierre-Louis Toutain

### ► To cite this version:

Alain Bousquet-mélou, Philippe Jacquet, Hervé Hoste, Julien Clément, Jean-Paul Bergeaud, et al.. Licking behaviour induces partial anthelmintic efficacy of ivermectin pour-on formulation in untreated cattle.. *Int J Parasitol*, 2011, 41 (5), epub ahead of print. 10.1016/j.ijpara.2010.12.007 . hal-00556815

**HAL Id: hal-00556815**

**<https://hal.science/hal-00556815>**

Submitted on 7 Feb 2011

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Licking behaviour induces partial anthelmintic efficacy of**  
2 **ivermectin pour-on formulation in untreated cattle**

3

4 **Alain Bousquet-Mélou** <sup>a,d, \*</sup>, **Philippe Jacquet** <sup>b,d</sup>, **Hervé Hoste** <sup>b,d</sup>, **Julien**  
5 **Clément** <sup>b,d</sup>, **Jean-Paul Bergeaud** <sup>b,d</sup>, **Michel Alvinerie** <sup>c,d</sup> and **Pierre-Louis**  
6 **Toutain** <sup>a,d</sup>

7

8 <sup>a</sup> UMR181 Physiopathologie et Toxicologie Expérimentales, INRA, ENVT, Ecole  
9 Nationale Vétérinaire de Toulouse, France

10 <sup>b</sup> UMR1225 Interactions Hôtes Agents Pathogènes, INRA, ENVT, Ecole Nationale  
11 Vétérinaire de Toulouse, France

12 <sup>c</sup> UR66 Pharmacologie-Toxicologie, INRA, Toulouse, France

13 <sup>d</sup> Université de Toulouse, Toulouse, France.

14

15 \*Corresponding author : Ecole Nationale Vétérinaire de Toulouse, 23 chemin des  
16 Capelles, BP 87614, 31076 Toulouse cedex 03, France

17 Tel : +33 561 193 925; Fax : +33 561 193 917 ; email : [a.bousquet-melou@envt.fr](mailto:a.bousquet-melou@envt.fr)

18 **ABSTRACT**

19

20 Licking behaviour in cattle has been reported to account for the disposition of  
21 topically administered macrocyclic lactones. However, its impact on anthelmintic  
22 efficacy remains to be established. Therefore, we evaluated the impact of ivermectin  
23 exchange between cattle on the reduction in the faecal egg count (FEC) after pour-  
24 on administration in a group of 10 heifers experimentally infected with *O. ostertagi*  
25 and *C. oncophora*. Four treated (500 µg/kg, pour-on) and six untreated animals were  
26 put together after treatment and plasma and faecal exposure to ivermectin as well as  
27 the FECs were evaluated before and over 40 days after pour-on. Ivermectin was  
28 detected in plasma and faeces of the six untreated heifers, with maximal exposures  
29 2-3-fold lower than the minimal exposures in treated animals. The interindividual  
30 variability of exposure was very high in untreated, a 10-fold difference between the  
31 upper and lower range limits, compared to treated heifers, where there was only a 2-  
32 fold difference. Anthelmintic efficacy, expressed as an average reduction of the FECs  
33 over the experimental period, was maximal in the treated group. In untreated heifers,  
34 anthelmintic efficacies ranged from zero to maximal efficacy, with intermediary values  
35 between 30 and 80%. The use of a classical pharmacodynamic model demonstrated  
36 a clear relationship between exposure and efficacy and enabled us to define the  
37 critical plasma or faecal ivermectin concentrations delimiting an exposure window  
38 associated with partial anthelmintic efficacy. This range of ivermectin plasma  
39 concentrations [0.1–1ng/mL] could be considered as a potential selection window for  
40 anthelmintic resistance. Finally, our results show that macrocyclic lactone exchange  
41 between cattle after pour-on administration, resulting from natural grooming  
42 behaviour, can significantly impact on anthelmintic efficacy. This raises several  
43 issues such as the design of comparative clinical trials and the occurrence of partial

44 efficacy which is considered a risk factor for the development of anthelmintic  
45 resistance.

46

47

48 **Key words:** Macrocyclic lactones; Ivermectin; topical ; allo-grooming ; Anthelmintic

49 resistance ; Cattle

## 50 **1. Introduction**

51  
52 Macrocyclic lactones (MLs) such as ivermectin (IVM), doramectin (DOR),  
53 eprinomectin (EPR) and moxidectin (MOX) are administered topically to conveniently  
54 treat different parasitic infections in cattle. The efficacy of pour-on (PO) formulations  
55 depends on systemic (blood) exposure for all internal parasites. We have reported  
56 that after a topical administration of DOR or IVM (500 µg/kg) the range of individual  
57 exposures was very large, with values of the areas under the plasma concentration-  
58 time curves (AUC) showing up to a 3.6-fold variation for IVM and up to a 2.2-fold  
59 variation for DOR, indicating a poor inter-individual reproducibility of this route of  
60 administration (Gayrard et al., 1999). Low and variable individual exposures after PO  
61 administration was also reported for MOX in calves (Sallovitz et al., 2002) and for  
62 EPR in dairy cows (Alvinerie et al., 1999). In addition, in a parallel trial involving two  
63 groups of 20 cattle dosed by PO (500 µg/kg) or subcutaneously (SQ, 200 µg/kg) with  
64 either DOR or IVM (Toutain et al., 1997), we showed that the SQ administration was  
65 associated with not only a higher systemic exposure for both products but also a  
66 much lower inter-animal variability than PO (coefficients of variation of 3.1% and  
67 4.7% vs. 25% and 37% for SQ vs. PO formulations of DOR and IVM respectively).  
68 These results indicated that the PO formulation was responsible for the poor  
69 reproducibility of plasma drug exposure. This phenomenon was better understood  
70 when it was demonstrated that the actual disposition of MLs poured on the backs of  
71 cattle was largely influenced by both self-and allo-grooming and that consequently a  
72 large fraction of the MLs was actually orally and erratically ingested by the animal  
73 itself (Laffont et al., 2001; Laffont et al., 2003) or exchanged with another animal in  
74 the herd (Bousquet-Mélou et al., 2004) rather than directly absorbed throughout the  
75 skin. The consequences of this largely overlooked “behavioural clearance”

76 mechanism for topically administered drugs in cattle are manifold, including  
77 inconsistency of drug efficacy, unexpected residue levels (Imperiale et al., 2009),  
78 contamination of the environment (Herd et al., 1996), or the design of clinical and  
79 bioequivalence trials (Barber and Alvinerie, 2003). It is often quoted that underdosing  
80 and subtherapeutic ML exposure are factors favouring reduced anthelmintic  
81 efficacies in the field (El-Abdellati et al., 2010), which can in turn favour the  
82 emergence of anthelmintic resistance (Smith et al., 1999) for which there is  
83 experimental evidence (Van Zeveren et al., 2007). As drugs poured on the back of  
84 treated animals can easily be exchanged between animals of a herd by allo-licking  
85 we hypothesized that some animals could easily be underexposed to MLs especially  
86 if only some animals within a herd are treated. Under these conditions, undesirable  
87 subtherapeutic concentrations could be anticipated in both treated and untreated  
88 animals.

89 In the present study using an experimental infection, we addressed the question of  
90 the occurrence of a possible underexposure window in a group of young cattle for  
91 which only a few of the animals were treated with an IVM PO dose. More precisely,  
92 we sought to establish a range of plasma and faecal IVM concentrations associated  
93 with partial anthelmintic efficacy.

94 **2. Materials and methods**

95

96 *2.1. Experimental Animals*

97

98 Ten heifers, nine Prim Holstein and one Limousine, which were 95-225-days old at  
99 the beginning of the trial, were first de-wormed with an oral dose of oxfendazole  
100 (5mg/kg) (Synanthic®, Merial, France). Faecal egg counts (FEC) were performed  
101 according to Raynaud (Raynaud, 1970) to check the efficacy of the oxfendazole  
102 drench. All the animals were negative at the time of the future L3 challenge. The  
103 heifers were housed together in a small yard (no grass) to prevent re-infestation.  
104 They were fed with a commercial concentrate diet (2 kg per day) and hay ad libitum.

105

106 *2.2. Experimental infection, treatment and sampling*

107

108 Three weeks after the de-worming, each heifer was orally infected with 20,000 L3  
109 of *Ostertagia ostertagi* and 20,000 L3 of *Cooperia oncophora* (adapted from  
110 (Vercruysse et al., 2000)). Four out of the ten animals were randomly selected to be  
111 treated 35 days after the experimental infection with an IVM PO dose (500µg/kg,  
112 Ivomec® bovine pour-on, Merial France). IVM was gently poured down the dorsal  
113 middle-line according to the manufacturer's recommendations. The six other  
114 untreated cattle were considered as controls and remained permanently housed with  
115 the group of the four treated animals. Before treatments, the mean and standard  
116 deviation of egg excretion in the treated and untreated groups were similar : 538 ±  
117 448 and 503 ± 392 eggs per gram (EPG), respectively. After the IVM treatment,  
118 efficacy was assessed by daily FEC measurements over two weeks and then three  
119 times weekly up until five weeks after the IVM treatment. Blood samples (n=21 per  
120 animal) were regularly collected from the jugular vein before (control value) and up to

121 day 36 post-IVM administration. Faecal samples (n=26 per animal) were obtained  
122 directly from the rectum between 08:00 am and 09:00 am. They were regularly  
123 collected before (4 control samples) and after IVM administration up to 41 days after  
124 IVM administration.

125

### 126 2.3. Analytical procedures

127

128 IVM (22, 23-dihydroavermectin B1a) concentrations in plasma and faeces were  
129 measured using a HPLC technique as previously described (Alvinerie et al., 1987).  
130 The limits of quantification of IVM were 0.05 ng/mL for plasma and 0.5 ng/g for wet  
131 faeces. Accuracy and precision (intra-assay variation) expressed as relative standard  
132 deviation were less than 8 and 6%, respectively.

133

### 134 2.4. FEC and efficacy measurements

135

136 FECs were carried out using the modified McMaster method (Raynaud, 1970) with  
137 a sensitivity of 7 eggs per gram of faeces.

138 The time development of individual efficacy was assessed using daily FECs. The  
139 percentage (from 0 to 100%) of reduction from the control FEC (geometric mean of  
140 the four control measures) was calculated daily using the following equation:

$$141 \quad FEC_{reduction(\%)}(dayi) = 100 \times \left( 1 - \frac{FEC_{dayi}}{FEC_{control}} \right) \quad \text{Equation 1}$$

142 where  $FEC_{dayi}$  is the FEC at day  $i$  post-IVM administration.

143 When the FEC after IVM administration was higher than control values, its value was  
144 arbitrarily fixed at the control value. The area under the  $FEC_{reduction(\%)}(dayi)$  versus  
145 time curve ( $AUC_{FECreduction(\%)[0-dayi]}$ ) was calculated using the trapezoidal rule and then  
146 the efficacy at a given day ( $dayi$ ) was expressed as the  $AUC_{FECreduction(\%)[0-dayi]}$  divided



147 by the time interval [0-*dayi*]:

$$148 \quad \text{Efficacy}(\%)_{\text{day}i} = \frac{AUC_{FEC\text{ reduction}(\%)[0-\text{day}i]}}{Time_{[0-\text{day}i]}} \quad \text{Equation 2}$$

149 *Efficacy*(%)<sub>dayi</sub> corresponds to the average *FEC*<sub>reduction(%)</sub> over the time interval [0-  
150 *dayi*]. Expressing efficacy this way gives at each day, not the result observed on that  
151 day, but an average efficacy from day 0 to *dayi*. The advantages of this method were  
152 obtaining a smooth curve that avoided the spurious rebound in some daily FECs, and  
153 taking into account the more or less rapid time development of efficacy associated  
154 with the unpredictable beginning of IVM exposure in untreated animals.

155

## 156 2.5. Pharmacokinetic measurements and pharmacodynamic modelling

157

158 Pharmacokinetic analyses were performed using WinNonlin Professional version  
159 5.2 (Pharsight Corporation, Cary, NC, USA). Areas under the plasma or faecal IVM  
160 concentration curves (*AUC*<sub>IVM</sub>) were obtained by the trapezoidal rule from time 0 to  
161 the last measurable concentration (Non-compartmental module of WinNonlin).

162 The exposure-efficacy relationship was described using the following sigmoid  
163 Emax model:

$$164 \quad \text{Efficacy}(\%)_{\text{day}30} = \frac{E_{\text{max}} \times (AUC_{IVM})^n}{(AUC_{IVM})_{50}^n + (AUC_{IVM})^n} \quad \text{Equation 3}$$

165 where *Efficacy*(%)<sub>day30</sub> is the individual efficacy at day 30 calculated using Equation 2,  
166 *E*<sub>max</sub> is the maximal estimated *Efficacy*(%), *AUC*<sub>IVM</sub> is the plasma or faecal IVM AUC  
167 as obtained for each individual, *n* is the Hill coefficient and (*AUC*<sub>IVM</sub>)<sub>50</sub> is the plasma  
168 or faecal IVM AUC giving 50% of *E*<sub>max</sub>. Descriptive statistics were obtained with  
169 WinNonlin and the results are reported as means (arithmetic or geometric) and SD.

170 **3. Results**

171

172 *3.1. Plasma and faecal exposure to IVM in treated and untreated heifers*

173

174 Figs 1 and 2 show the semi-logarithmic plot of individual plasma and faecal  
175 exposures in the ten heifers. Visual inspection of the graphs indicates that all the  
176 cattle (treated and untreated) were exposed to IVM, but that treated cattle were  
177 always more exposed to IVM than untreated ones. Mean and extreme  $AUC_{IVM}$  values  
178 are given in Table 1.

179 Table 1 shows that the mean plasma  $AUC_{IVM}$  of untreated cattle was 9.3% of the  
180 mean plasma  $AUC_{IVM}$  of treated cattle. However, on an individual basis, the highest  
181 value for an untreated heifer reached 36.5% of the lowest value for the treated cattle.

182 Faecal IVM exposure confirmed that the six untreated cattle ingested by allo-licking  
183 some of the IVM poured on the back of the four treated animals. The mean faecal  
184  $AUC_{IVM}$  of untreated cattle represented 14.8% of the mean faecal  $AUC_{IVM}$  of treated  
185 cattle but the most exposed of the untreated heifers reached 53.6% of the value of  
186 the least exposed treated cattle. It is worth to note that the faecal excretion of IVM at  
187 detectable levels was shorter in the untreated heifers than in the treated ones.

188

189 *3.2. FEC reduction in treated and untreated heifers*

190

191 The time development of IVM anthelmintic activity from day 1 to day 30 post-IVM  
192 administration is shown in Fig. 3. Individual FEC expressed as percent of control  
193 values clearly shows partial and highly variable FEC reductions in the untreated  
194 group whereas FEC reductions were almost total in the treated group (Fig. 3, panel  
195 A). It should be noticed that FEC reduction was also total for one untreated animal  
196 (open squares). In untreated animals, *Efficacy(%)* ranged from 0% (one heifer) to

197 approximately 95% (one heifer), with intermediary values from around 30% to 80% in  
198 the four other untreated animals (Fig. 3, panel B). It should be noted that the heifer  
199 exhibiting no efficacy (Fig. 3, star symbols) was the one for which plasma and faecal  
200 exposures were the lowest (2.1 ng\*day/mL and 89.5 ng\*day/g), while the highest  
201 efficacies among untreated cattle were observed for the two heifers exhibiting the  
202 highest plasma and faecal IVM exposures (Fig. 3, open square and triangle).

203 In treated animals, *Efficacy*(%) was about 95% at day 30, but the time development  
204 of *Efficacy*(%) showed some variability. This variability can be exemplified when  
205 considering the efficacy level of 80%, which was achieved in the four treated and two  
206 untreated animals: the times to reach this level were 3 days for three heifers (two  
207 treated, one untreated) and 9 days for the three others (two treated, one untreated).

208

### 209 3.3. Modelling of the exposure – efficacy relationship

210

211 In order to better characterize the relationship between efficacy and IVM exposure,  
212 individual IVM efficacies at day 30 were plotted against the corresponding plasma or  
213 faecal  $AUC_{IVM}$  (Fig. 4). The observed data were fitted using the sigmoid Emax model  
214 described by Equation 3, and the estimated parameters of the fitting are presented in  
215 Table 2. This model allowed us to compute the plasma or faecal  $AUC_{IVM}$   
216 corresponding to selected levels of *Efficacy*(%), that are presented in Table 3. By  
217 dividing these  $AUC_{IVM}$  by a standard duration of exposure of 21 days, we computed  
218 the corresponding average plasma or faecal concentrations associated with the  
219 selected levels of *Efficacy*(%). Inspection of Table 3 indicates that an average  
220 plasma IVM concentration over 1.2 ng/mL maintained for 21 days was fully  
221 efficacious ( $Efficacy(\%)_{day30} > 90\%$ ), while lower concentrations between 0.1 and 0.6  
222 ng/mL were associated with efficacies ranging from 20% to 80%.

#### 223 4. Discussion

224

225 The present experiment confirms that the natural grooming behaviour of cattle  
226 accounts for the pharmacokinetic disposition of topical MLs and that allo-licking  
227 allows significant exchanges of IVM between animals in a group (Bousquet-Mélou et  
228 al., 2004). More importantly, we report here the consequences of this phenomenon in  
229 terms of anthelmintic efficacy, showing that an untreated animal may be partially or  
230 totally cured from an experimental parasitic challenge. This raises several issues  
231 related to the design of comparative clinical trials and to the possible drug  
232 underexposure, a claimed risk factor favouring the emergence of resistance.

233 The design of the present experiment was selected to represent a worst-case  
234 scenario for ML treatment namely a situation where only a part (40%) of a herd is  
235 treated. Due to the apportionment of the total administered dose ( $4 \times 500 \mu\text{g}/\text{kg}$ )  
236 between the different members of the group, a possible underexposure was  
237 anticipated in both treated and untreated animals. In the present experiment, all  
238 treated animals were in fact sufficiently exposed to obtain maximal IVM efficacy,  
239 despite the fact that they shared part of their dose with their untreated congeners as  
240 a result of allo-licking activity. It should be pointed out here that expressing efficacy  
241 as an average  $FEC_{reduction(\%)}$  over a time period (Equation 2) led to maximal values at  
242 30 days lower than 100%, even if daily FEC reductions could reach 100% during the  
243 last days (data not shown). This was due to the integration in the calculation of the  
244 progressive increase of FEC reduction during the first days post-administration  
245 (Fig.3). Moreover, taking into account the time development of the reduction in the  
246 FECs highlighted its clear slowing down in half of the treated cattle (time to reach  
247 80% efficacy increasing from 3 days to 9 days), which might be attributed to the fact  
248 that IVM loss by allo-licking occurred, mainly during the first days post-administration

249 (Laffont et al., 2003).

250 The average plasma IVM exposure values ( $AUC_{IVM}$ ) of treated animals ( $121\pm 43$   
251 ng.day/mL) was similar to those reported in a trial (Gayrard et al., 1999) in which all  
252 the animals were treated ( $115\pm 43$  ng.day/mL), and in a trial (Bousquet-Mélou et al.,  
253 2004) where two treated cattle were pooled with six non-treated congeners ( $81\pm 27$   
254 ng.day/mL). In addition, by using the sigmoid Emax model to describe the exposure-  
255 efficacy relationship, we evaluated the critical plasma IVM exposure corresponding to  
256 90% efficacy to 25 ng.day/mL (Table 3). This value is much lower than those we  
257 observed both in the treated cattle of the present study (78-181 ng.day/mL, Table 1)  
258 and in a previous experiment where two out of eight cattle had been treated (61-100  
259 ng.day/mL, (Bousquet-Mélou et al., 2004). These results indicate that it is unlikely  
260 that under field conditions allo-licking will be responsible for a loss of IVM  
261 anthelmintic efficacy in treated cattle. In contrast, plasma IVM exposure in untreated  
262 cattle ranged from 2.1 to 28.4 ng.day/mL in the present experiment, in agreement  
263 with the range of plasma IVM exposures [3-43 ng.day/mL] observed in the six  
264 untreated cattle from the same previous experiment (Bousquet-Mélou et al., 2004).  
265 Such plasma IVM exposures cover the range from 10-20% to maximal efficacy  
266 (Table 3). Thus the population at risk of underexposures leading to partial  
267 anthelmintic efficacy appears to be the untreated rather than the treated animals.  
268 This merits attention when considering the potential factors responsible for reduced  
269 anthelmintic efficacies observed in the field, which as recently pointed out (El-  
270 Abdellati et al., 2010), should not be systematically attributed to resistant parasites.  
271 However, as it has been shown that an exposure of 14 ng.day/mL was able to initiate  
272 a process of resistance selection (Van Zeveren et al., 2007), our results are also of  
273 relevance to the discussion concerning the potential emergence of anthelmintic

274 resistance.

275 Many comparative clinical trials have been carried out to compare, in the same  
276 setting, different topical formulations or to compare topical versus non-topical  
277 formulations. Such trials can be severely biased if the two groups of animals are not  
278 totally separated. For example, a slow release bolus of fenbendazole was compared  
279 to a PO formulation of doramectin and a control non-treated group (Houffschmitt et  
280 al., 2003). After treatment, all the cattle of the three groups were turned out on the  
281 same pasture. It was shown that the bolus performed better in terms of FEC  
282 reduction, with no significant difference between the control and the doramectin  
283 groups at day 56 (mean $\pm$ SD of FECs were 10 $\pm$ 21% of pre-treatment FECs for the  
284 fenbendazole group, 70 $\pm$ 111% for the PO doramectin group and 80 $\pm$ 95% for the  
285 control group). Considering the results of the present experiment, these data could  
286 be interpreted as follows: 1) the bolus group was fully exposed to fenbendazole and  
287 likely to a fraction of the doramectin doses, thus contributing to its better activity, 2)  
288 the anthelmintic activity in the doramectin group was declining at day 56 as the claim  
289 warrant efficacy for a lower period, 3) the control group was exposed to doramectin  
290 thanks to allo-licking activity in some animals, thus promoting a partial activity in  
291 these animals and contributing to the large inter-individual variability of FECs and to  
292 a mean not different from the doramectin group. Barber and Alvinerie (2003) drew  
293 attention to this possible cross-contamination during a comparative clinical trial,  
294 where individuals from the treated groups (four MLs administered as PO) and from  
295 the control group, were put together in the same paddocks. They showed that 7 days  
296 after treatment, the faeces of 80% of the animals in the control group contained  
297 detectable amounts of at least one ML, with 55% of the animals having two or more  
298 MLs. At the same time, they observed that post-treatment FECs were reduced in the

299 control group.

300 Emergence of anthelmintic resistance is another issue for PO formulations  
301 because underexposure of worms is often quoted as one of the risk factors (Smith et  
302 al., 1999), even if “underexposure” was classically referring to under-dosing of  
303 treated animals rather than the “unexpected dosing” of untreated animals. In the  
304 present experiment, we assessed the range of plasma IVM concentrations  
305 associated with a partial efficacy, i.e. a situation putatively able to develop a selective  
306 pressure by eliminating the most susceptible subpopulations of worms. In our  
307 conditions of worm load, we estimated this possible selection window between 0.1  
308 and 1 ng/mL of plasma IVM concentrations. Above 1 ng/mL, efficacy tends to be  
309 maximal ( $EC_{90}=1.2$  ng/mL) and under 0.1 ng/mL ( $EC_{20}=0.114$  ng/mL) anthelmintic  
310 efficacy is probably not sufficient to eliminate all susceptible worms. The question of  
311 what is the most dangerous exposure level to promote anthelmintic resistance, i.e. to  
312 promote selection for parasites possessing genes that confer survival fitness, was  
313 addressed using a mathematical model and it was shown that no simple  
314 recommendation could be made to reduce the selection pressure for anthelmintic  
315 resistance (Smith et al., 1999). Indeed, the extent of the selection window could not  
316 be a simple drug property but more probably depends on a complex interaction  
317 between drug exposure and the genetic status of the initial worm population.

318 Nevertheless, the most important recent concept in preventing the development of  
319 anthelmintic resistance does not seem to be related to dosing rate but rather to the  
320 concept of refugia, i.e. a proportion of the worm population that is not exposed to the  
321 drug (Kaplan, 2004). In this context, our results question the use of pour-on  
322 formulations of anthelmintic drugs for the development of targeted selective  
323 treatment in cattle as a strategy for maintaining refugia (Hoglund et al., 2009; Gaba

324 et al., 2010). Indeed, such strategy implies the co-habitation of treated and untreated  
325 animals, which is precisely the situation at risk for the occurrence of underexposures  
326 consecutive to licking-driven drug transfer in the untreated ones.

327 To conclude, the present experiment confirms that the disposition of macrocyclic  
328 lactones administered as PO formulations is influenced by the social behaviour of  
329 cattle, explaining a poor inter-individual reproducibility of plasma exposure. In terms  
330 of risk of underexposure of worms to the drugs, the worst situation seems when only  
331 a proportion of the animals within a herd are treated, leading the untreated animals to  
332 have plasma drug exposures associated with a partial anthelmintic efficacy and  
333 corresponding to a possible anthelmintic resistance selection window.



334 **Acknowledgments**

335 The authors wish to express their thanks to Pr. J. Vercruysse from Ghent University  
336 who kindly provided the infective larvae of both parasite species. The authors also  
337 thank J.F. Sutra for technical assistance.

338 **References**

339

- 340 Alvinerie, M., Sutra, J.F., Galtier, P., Toutain, P.L., 1987. Determination of ivermectin  
341 in milk by high performance liquid chromatography. *Ann Rech Vet* 18, 269-274.
- 342 Alvinerie, M., Sutra, J.F., Galtier, P., Mage, C., 1999. Pharmacokinetics of  
343 eprinomectin in plasma and milk following topical administration to lactating  
344 dairy cattle. *Res Vet Sci* 67, 229-232.
- 345 Barber, S., Alvinerie, M., 2003. Comment on "A comparison of persistent anthelmintic  
346 efficacy of topical formulations of doramectin, eprinomectin, ivermectin and  
347 moxidectin against naturally acquired nematode infections of beef calves" and  
348 problems associated with mechanical transfer (licking) of endectocides in  
349 cattle. *Vet Parasitol* 112, 255-257.
- 350 Bousquet-Mélou, A., Mercadier, S., Alvinerie, M., Toutain, P.L., 2004. Endectocide  
351 exchanges between grazing cattle after pour-on administration of doramectin,  
352 ivermectin and moxidectin. *Int J Parasitol* 34, 1299-1307.
- 353 El-Abdellati, A., Charlier, J., Geldhof, P., Levecke, B., Demeler, J., von Samson-  
354 Himmelstjerna, G., Claerebout, E., Vercruysse, J., 2010. The use of a  
355 simplified faecal egg count reduction test for assessing anthelmintic efficacy  
356 on Belgian and German cattle farms. *Vet Parasitol* 169, 352-357.
- 357 Gaba, S., Cabaret, J., Sauve, C., Cortet, J., Silvestre, A., 2010. Experimental and  
358 modeling approaches to evaluate different aspects of the efficacy of Targeted  
359 Selective Treatment of anthelmintics against sheep parasite nematodes. *Vet*  
360 *Parasitol* 171, 254-262.
- 361 Gayrard, V., Alvinerie, M., Toutain, P.L., 1999. Comparison of pharmacokinetic  
362 profiles of doramectin and ivermectin pour-on formulations in cattle. *Vet*  
363 *Parasitol* 81, 47-55.
- 364 Herd, R.P., Sams, R.A., Ashcraft, S.M., 1996. Persistence of ivermectin in plasma  
365 and faeces following treatment of cows with ivermectin sustained-release,  
366 pour-on or injectable formulations. *Int J Parasitol* 26, 1087-1093.
- 367 Hoglund, J., Morrison, D.A., Charlier, J., Dimander, S.O., Larsson, A., 2009.  
368 Assessing the feasibility of targeted selective treatments for gastrointestinal  
369 nematodes in first-season grazing cattle based on mid-season daily weight  
370 gains. *Vet Parasitol* 164, 80-88.
- 371 Houffschmitt, P., Marciat, D., Roy, O., Skowronski, V., Holland, W., 2003. Continuous  
372 release bolus vs. pour-on application of anthelmintic during first grazing  
373 season: comparison of growth performance and egg shedding. In, EAVPT  
374 Congress, Vol. 26, *J Vet Pharmacol Therap*, pp. 126-127.
- 375 Imperiale, F., Sallovitz, J., Farias, C., Lifschitz, A., Lanusse, C., 2009. Licking  
376 induced changes to the pattern of moxidectin milk elimination after topical  
377 treatment in dairy cows. *J. Vet. Pharmacol. Therap.* 32, 534-540.
- 378 Kaplan, R.M., 2004. Drug resistance in nematodes of veterinary importance: a status  
379 report. *Trends Parasitol* 20, 477-481.
- 380 Laffont, C.M., Alvinerie, M., Bousquet-Mélou, A., Toutain, P.L., 2001. Licking  
381 behaviour and environmental contamination arising from pour-on ivermectin  
382 for cattle. *Int J Parasitol* 31, 1687-1692.
- 383 Laffont, C.M., Bousquet-Mélou, A., Bralet, D., Alvinerie, M., Fink Gremmels, J.,  
384 Toutain, P.L., 2003. A pharmacokinetic model to document the actual  
385 disposition of topical ivermectin in cattle. *Vet Res* 34, 445-460.

- 386 Raynaud, J.P., 1970. [Study of the efficiency of a quantitative coproscopic technic for  
387 the routine diagnosis and control of parasitic infestations of cattle, sheep,  
388 horses and swine]. *Ann Parasitol Hum Comp* 45, 321-342.
- 389 Sallovitz, J., Lifschitz, A., Imperiale, F., Pis, A., Virkel, G., Lanusse, C., 2002. Breed  
390 differences on the plasma availability of moxidectin administered pour-on to  
391 calves. *Vet J* 164, 47-53.
- 392 Smith, G., Grenfell, B.T., Isham, V., Cornell, S., 1999. Anthelmintic resistance  
393 revisited: under-dosing, chemoprophylactic strategies, and mating probabilities.  
394 *Int J Parasitol* 29, 77-94.
- 395 Toutain, P.L., Upson, D.W., Terhune, T.N., McKenzie, M.E., 1997. Comparative  
396 pharmacokinetics of doramectin and ivermectin in cattle. *Vet Parasitol* 72, 3-8.
- 397 Van Zeveren, A.M., Casaert, S., Alvinerie, M., Geldhof, P., Claerebout, E.,  
398 Vercruysse, J., 2007. Experimental selection for ivermectin resistance in  
399 *Ostertagia ostertagi* in cattle. *Vet Parasitol* 150, 104-110.
- 400 Vercruysse, J., Dorny, P., Claerebout, E., Demeulenaere, D., Smets, K., Agneessens,  
401 J., 2000. Evaluation of the persistent efficacy of doramectin and ivermectin  
402 injectable against *Ostertagia ostertagi* and *Cooperia oncophora* in cattle. *Vet*  
403 *Parasitol* 89, 63-69.

## Legends of the figures

**Fig. 1.** Semi-logarithmic plot of IVM plasma concentration-time profiles in four treated (filled symbol) and six non-treated (open symbol) heifers over a 40-day period. The four treated heifers received a single 500 µg/kg topical administration of IVM. Similar slopes for the plasma terminal phases were observed in treated and untreated animals.

**Fig. 2.** Semi-logarithmic plot of IVM faecal concentration-time profiles in four treated (filled symbol) and six untreated (open symbol) heifers over a 40-day period. The four treated heifers received a single 500 µg/kg topical administration of IVM. Similar slopes for the faecal terminal phase were observed in treated and untreated animals.

**Fig. 3.** Time development of IVM anthelmintic activity in ten heifers including four treated animals (filled symbols) and six untreated animals (open symbols). Panel A: individual FEC expressed as percent of pre-treatment values are presented over a 30 days period. Panel B: at each day, efficacy (from 0 to 100%) represents the average cumulated  $FEC_{reduction(\%)}$  from time 0 (the time of IVM administration) to that day.

**Fig. 4.** Average IVM efficacy over 30 days versus plasma (panel A) or faecal (panel B) IVM exposure ( $AUC_{IVM}$  from time 0 to the last quantifiable plasma concentration). Efficacy was assessed from daily faecal egg counts (FECs) and was expressed as a percentage (from 0 to 100%) corresponding to the average efficacy over the first 30 days post IVM administration.

**Table 1**

Descriptive statistics for plasma and faecal IVM exposure in a group of ten heifers of which four were treated with a pour-on dose of IVM (500 µg/kg) and six were untreated but housed in the same yard.

	<b>Plasma exposure</b>			<b>Faeces exposure</b>		
	ng*day/mL			ng*day/g		
	Mean (SD)	Min-Max	CV%	Mean (SD)	Min-Max	CV%
Control	11.3 (11.1)	2.1 - 28.4	98.4	365 (340)	89.5 - 899	93.2
Treated	121 (43.0)	77.8 - 181	35.4	2458 (1063)	1676 - 3998	43.2

Exposures are expressed as area under the concentration curve computed from time 0 to the last measured concentration.

**Table 2**

Pharmacodynamic parameters of a sigmoid Emax model describing the exposure versus efficacy relationship.

<b>Parameters</b>	<b>Units</b>	<b>Plasma</b>	<b>Faeces</b>
Emax	(%)	93.3	95.5
AUC <sub>50</sub>	ng*days/mL (plasma) or ng*days/g (faeces)	4.56	173.6
Gamma	No unit	1.94	1.64

Emax is the predicted maximal efficacy. The estimated Emax was not 100% due to the way efficacy was expressed (see Material and Methods). AUC<sub>50</sub> is the AUC corresponding to Emax/2. Gamma is the slope of the exposure versus efficacy relationship.

**Table 3**

Average plasma or faecal concentrations of IVM corresponding to different percentages of anthelmintic efficacy.

Efficacy (%)	Plasma exposure		Faecal exposure	
	AUC (ng*day/mL)	Concentration (ng/mL)	AUC (ng*day/g)	Concentration (ng/g)
10	1.6	0.076	48.1	2.29
20	2.4	0.114	80.0	3.81
50	5.0	0.238	188	8.95
80	11.6	0.552	472	22.48
90	25.0	1.190	952	45.33

The concentrations were obtained from the corresponding (plasma or faeces) AUC predicted by the sigmoid Emax model, scaled by a standard duration of 21 days.

Fig. 1.

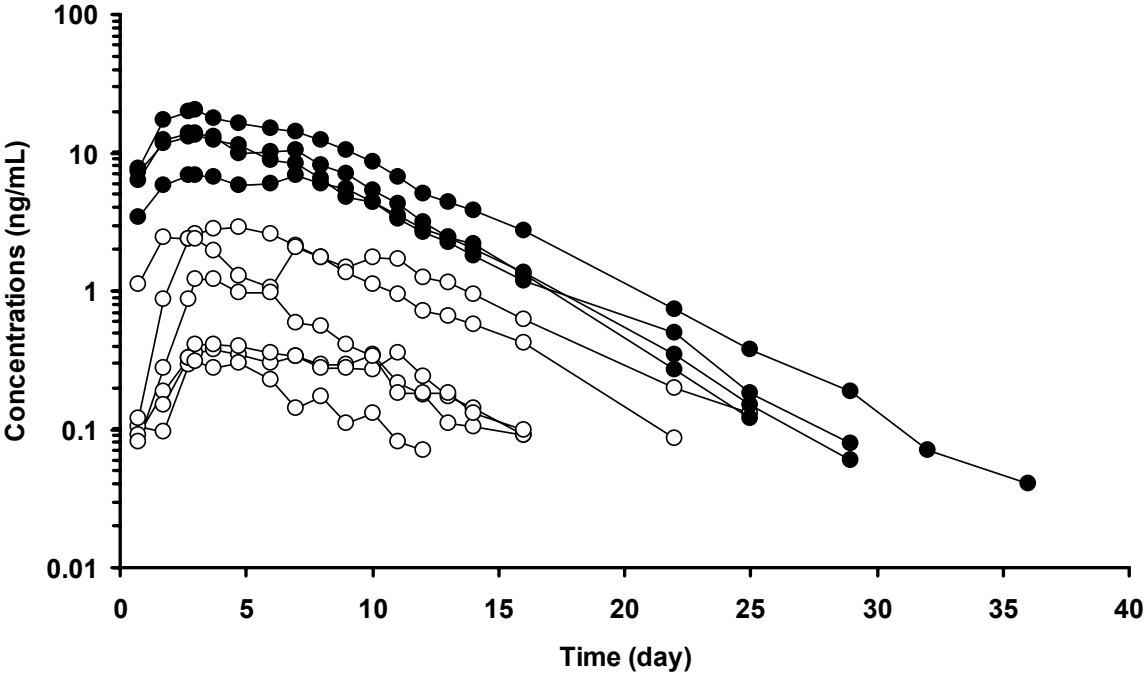




Fig. 2.

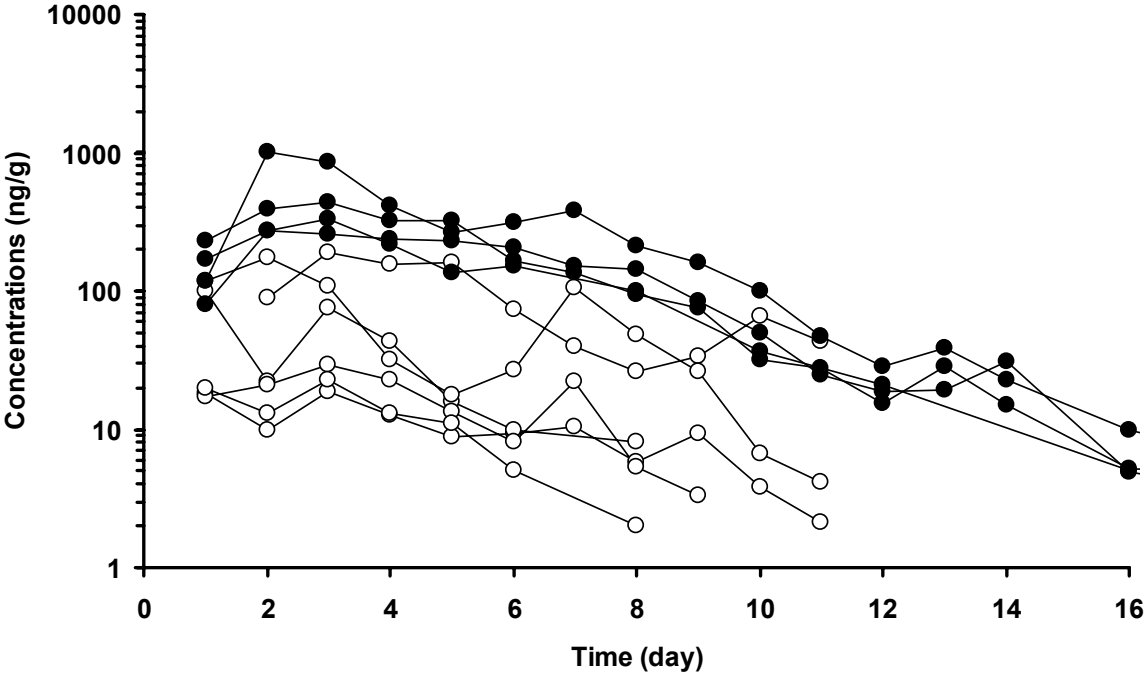
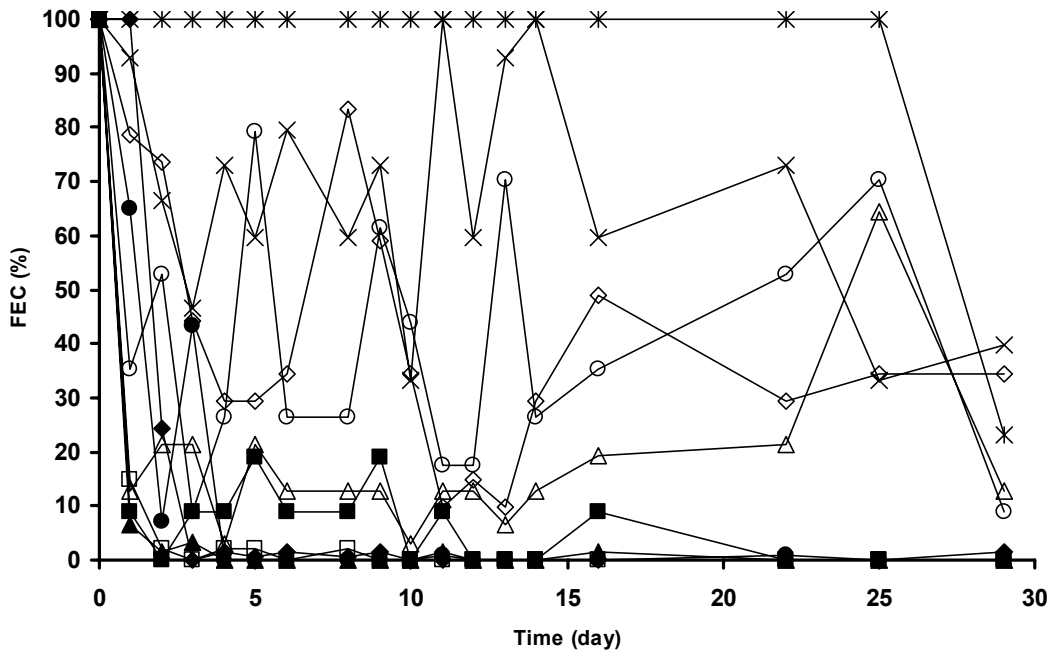


Fig. 3.

A



B

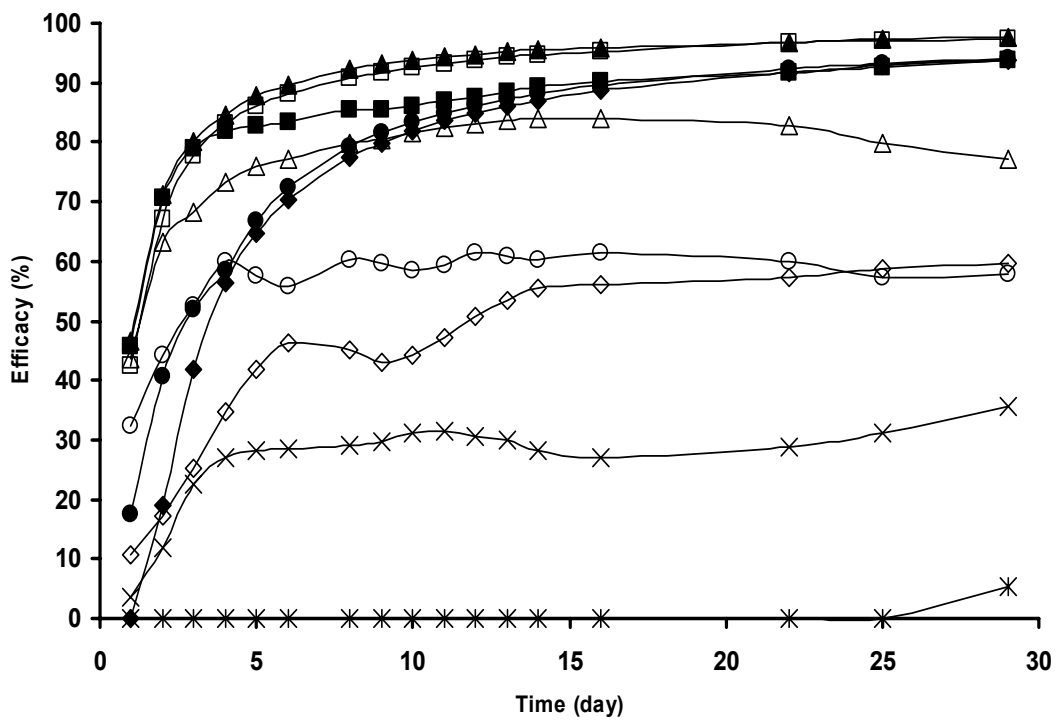


Fig. 4.

