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ABSTRACT

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

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

Key words: Macrocyclic lactones; Ivermectin; topical ; allo-grooming ; Anthelmintic resistance ; Cattle

1. Introduction

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

2. Materials and methods

2.1. Experimental Animals

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

2.2. Experimental infection, treatment and sampling

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

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

2.3. Analytical procedures

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

2.4. FEC and efficacy measurements

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

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

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

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

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

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

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

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

2.5. Pharmacokinetic measurements and pharmacodynamic modelling

Pharmacokinetic analyses were performed using WinNonlin Professional version 5.2 (Pharsight Corporation, Cary, NC, USA). Areas under the plasma or faecal IVM concentration curves (AUC_{IVM}) were obtained by the trapezoidal rule from time 0 to the last measurable concentration (Non-compartmental module of WinNonlin).

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

$$Efficacy(\%)_{day30} = \frac{E\max \times (AUC_{IVM})^n}{(AUC_{IVM})_{50}^n + (AUC_{IVM})^n} \quad \text{Equation 3}$$

where *Efficacy(%)*$_{day30}$ is the individual efficacy at day 30 calculated using Equation 2, *E*max is the maximal estimated *Efficacy(%)*, AUC_{IVM} is the plasma or faecal IVM AUC as obtained for each individual, *n* is the Hill coefficient and $(AUC_{IVM})_{50}$ is the plasma or faecal IVM AUC giving 50% of Emax. Descriptive statistics were obtained with WinNonlin and the results are reported as means (arithmetic or geometric) and SD.

3. Results

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

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

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

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

3.2. FEC reduction in treated and untreated heifers

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

4. Discussion

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

The design of the present experiment was selected to represent a worst-case scenario for ML treatment namely a situation where only a part (40%) of a herd is treated. Due to the apportionment of the total administered dose (4×500 µg/kg) between the different members of the group, a possible underexposure was anticipated in both treated and untreated animals. In the present experiment, all treated animals were in fact sufficiently exposed to obtain maximal IVM efficacy, despite the fact that they shared part of their dose with their untreated congeners as a result of allo-licking activity. It should be pointed out here that expressing efficacy as an average $FEC_{reduction(\%)}$ over a time period (Equation 2) led to maximal values at 30 days lower than 100%, even if daily FEC reductions could reach 100% during the last days (data not shown). This was due to the integration in the calculation of the progressive increase of FEC reduction during the first days post-administration (Fig.3). Moreover, taking into account the time development of the reduction in the FECs highlighted its clear slowing down in half of the treated cattle (time to reach 80% efficacy increasing from 3 days to 9 days), which might be attributed to the fact 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±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±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±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 then the treated animals.
268 This merits attention when considering the potential factors responsible for reduced
269 anthelmentic 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±SD of FECs were 10±21% of pre-treatment FECs for the
284 fenbendazole group, 70±111% for the PO doramectin group and 80±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

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

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

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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.

	Plasma exposure ng*day/mL			**Faeces exposure** 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.

Parameters	Units	Plasma	Faeces
Emax	(%)	93.3	95.5
AUC_{50}	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_{50} 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.

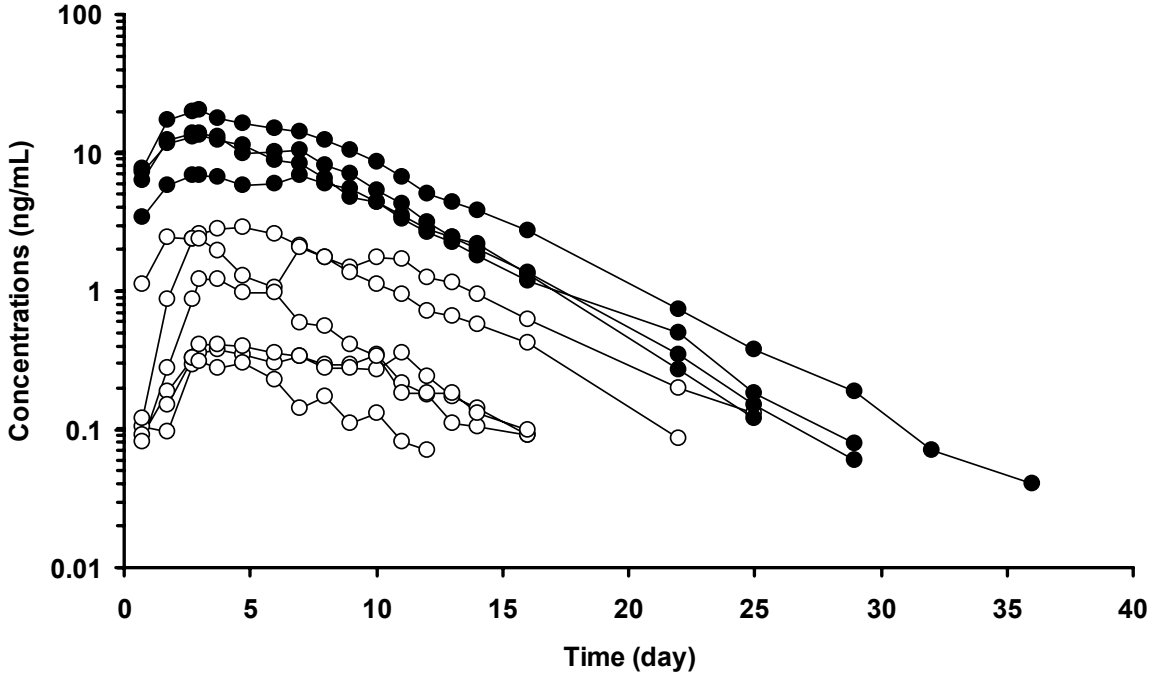

100
10
1
0.1
0.01
Concentrations (ng/mL)
0
5
10
15
20
25
30
35
40
Time (day)

Fig. 2.

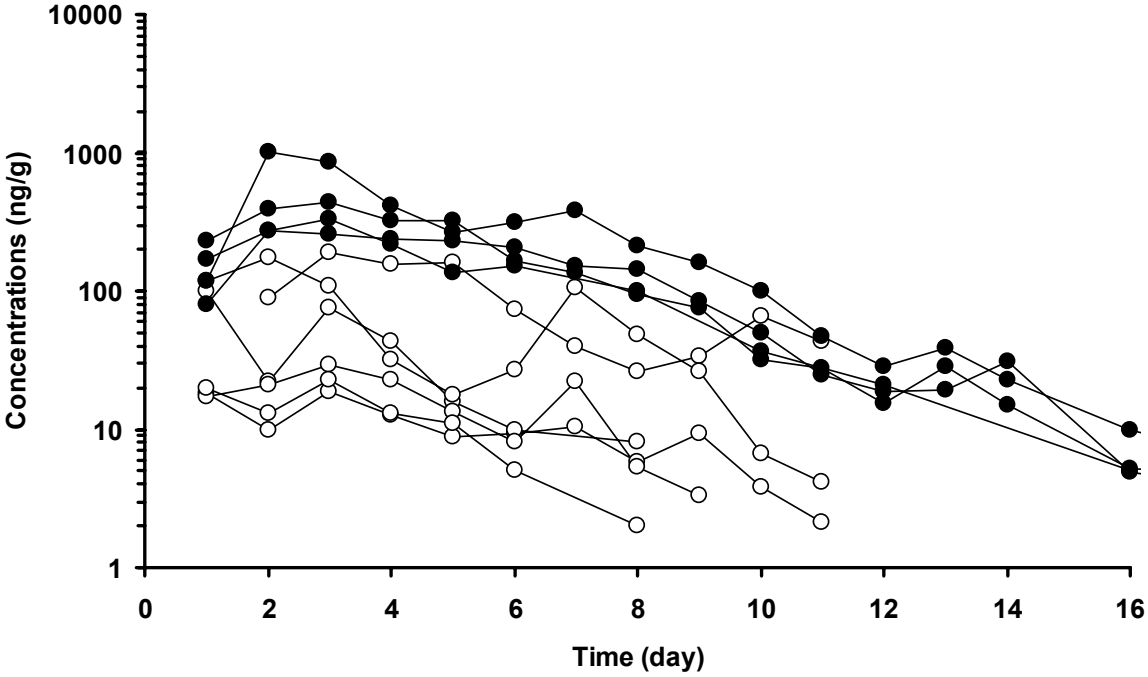
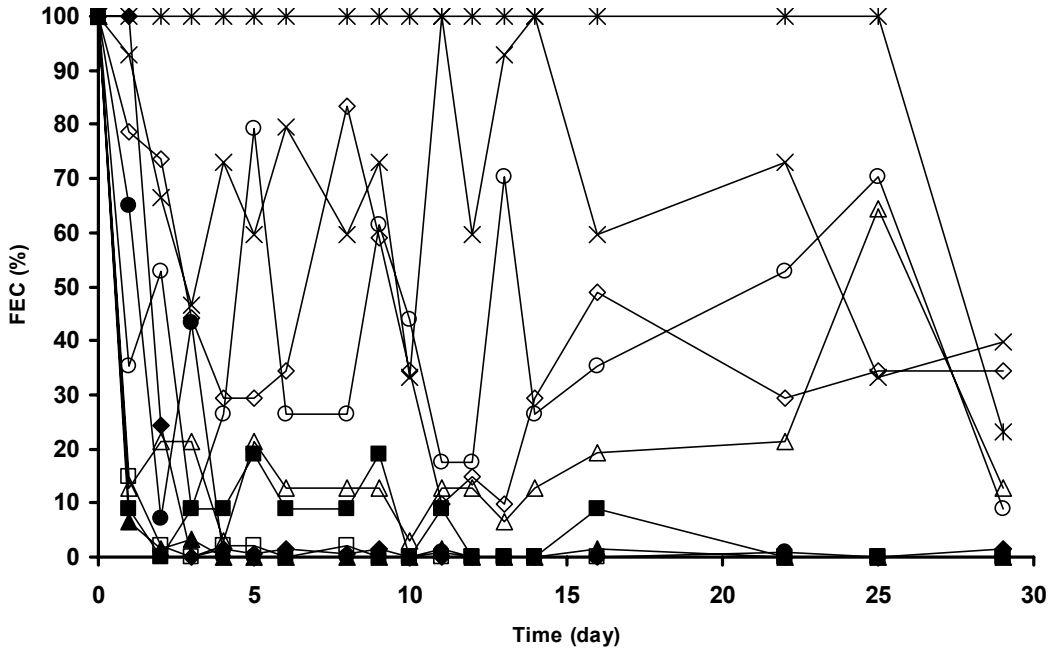

10000
1000
100
10
1
Concentrations (ng/g)
0
2
4
6
8
10
12
14
16
Time (day)

Fig. 3.

A


FEC (%)
Time (day)
0
10
20
30
40
50
60
70
80
90
100
0
5
10
15
20
25
30

B

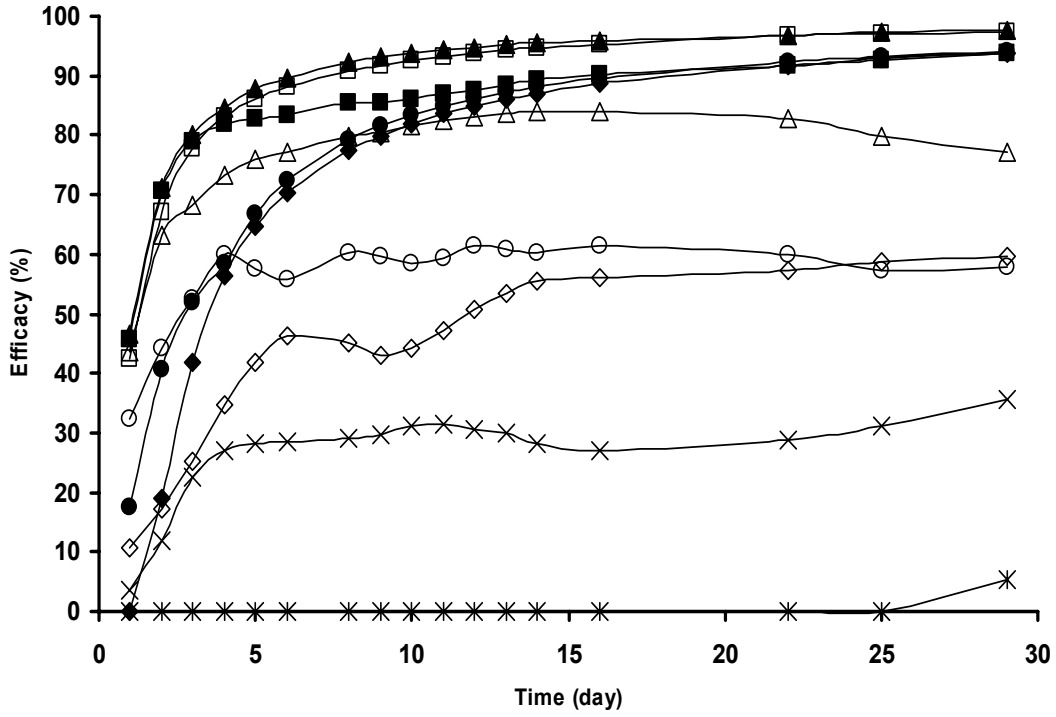
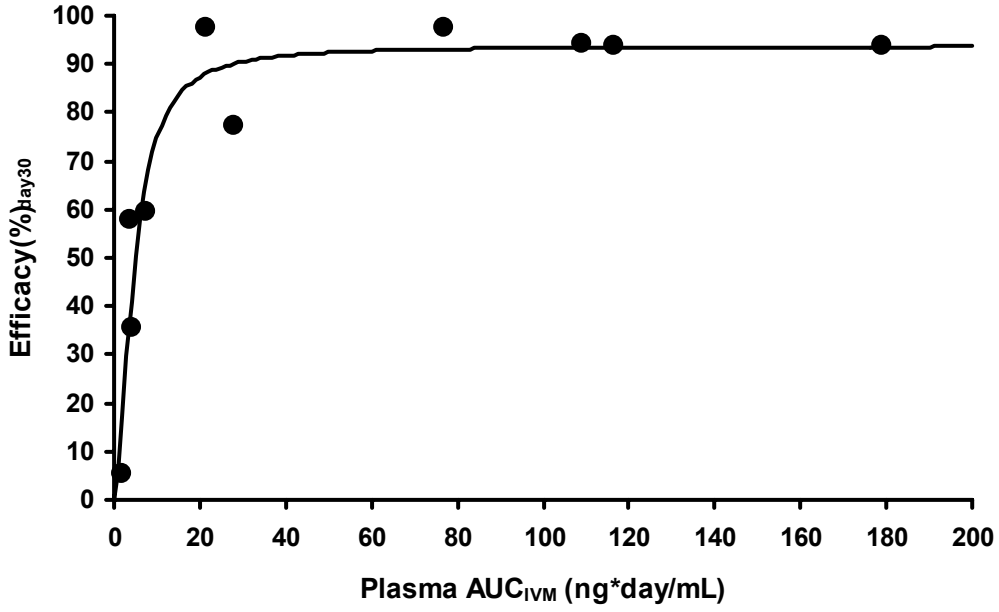
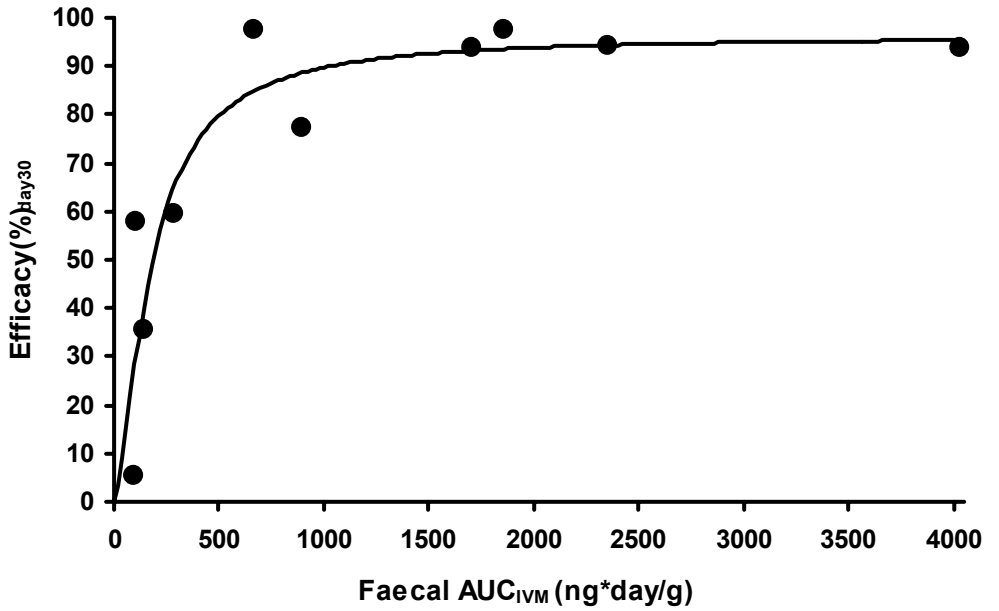

Efficacy (%)
Time (day)
0
10
20
30
40
50
60
70
80
90
100
0
5
10
15
20
25
30

Fig. 4.


Efficacy(%)Day30
100
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
120
140
160
180
200
Plasma AUCIVM (ng*day/mL)


Efficacy(%)Day30
100
90
80
70
60
50
40
30
20
10
0
0
500
1000
1500
2000
2500
3000
3500
4000
Faecal AUCIVM (ng*day/g)