CHAPTER 5

Licking Behaviour and Environmental Contamination Arising from Pour-on Ivermectin for Cattle

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Abstract

Pour-on formulations of endectocides are extensively used to treat and control systemic parasitic diseases in cattle, worldwide. The purpose of the present study was to investigate the influence of the natural licking behaviour of cattle on the plasma and faecal disposition of topically-administered ivermectin. Twelve Holstein cattle were given one single i.v. (200 µg/kg) and topical (500 µg/kg) administration of ivermectin at a 5-month interval. For the pour-on administration, the animals were allocated into two groups (n = 6): one control group (lickers) and one group where licking was prevented (non-lickers). Ivermectin plasma (total) clearance (270 ± 57.4 mL/kg/day) was very homogeneous among the 12 cattle. In contrast, major differences between lickers and non-lickers were observed following pour-on administration. Prevention of licking resulted in an extended terminal plasma half-life (363 ± 16.2 *vs* 154 ± 7.4 h in lickers) and in a lower and less variable systemic availability of ivermectin (19 ± 4.9 *vs* 33 ± 18.5% in lickers). More importantly, nearly 70% of the pour-on dose was recovered as parent drug in the faeces of lickers *vs* only 6.6% in non-lickers. Altogether, these results are consistent with an oral rather than percutaneous absorption of topical ivermectin in control animals, the non-systemically available fraction of ingested ivermectin providing a major contribution (80%) to the drug faecal output. The consequences of licking on the disposition of pour-on ivermectin are discussed in terms of environment, given the known ecotoxicity of this drug, and of cross-contamination. Animals licking themselves and each other could result in unexpected residues in edible tissues of untreated animals and in possible subtherapeutic drug concentrations, a factor in drug resistance. According to the Precautionary Principle, these considerations elicit concern over the use of topical drug formulations in cattle.

Introduction

In both livestock and companion animals, many different antiparasitic drugs, including pyrethroid compounds, organophosphates and later, endectocides (such as ivermectin, doramectin, eprinomectin and moxidectin) are administered topically to treat different parasitic conditions. Pour-on formulations of endectocides limit the risk of injury to user and animal and are particularly convenient for farmers who can apply the product easily themselves (Hennessy, 1997). For these reasons, pour-on have largely displaced therapeutically equivalent injectable formulations in farming practice and are routinely used to treat millions of cattle per year, worldwide.

Ivermectin and structurally related drugs are extensively excreted as metabolites and parent drug in the faeces of treated animals, regardless of administration route (Campbell, 1985; Chiu et al., 1990*)*. Unchanged (active) compound in the faeces may be toxic to non-target organisms such as dung-breeding insects and the fauna involved in the degradation of livestock dung on the pasture (Wall and Strong, 1987*)*. The issue of environmental impact of endectocides used in large scale has been debated for almost 20 years (Fincher, 1992, 1996; Sommer et al., 1993), and there is still little sign of consensus since different studies have shown conflicting results with varying degrees of ecotoxicity (Strong and Wall, 1994*)*. Contradictory data can be partly explained by the observation that different routes of administration (s.c., topical, oral) lead to different ivermectin excretion profiles (Herd et al., 1996*)*. Interestingly, higher faecal concentrations of ivermectin have been reported following pour-on application than following s.c. injection (Herd et al., 1996*)*, which is unexpected considering the lower plasma concentrations. Indeed, if it is assumed that the plasma concentration of ivermectin is the only driving force for ivermectin excretion into faeces, lower faecal concentrations of parent drug should be expected after the pour-on administration. To explain this apparent inconsistency, we decided to explore the influence of the natural grooming behaviour of domestic cattle on the disposition of ivermectin pour-on formulation. This grooming behaviour consists predominantly of self-licking or licking another animal (so called allo-licking). It serves an important physiological function in skin and hair hygiene, can be stimulated by the presence of ectoparasites and is also a factor in the establishment and cohesion of herd social structure (Simonsen, 1979; Sato et al., 1991, 1993; Krohn, 1994*)*. The present experiment was designed to test the hypothesis that a relevant fraction of the ivermectin topically-administered to cattle was actually ingested by licking.

Materials and Methods

Experimental design

Six pairs of monozygotic twin Holstein cattle (567 ± 24 kg body weight, 3 years old), obtained by micro-manipulation (Ozil et al., 1982*)* and maintained under identical conditions, were given a single i.v. administration of injectable ivermectin (Ivomec® injectable, Merial; 200 µg/kg). After a 5-month washout period, each animal was given one single administration of topical ivermectin (Ivomec® pour-on bovin, Merial) at the recommended dose of 500 µg/kg. For the pour-on administration, each pair of twins was separated into two groups of six animals. One group (the lickers) was kept in individual tie-stalls, each animal being tethered with a loose chain so that it could lick itself and its immediate neighbours. In the other group (the non-lickers), each animal was isolated from the others by a screen and was fitted with a wooden neck collar to prevent self-licking. The collar was removed 44 days after administration. Blood was collected regularly for 44 days and 31 days following pour-on and i.v. administration, respectively. An additional blood sample was obtained in the 12 cattle on day 56 following pour-on application (12 days after removal of collars). Once collected, the blood samples were chilled on wet ice and promptly centrifuged. The plasma was removed from the tubes, and stored at – 20°C until analysis. Total faeces were collected over 24 h on days 4, 7 and 14 after the i.v. administration. Following the pour-on administration, faeces were collected for 6 h (from 09:00 to 15:00 h) on days 1, 2, 3, 4, 7, 14, 18, 22 and 28 after application. Wet faeces were weighed, homogenized, and a 50-g aliquot was collected and stored at – 20°C until analysis.

Analytical method

Ivermectin (22,23-dihydroavermectin B1a) concentrations in plasma and faeces were measured using a high-performance liquid chromatography (HPLC) technique (Alvinerie et al., 1987*)*. The lower limit of quantification for ivermectin was 0.05 ng/mL for the plasma and 0.5 ng/g for the wet faecal samples. Accuracy and precision (intra-assay variation) expressed as relative standard deviation were less than 8 and 6%, respectively.

Pharmacokinetic analysis

Data were analysed using a non-compartmental approach. The areas under the plasma concentration-time curve AUC ($0\text{-}t_{last}$) (from 0 to the last sample *e.g.* 31 days (i.v.) or 44 days (pour-on)) were computed using the trapezoidal rule.

Ivermectin total (plasma) clearance was calculated by dividing the administered dose by the AUC (0-t_{last}) obtained for the i.v. route (Eq. 1):

$$Cl_{tot} = \frac{Dose^{i.v.}}{AUC^{i.v.}_{0-tlast}} \quad \text{Eq.1}$$

The systemic availability for topical ivermectin was calculated using the ratio of the AUC (0-t_{last}) obtained after topical ($AUC^{pour-on}_{0-tlast}$) and i.v. administration ($AUC^{i.v.}_{0-tlast}$), corrected by the ratio of the administered doses (Eq. 2):

$$F(\%) = \frac{AUC^{pour-on}_{0-tlast}}{AUC^{i.v.}_{0-tlast}} \times \frac{Dose^{i.v.}}{Dose^{pour-on}} \times 100 \quad \text{Eq. 2}$$

The faecal excretion rate of ivermectin at each time point (t) was obtained by dividing the total amount of parent drug eliminated in faeces within the collection interval ($Q_{faeces,\,\tau}$) by the time of collection τ (6 or 24 h):

$$faecal\ excretion\ rate\ (t) = \frac{Q_{faeces,\,\tau}}{\tau} \quad \text{Eq. 3}$$

where $Q_{faeces,\,\tau}$ was the product of the weight of wet faeces and the faecal concentration of ivermectin (µg/g wet faeces) over the collection period. The total amount of parent drug eliminated in faeces within 28 days post-topical administration was estimated by integration of the faecal excretion rate profile in function of time between 0 and 28 days using the trapezoidal rule.

Faecal clearances were calculated at each time point (t) following the equation given below:

$$Cl_{faecal}\ (t) = \frac{faecal\ excretion\ rate\ (t)}{C_{plasma,\,\tau}} \quad \text{Eq. 4}$$

where $C_{plasma,\,\tau}$ was the corresponding plasma concentration over τ.

Statistics

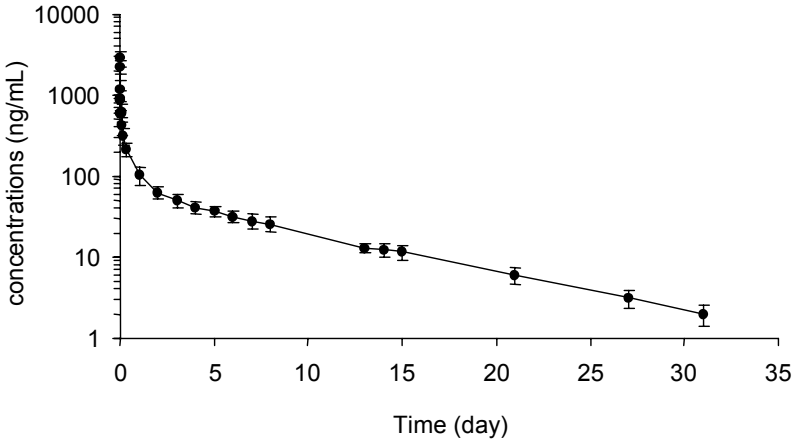
The arithmetic means and standard deviations (SD) of the different parameters were calculated. For terminal plasma half-life, the harmonic means and SD were computed using the Jackknife technique (Lam et al., 1985). Comparison between lickers and non-lickers was carried out using paired *t*-test for terminal half-lives, AUC (0-t_{last}), total (plasma) clearance and C_{max}, and a using non-parametric paired test (Wilcoxon) for T_{max} and F% (SYSTAT ® 8.0, SPSS Inc., Chicago, IL). A $p < 0.05$ was considered as significant.

Results

Plasma disposition

Ivermectin plasma concentration-time profiles obtained in cattle following i.v. and pour-on administrations are presented in Figs. 1 and 2, respectively. Table 1 gives the mean values of ivermectin pharmacokinetic parameters for both i.v. and pour-on administrations in the licker and non-licker groups. Ivermectin total (plasma) clearance following i.v. administration was found to be homogeneous among the 12 cattle, and equal to 270 ± 57.4 mL/kg/day (n = 12). The terminal plasma half-life was similar between pour-on and i.v. administrations in lickers, but was much longer after pour-on (363 ± 16.2 h) than following i.v. administration

Figure 1. *Ivermectin mean plasma concentration-time profile over 31 days in the 6 pairs of monozygotic twin cattle simultaneously administered with 200 µg/kg ivermectin i.v.*



Each point represents the mean ± SD obtained in the 12 animals.

Table 1. *Pharmacokinetic parameters (mean ± SD) of ivermectin following intravenous and topical administration of ivermectin (200 and 500 μg/kg, respectively) to six licking and six non-licking monozygotic twin cattle.*

Parameter	Lickers (n = 6)		Non-lickers (n = 6)	
	i.v.	pour-on	i.v.	pour-on
$t_{1/2}$ (h)	137 ± 2.7	154 ± 7.4	144 ± 3.0	363 ± 16.2 **, #
AUC (ng.h/mL)	18429 ± 3652	14283 ± 6424	18749 ± 3036	9146 ± 3078 *
Cl (mL/kg/day)	274 ± 68.8	—	264 ± 47.4	—
F (%)	—	33 ± 18.5	—	19 ± 4.9 *
C_{max} (ng/mL)	—	39 ± 20.9	—	16 ± 6.4 ***
T_{max} (day)	—	147 ± 43.6	—	191 ± 15.2

* ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$) indicate a significant difference between the licker and non-licker groups for this parameter.

The plasma terminal half-life ($t_{1/2}$) differed significantly between i.v. and pour-on administrations ($p < 0.01$).

A paired *t*-test was used for plasma terminal half-lives, AUC, Cl, and C_{max}, whereas a non-parametric paired test (Wilcoxon) was used for F(%) and T_{max}.

(144 ± 3.0 h) in the non-licker group, indicating a flip-flop phenomenon in non-licking cattle. The systemic availability for topical ivermectin was higher and more variable in lickers than in non-lickers (33 ± 18.5 % *vs* 19 ± 4.9 %).

At the end of the 44-day trial, the collars were removed from the six non-licking cattle, and measurement of ivermectin plasma concentrations 12 days later indicated an obvious rebound in three of the six animals. In these three animals, the plasma concentrations on day 56 were increased by a factor of 39, 56 and 135% compared to the plasma concentrations measured on day 44, which could not be attributed to the variability of the analytical method.

Faecal disposition

Comparison of ivermectin excretion profiles in faeces (Fig. 3) showed a major difference between lickers and non-lickers. On day 4 post-administration, for example, the faecal elimination rate of ivermectin in the licker group was 33-times

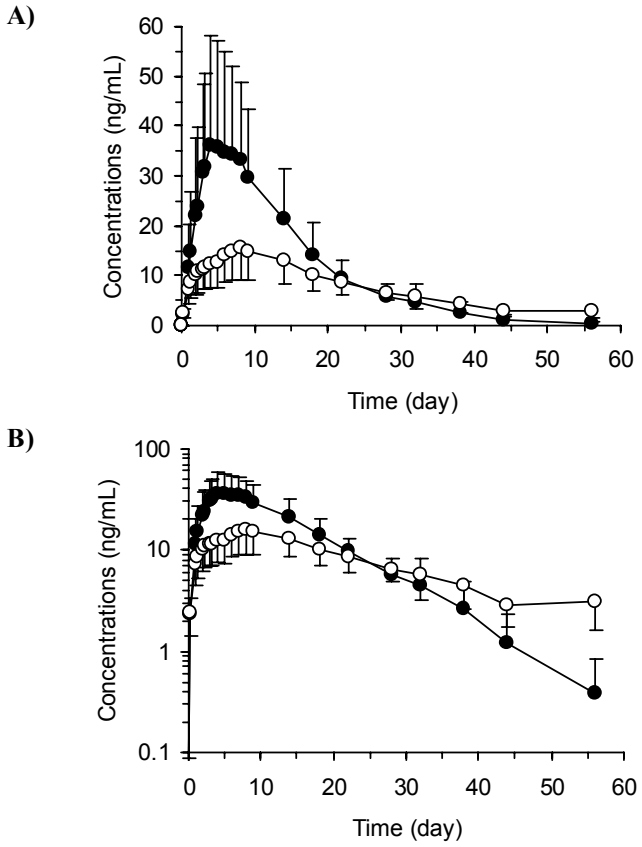


Figure 2. Comparative ivermectin plasma concentration-time profiles in six licking (filled symbol) and six paired non-licking (open symbol) monozygotic twin cattle over a 56-day period, following a single 500 µg/kg topical administration with ivermectin pour-on formulation. Each point represents the mean ± SD obtained in the six animals of each group. **A)** Arithmetic scale shows a higher and more variable ivermectin bioavailability in lickers than in non-lickers. **B)** Semi-logarithmic scale shows a different slope for the plasma terminal phase in lickers and non-lickers.

higher than in the non-licker group (825 ± 227.5 *vs* 25 ± 10.0 µg/h) and 10-times higher than after the i.v. administration (83 ± 10.4 µg/h). The estimated amount of ivermectin eliminated in the faeces over 28 days was 346 ± 60.5 µg/kg body weight (69% of the administered dose; Fig. 3B) in the lickers *vs* 33 ± 11.7 µg/kg body weight in non-lickers (6.6% of the dose; Fig. 3B).

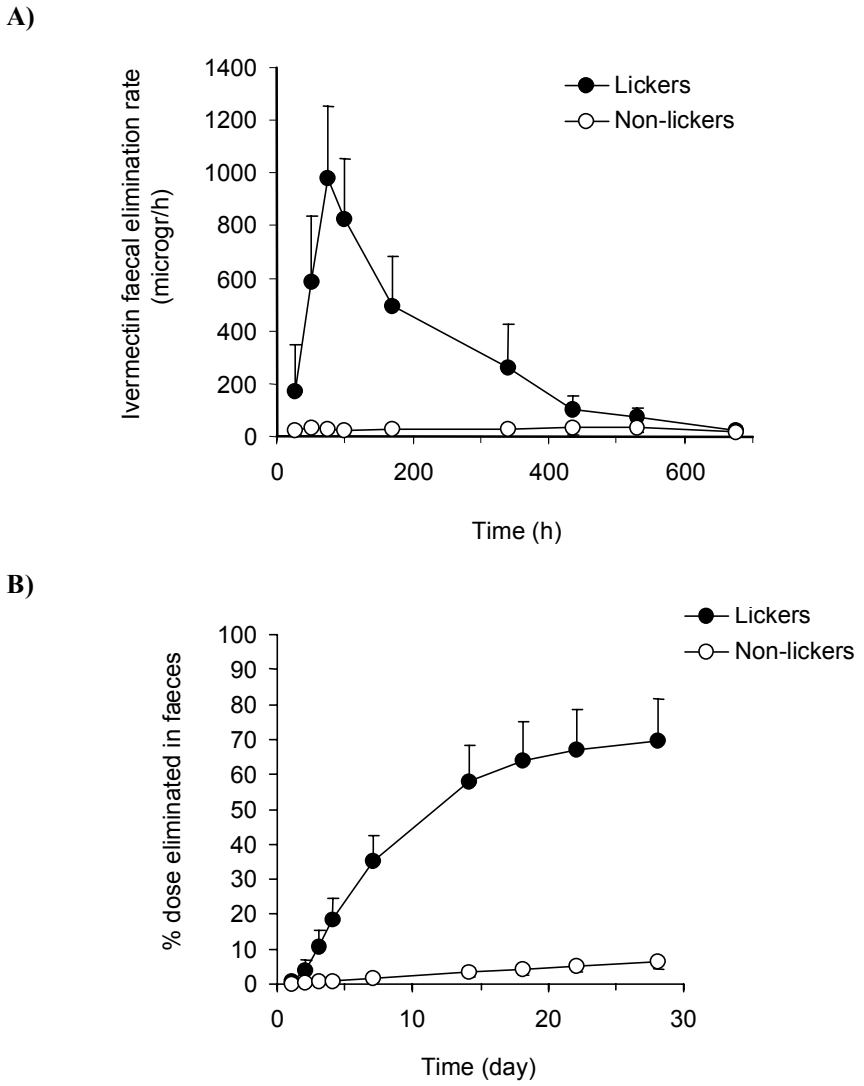


Figure 3. Comparative excretion profiles of unchanged ivermectin in the faeces of six licking (filled symbol) and six paired non-licking (open symbol) monozygotic twin cattle over 28 days, following a single 500 µg/kg administration of pour-on ivermectin. **A)** Faecal elimination rates (mean ± SD) of ivermectin as parent drug. **B)** Cumulative amounts of ivermectin eliminated in faeces expressed as percentages of the administered dose (mean ± SD).

In non-lickers (Fig. 4A), individual faecal clearances of ivermectin were similar after pour-on (89 ± 24.5 mL/kg/day) and i.v. administrations (102 ± 23.5 mL/kg/day). In contrast, in the licker group (Fig. 4B), the individual apparent faecal clearances of ivermectin following pour-on administration (ranging from 203 ± 170.6 to 1671 ± 724.1 mL/kg/day) were much higher than the faecal clearance obtained for the i.v. route (106 ± 33.5 mL/kg/day) throughout the 28 days of investigation.

The values of ivermectin faecal clearance obtained in the 12 cattle on day 4, 7 and 14 after i.v. administration were very homogeneous (104 ± 28.6 mL/kg/day, n = 12), representing 38% ($CI_{95\%}$: [36%;40.5%]) of the plasma (total) clearance (270 ± 57.4 mL/kg/day, n = 12).

Discussion

Our results indicated that i.v. faecal clearance accounted for 38% of total (plasma) clearance following i.v. administration, which implies that 38% of the i.v. dose was actually excreted as parent drug in the faeces. This is consistent with the

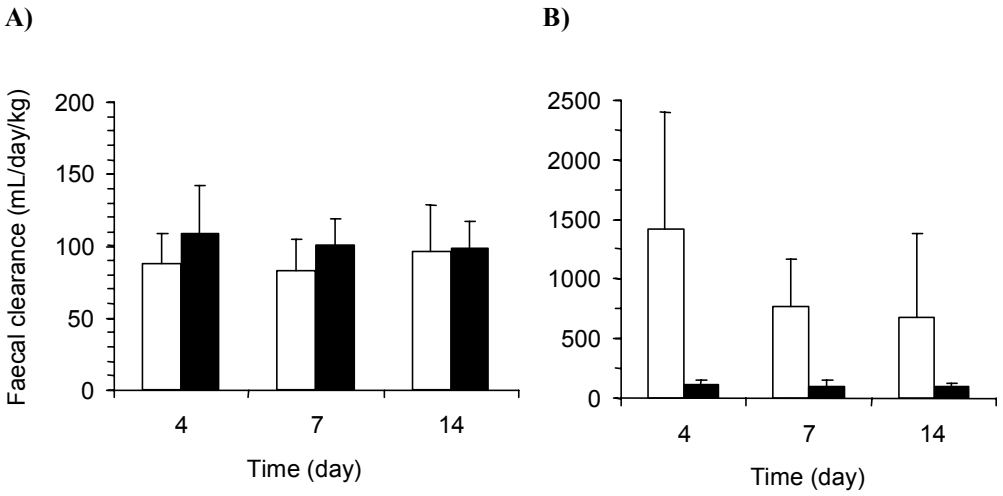


Figure 4. Ivermectin faecal clearance on days 4, 7 and 14 post-administration, following an i.v. injection of ivermectin at 200 µg/kg (filled column) and a pour-on application of ivermectin at 500 µg/kg (open column). **A)** mean ± SD in the non-licker group. **B)** mean ± SD in the licker group. The figures show that ivermectin faecal clearance was similar after i.v. and pour-on administrations in non-lickers, but much higher after pour-on than following i.v. administration in lickers due to the large ingestion of ivermectin by licking.

study performed in cattle and with radiolabelled drug by Halley et al. (1989), who showed that 39-45% of the faecal output consisted of parent drug and 59% of metabolites after subcutaneous administration.

After the pour-on administration, a marked difference was observed between licker and non-licker groups for both plasma and faecal disposition of ivermectin. In the non-licker group, faecal clearance of ivermectin following pour-on application was similar to faecal clearance found after i.v. administration, which is consistent with the assumption that the plasma concentration was the only driving force controlling ivermectin faecal elimination in non-lickers. The percentage of the administered dose eliminated in faeces from systemic blood can be theoretically assessed as the product of the faecal-to-total clearance ratio (0.38) and the fraction systemically available. In non-lickers, this theoretical percentage was 7% (0.38×0.19), which is very close to the value estimated by integration of the excretion rate profile against time (6.6%). In lickers, we would expect 13% of the dose (0.38×0.33) to be removed as parent drug from blood in faeces. In contrast, we observed that nearly 70% of the pour-on dose (500 µg/kg) was eliminated unchanged in the dung. In addition, pour-on faecal clearance in lickers was much higher than the faecal clearance obtained for the i.v. route throughout the 28 days of investigation, and could exceed up to nine times the i.v. plasma (total) clearance in the same animals, which is theoretically impossible. Altogether, these findings demonstrate that over this time period, a large fraction of the ivermectin eliminated in the faeces of licking animals (nearly 57% of the dose *e.g.* 80% of the faecal output) could not be of plasma origin. It is concluded that an important amount of topically administered drug was actually ingested by licking and transited directly through the digestive tract into faeces.

The systemic availability for topical ivermectin was highly variable in lickers (coefficient of variation of 56%), which is in line with previous studies (Gayrard et al., 1999). The prevention of licking resulted in a lower and less variable systemic availability (coefficient of variation of 26% in non-lickers) and in an extended elimination half-life (363 h in non-lickers *vs* 154 h in lickers). The terminal plasma half-life in the non-licker group was also much longer than after the i.v. administration (144 h). This indicates that ivermectin absorption through the skin was a very slow process limiting the drug plasma elimination. In contrast, the terminal plasma half-life of ivermectin in the licker group was very similar to that observed after the i.v. administration. These results conclusively demonstrate that the rate and extent of ivermectin absorption differed between licking and non-licking animals, which is consistent with a difference in absorption mechanism. Considering that a large amount of drug transited through the digestive tract, it is

strongly suggested that a large fraction of topical ivermectin gained access to systemic circulation by the oral route, rather than percutaneous absorption, as a consequence of the licking behaviour. We found a relatively high systemic availability in the licker group (33%). By comparison with the s.c. route, the systemic availability for the oral route was estimated to be 12.5% for the sustained release bolus (Alvinerie et al., 1998) and 26% for the intra-ruminal bolus (Chiu et al., 1990). This suggests that perlingual absorption of ivermectin cannot be ruled out in licking cattle.

The obvious rebound in plasma concentration observed in three of the six animals (non-licking group) 12 days after removal of the collar suggests that some ivermectin was still present on the skin of the animals and available for licking. This implies that ivermectin did not undergo complete degradation over 44 days, which is surprising given the alleged photolability of the drug.

The present findings are consistent with the observations reported by others but not previously understood (Sommer et al., 1992; Herd et al., 1993, 1996). Our results provide clear evidence that the natural grooming behaviour of cattle has a major influence on the plasma disposition of topical ivermectin. Self- and allo-grooming are governed by various social, nutritional, physiological, pathological, environmental and managerial factors (Sato et al., 1991, 1993), which makes the systemic availability of topical ivermectin more variable and unpredictable. More importantly, allo-grooming might result in cross-contamination of animals, giving rise to unexpected drug residues in edible tissues of untreated cattle, and undesirable subtherapeutic concentrations in both treated and untreated cattle, which can contribute to the development of drug resistance. Finally, our study demonstrates that the prevention of licking can lead to 10-times lower amounts of parent drug in faeces, under our experimental conditions. This suggests that the licking behaviour of cattle should be taken into consideration in the environmental risk assessment of endectocides. It must also be stressed that the poor and erratic bioavailability of pour-on formulations has led to increased dose rates for ivermectin, doramectin and moxidectin by a factor of 2.5 compared with the s.c. formulation. However, with approximately 70% of the dose recovered in the faeces of licking cattle, increasing the dose contributes to a higher and unnecessary environmental burden of parent drug. In contrast, the therapeutically equivalent s.c. formulation would provide a lower faecal output of ivermectin. Indeed, the maximum faecal excretion of parent drug following s.c. administration (200 µg/kg) can be estimated using our i.v. faecal clearance to be about 78 µg/kg *vs* 346 ± 60.5 µg/kg in lickers given topical ivermectin. Altogether, these considerations elicit concern over the topical route for endectocide administration in cattle, and it is

strongly suggested that the use of safe and efficacious injectable preparations be encouraged.

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