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ORIGINAL PAPER

Patterns of doramectin tissue residue depletion in parasitized vs nonparasitized lambs

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Abstract The effect of gastrointestinal parasitism on patterns of edible tissue depletion of doramectin was studied in greyface Suffolk lambs. Twelve weight-matched pairs of lambs were allocated into group I (nonparasitized, pretreated with three administrations of 5 mg/kg fenbendazole) and group II (parasitized, did not receive anthelmintic treatment). Both groups were maintained together under similar conditions for 70 days, when they were treated with a subcutaneous dose of 0.2 mg/kg bw doramectin. At 7, 14, 21, and 28 days after doramectin administration, three lambs from each group were slaughtered and samples of liver, kidney, muscle, and fat were obtained. Pre-treatment with fenbendazole significantly reduced the nematode fecal egg count and significantly increased lamb body weight compared to the parasitized group. Doramectin was detected in all of the tissues up to 28 days post-treatment. Significantly higher and more persistent doramectin concentrations were found in the nonparasitized lambs compared to the parasitized animals. Considering the EMEA maximum residue limits for doramectin in fat, the calculated withdrawal period for the healthy lambs (43 days) was significantly higher than that for the parasitized animals (26 days).

Introduction

Infection with gastrointestinal nematodes, particularly *Ostertagia* species, continues to be an important cause of impaired ruminant productivity in temperate climates. The mechanisms responsible for such losses include changes in animal feed intake, gastrointestinal function, and protein and energy metabolism combined with changes in mineral and water balance, which in turn lead to alterations in body composition and carcass quality (Steel 1974; Fox 1997). The knowledge that subclinical and production parasitism adversely affect productivity has increased producer use of anthelmintics (Stromberg et al. 1997). Current production systems rely largely upon chemotherapy and chemoprophylaxis for parasite treatment and control (Coop et al. 2002). Because of the widespread prevalence of anthelmintic resistance in nematodes of small ruminants, however, the use of macrocyclic lactones (MLs) has grown in importance. In many countries, MLs are crucial in combating parasitic disease in small ruminants because of their unique broad spectrum of efficacy against both endo- and ectoparasites, the ML family's characteristics of drug persistence, and the low prevalence of resistance against these drugs (Coop et al. 2002).

Doramectin (DRM) is a broad-spectrum ML anthelmintic belonging to the avermectin class drug family (Lanusse et al. 1997). The drug has high lipid solubility and is formulated as an oil-based preparation containing sesame oil/ethyl oleate (90:10 *v*/*v*), which results in lower and later peak concentrations with more persistent plasma levels when injected subcutaneously in cows (Wicks et al. 1993). The high lipid solubility of DRM facilitates deposition in adipose tissue. Gottschall (1997) demonstrated that the subcutaneous administration of this drug results in higher DRM concentrations in fat than in other edible tissues. Therefore, it has been suggested that the high lipid solubility of avermectins and a large fat reservoir in sheep may contribute to longer persistence of DRM in plasma at low concentrations, probably because of the lower blood supply to fat tissues (Atta and Abo-Shihada 2000).

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Gastrointestinal parasitic burden is associated with dramatic pathophysiological changes, such as intestinal dysfunction and nutritional stress, leading to a poor body condition (Steel 1974; Holmes 1987; Fox 1997). These changes may have a major impact on the plasma, tissue, and gastrointestinal disposition of anthelmintic drugs and consequently on their anthelmintic efficacy (Lespine et al. 2004). As previously demonstrated (Pérez et al. 2007), gastrointestinal parasitism induces significant changes in plasma disposition and DRM availability in subcutaneously treated lambs. Similar effects in sheep have been described for moxidectin (Lespine et al. 2004) and ivermectin (Pérez et al. 2006).

The present study examined the effect of gastrointestinal parasitism on the patterns of edible tissue depletion of DRM in two groups of lambs. The aim of the study was to test the hypothesis that parasitic disease can have significant influences on the patterns of tissue depletion of DRM because of the changes induced on the host's gastrointestinal tract and nutritional condition in comparison with the patterns observed in healthy nonparasitized lambs.

Studies of the pharmacokinetic and tissue residues depletion of anthelmintics are normally carried out in healthy nonparasitized animals. As we demonstrate in the current work, however, the administration of DRM to parasitized lambs produces lower and less-persistent drug tissue concentrations, resulting in a significant decrease in the withdrawal period (WP). These values are lower than the commonly licensed WP for DRM of 60 days.

Materials and methods

Twenty-four parasitized Suffolk down lambs (23.3± 0.7 kg bw) were selected for the study. During the experimental period, the lambs were maintained together, outdoors during the day and housed at night. They were fed daily with a rye grass and clover hay mix and a supplementary concentrate. Water and hay were provided ad libitum. They also had access to a paddock with a natural grass pasture. All lambs were weighed prior to treatment using a digital scale.

A serum clinical biochemistry panel including hepatic function tests was performed to evaluate the animals' health conditions, and their values were within the normal ranges described for the ovine species under basal conditions (Meyer et al. 1992). To identify the natural infection level, fecal examinations were performed on all lambs to determine fecal egg counts (FECs). Quantitative pre- and post-treatment FECs were performed using a modified McMaster technique (Zajac 1994) during the 70-day period prior to DRM treatment. All fecal samples were obtained from the rectum during the 7-day interval between the two treatment periods.

The lambs were ranked in descending order of body weight and sequentially paired from heaviest to lightest. Twelve pairs of lambs were allocated into two groups equally balanced in body weight. Once the pairs of animals were established, they were distributed to the experimental groups.

The animals in group I were treated three times with an oral administration of 5 mg/kg fenbendazole (Panacur, Intervet) at 21-day intervals to maintain a healthy, low level of parasitism for 70 days. In addition to its good efficacy against the main gastrointestinal sheep nematodes, this drug was selected because of its faster elimination rate and the short persistence of active metabolite concentrations in plasma after oral administration in this species (Lanusse et al. 1995). Considering these characteristics, we assumed that fenbendazole did not produce any effect on the DRM disposition. The animals in group II did not receive any anthelmintic treatment in order to maintain their parasitized condition for the same period.

After the 70-day pre-treatment period, both groups were treated with DRM by subcutaneous injection in the shoulder area at 200 μg/kg bw, using commercially available formulations (Dectomax, Pfizer). After treatment, the animals were observed continuously for a 4-h period and at least twice daily within 2 days after treatment for any signs of adverse reaction. During the entire experimental period, both groups were maintained under similar feeding and management conditions.

Tissue sample collection

To validate the analytical methodology, one untreated animal was euthanized on day 21 to obtain blank tissues. All experimental animals (groups treated with DRM) were euthanized at different times post-treatment to characterize the profile of DRM tissue residues. Three animals were randomly chosen to be slaughtered on each of the following days post-treatment: 7, 14, 21, and 28. The animals were stunned by captive bolt and exsanguinated immediately according to internationally accepted animal welfare guidelines. At sacrifice, liver, kidney, and muscle samples (approximately 100–200) and the amount of abdominal fat that was available were taken. All tissues were individually weighed, coarsely chopped, mixed, and divided into duplicate samples and then stored at −18°C prior to analysis.

Analytical procedures

DRM was assayed by high-performance liquid chromatography (HPLC) with fluorescence detection after solid phase

extraction, according to procedures described previously (Nowakowski et al. 1995; Gottschall 1997).

Drug extraction and derivatization

Drug-free tissue samples, 1.0 g for liver, kidney, and muscle and 0.1 g for fat, were fortified with DRM to reach final concentrations of 5.0, 10.0, 25.0, 50.0, 100, and 200 ng/g. Fortified and experimental tissue samples were homogenized and solid phase extraction was performed after 15 min of incubation at room temperature. Briefly, 2 ml of acetonitrile and 0.5 ml of water were added to 1 g of tissue, which was vortexed (30 s) and shaken vigorously for 20 min. The mixture was sonicated in an ultrasound bath (Aquasonic, VWR Scientific) for 20 min at 35°C and then centrifuged at 2,000×*g* for 5 min. The solid residue was resuspended in 2 ml of acetone, vortexed, sonicated, and centrifuged. The supernatants were collected and combined in an assay tube, mixed with 2 ml of hexane (HPLC grade), and shaken for 10 min. The upper *n*-hexane phase was discarded, and the supernatant was transferred to a Supelco C18 cartridge (Supelco Inc.) for solid phase extraction; the cartridge was flushed with 2 ml of water/methanol (75:25 *v*/*v*). The analyte was eluted with 1.0 ml methanol and concentrated to dryness under a gentle stream of nitrogen, and then the residue was dissolved in 100 μl of *N*-methylimidazole solution in acetonitrile (1:1 *v*/*v*). To initiate the derivation, 150 μl of trifluoroacetic anhydride solution in acetonitrile (1:2 *v*/*v*) was added. After completion of the reaction (<30 s), a 100-μl aliquot of this solution was injected directly into the chromatograph. To avoid photoisomerization of the derivatives under ambient light conditions (Sklavounos et al. 1994), solutions were stored in amber glassware during HPLC analysis (Nowakowski et al. 1995). The solvents used for sample extraction and drug analysis were of HPLC grade (Merck).

Drug analysis

DRM tissue concentrations were determined by HPLC. The mobile phase consisted of acetic acid (0.2% in water), methanol, and acetonitrile (4:32:64, *v*/*v*/*v*) at a flow rate of 1.5 ml/min through a reverse-phase Supelcosil C18 column (3 μm; 4.6 mm id×150 mm, Supelco Inc., Bellefonte) with fluorescence detection at an excitation wavelength of 383 nm and an emission wavelength of 447 nm (RF 551 Fluorescence Detector, Shimadzu). Pure reference standards (provided by Pfizer Laboratories) were used to prepare calibration curves in the range of 5.0–200 ng/g, using drug-free tissues of an untreated lamb. Pooled tissues samples were taken through the procedure, and calibration curves were plotted using the peak area as a function of analyte concentration. Linear regression analysis was performed to determine the slopes and correlation coefficients of the different calibration curves.

Validation procedures

Linearity, recovery percentages, method precision, and theoretical detection and quantification limits for liver, kidney, muscle, and fat tissues samples were carried out before the experimental sample analysis (Lifschitz et al. 2000) to validate the complete method.

Linearity DRM was identified based on the retention times of a pure reference standard (94.7% pure). Linearity was established to determine the concentration–detector response relationship. The linearity was determined by injection of spiked DRM standards into liver, kidney, muscle, and fat at different concentrations (triplicate determinations). Calibration curves were prepared using least squares linear regression analysis. Correlation coefficients (r) and CVs were calculated following routine procedures.

Recovery The extraction efficiency of DRM was measured by comparing the peak area from the spiked tissue samples with the peak area resulting from direct injections of DRM standard in methanol carried through the derivation procedure. DRM recovery percentages for the different tissues samples were obtained in the range of 5 to 200 ng/g (triplicate determinations). The mean percentage of recovery and the CV were calculated.

Precision Interassay precision for the extraction and chromatography procedures was evaluated by processing replicate aliquots of each tissue (sextuplicate determinations) containing known amounts of DRM on different days.

Detection and quantification limits The limit of drug detection was established by injecting tissue blanks and measuring the baseline noise at the retention time of the DRM peak. The mean baseline noise at the DRM retention time plus three standard deviations was defined as the detection limit. The mean baseline noise at the DRM retention time plus six standard deviations was defined as the theoretical quantification limit.

Estimation of withdrawal periods

The WPs were estimated by linear regression calculations at the point when the upper one-sided tolerance limit (95%) with a given confidence of 95% was below the maximum residue limit. A computer program was used to estimate

WPs based on guidelines from the European Agency for the Evaluation of Medicinal Products (EMEA 1996).

Data analysis

Results were compared using the paired Student's *t* test. Mean values were considered significantly different at $P<0.05$.

Results

Analytical methodology validation

The analytical method was appropriately validated (Table 1). Calibration curves were prepared in the range between 5 and 200 ng/g using least squares linear regression analysis. The percentage of DRM recovery from liver, muscle, kidney, and adipose tissue ranged from 76.8% to 85.7%. The theoretical quantification limit obtained for the different tissues ranged from 0.2 ng/g (kidney) to 2.5 ng/g (adipose). The linearity of the method was confirmed by the estimated values of the correlation coefficient ($r=0.9993$–0.9998 for the different tissues). The interassay precision of the analytical method showed a variation coefficient <6% (range: 0.8–5.1%). Data on the validation of the analytical method for each tissue are summarized in Table 1.

Fecal egg counts and body weight in parasitized and nonparasitized lambs

Tables 2 and 3 present data on the geometric mean values of FEC and mean body weight in both groups of lambs. Pre-treatment with fenbendazole in the healthy nonparasitized animals reduced FEC (Table 2) and increased the lambs' body weights (Table 3), changes that were significantly different ($P<0.05$) from those observed in parasitized lambs. Post-mortem examinations performed on three lambs not treated with fenbendazole revealed a high frequency of nematode species of the genera *Cooperia*, *Ostertagia*, *Trichostrongylus*, and *Nematodirus* in the abomasum and small intestine.

Tissue residues of doramectin in parasitized and nonparasitized lambs

The mean DRM concentrations measured at 7, 14, 21, and 28 days after treatment are shown in Table 4. DRM was detected in all of the tissues analyzed up to 28 days after subcutaneous administration. For both groups, higher DRM concentrations were obtained in adipose tissue in comparison with the other tissues analyzed (Table 4).

Comparing the results of DRM tissue concentrations obtained for both groups 7 days after treatment, significantly higher and more persistent concentrations were observed in the liver, kidney, muscle, and fat of the nonparasitized group compared to the parasitized lambs. Considering maximum residue limit established by the EMEA for DRM in the liver (50 μg/kg) and fat (100 μg/kg), the calculated WPs for the group of healthy lambs were higher than those obtained for the parasitized animals: 43 vs 26 days in fat and 32 vs 22 days in liver.

Discussion

Analytical methodology validation

Method validation is a procedure whereby every stage of an analytical method is subjected to a series of tests to ensure that the method provides the outcomes required. Confidence that the method can deliver these outcomes is expressed in terms of the statistical probability for the entire analyte concentration range established during the validation process (Wells 2000). Whether an analytical method is adequate depends on the objective of its use. A quantitative method allows the user to reliably establish whether the residue level exceeds the maximum residue limit (Aerts et al. 1995). The limits of quantification obtained in the current trial for liver and fat were 1.4 and 2.5 ng/g, respectively, values that are 30–40 times lower than the maximum residue limit established for DRM. These findings indicate the method's high sensitivity in detecting DRM concentrations that are below the maximum residue limit.

Table 1 Validation of the analytical methodology used to determine doramectin concentrations in liver, kidney, muscle, and adipose tissue of sheep

Parameter	Liver	Kidney	Muscle	Adipose tissue
Quantification limit (ng/g)	1.47	0.51	0.19	2.51
Recovery ± SD (%)	81.2±8.6	85.7±8.4	81.9±5.3	81.8±8.6
Linearity (r^2) (range)	0.9996 (0.9993–0.9998)	0.9997 (0.9996–0.9998)	0.9996 (0.9995–0.9998)	0.9996 (0.9994–0.9998)
Precision (CV) (range)	1.7% (0.8–2.7)	2.3% (0.8–5.0)	1.16% (0.84–1.7)	2.32% (1.02–5.08)

Table 2 Geometric mean of fecal eggs count in parasitized and nonparasitized lambs before and after doramectin administration

Group	Day −70 (n=12)	Day 0 (n=12)	Day 7 (n=3)	Day 14 (n=3)	Day 21 (n=3)	Day 28 (n=3)
Parasitized	577	1078	167	133	267	367
Nonparasitized	271*	128*	67	166	67*	33*

n Number of animals per group, *Day 0* doramectin administration
*P<0.05, paired Student's t test

The validation procedure indicated that the analytical method used to extract, derivatize, and quantify DRM in sheep tissue by HPLC using fluorescence detection is appropriate for the study of patterns of tissue residue depletion of DRM in subcutaneously treated sheep. The method presented levels of linearity, recovery, and precision for DRM detection in the range of 1.5–200 ng/g for liver and 2.5–200 ng/g for fat samples from sheep tissues.

Tissue residues of doramectin in parasitized and nonparasitized lambs

DRM was detected in all of the tissue analyzed up to 28 days after subcutaneous administration to lambs, which is in agreement with this compound's high lipophilicity and extensive distribution. The tissue residue pattern observed in the present study agrees with those obtained for 10-month-old Suffolk cross lambs (Gottschall 1997). The results of the DRM concentrations in liver, muscle, and kidney observed in the healthy lambs were similar to those previously described in sheep by Gottschall (1997). Higher DRM concentrations were obtained in the adipose tissue than in the other tissues analyzed between 7 and 28 days post-treatment. According to the EMEA (1999) proposal, considering the parent drug concentrations and the metabolites observed in different tissues, it is evident that liver and fat tissues are the most suitable for monitoring DRM residues and that the unchanged drug is the most appropriate compound for use as a marker residue.

Table 3 Mean ± SEM body weight in parasitized and nonparasitized lambs before and after doramectin administration

Time (days)	Nonparasitized	Parasitized	P value
−70 (n=12)	23.2±1.1	23.3±0.8	0.46
0 (n=12)	34.9±1.3	27.4±0.8	0.01*
7 (n=3)	32.8±2.1	26.2±1.2	0.02*
14 (n=3)	34.2±2.5	28.17±0.8	0.04*
21 (n=3)	36.8±3.6	30.0±1.1	0.07
28 (n=3)	40.7±2.9	33.27±2.6	0.06

n Number of animals per group, *Day 0* doramectin administration
*P<0.05, paired Student's t test

Table 4 Tissue residue concentrations of doramectin (mean ± SEM) in parasitized and nonparasitized lambs (n=3 at each time)

Tissue	Time (days)	Nonparasitized (ng/g)	Parasitized (ng/g)	P values
Liver	7	204.8±8.7	118.6±35.6	0.05*
	14	47.8±5.2	26.9±5.0	0.027*
	21	12.2±5.9	10.6±1.5	0.803
	28	10.1±2.5	2.2±1.1	0.025*
Kidney	7	64.8±1.4	37.1±8.5	0.018*
	14	12.7±0.9	10.8±2.9	0.514
	21	5.1±2.2	3.4±0.8	0.485
	28	2.4±0.8	1.5±0.5	0.37
Muscle	7	72.7±16.1	23.7±5.1	0.027*
	14	11.3±1.4	8.3±2.7	0.368
	21	13.7±2.3	4.2±1.0	0.008*
	28	2.6±0.6	0.4±0.1	0.015*
Adipose	7	530.0±34.4	199.1±44.5	0.001*
	14	155.1±23.9	65.1±14.8	0.018*
	21	40.5±6.3	25.9±6.3	0.1546
	28	31.3±2.8	13.7±3.6	0.085

*P<0.05, paired Student's t test

The results of the current study indicate that the body weight of the nonparasitized group increased significantly, whereas the parasitized lambs remained at values similar to those observed at the beginning of the assay (day −70). These body weight differences probably indicate important differences in the body composition of parasitized lambs. Gastrointestinal parasite infection in growing lambs can lead to significant production losses attributable to reduced efficiency in metabolizable energy use and reduced voluntary feed intake (Dynes et al. 1998; Coop and Kyriazakis 1999). The severe reduction in the efficiency of digested energy use is mainly indicated by reduced fat deposition (Sykes 1978).

According to our results, it is probable that the body weight changes observed in parasitized lambs can have a significant influence on DRM tissue distribution, considering that avermectins like DRM are highly lipophilic drugs with a high affinity for body fat. Thus, it is possible that some changes in lipid turnover may occur in parasitized lambs, which may account for the low DRM levels. It has been proposed that the residence time of subcutaneously administered ML compounds is influenced by the animal's body condition, and the volume of distribution and elimination half-lives of ML compounds are generally higher in animals with high body fat content, such as pigs and sheep, in comparison with cattle (Hennessy and Alvinerie 2002).

Among other factors, nutritional status and body composition have been shown to substantially affect the systemic availability of ivermectin in cattle (Lifschitz et al.

1997) and sheep (Bogan and McKellar 1988). In the current trial, the differences observed in body weight between the two groups of lambs resulted in different DRM distribution in tissues, with significantly higher and more persistent drug concentrations observed in the nonparasitized group. These results are in agreement with our previous study, which demonstrated that parasitism induced significant changes in DRM disposition and availability, changes that are associated with a greater drug clearance, in comparison with the parasite-naive animals (Pérez et al. 2007). Similarly, other studies have shown that the duration for moxidectin action may be reduced when the nutritional stress of parasitic disease affects body condition (Hennessy and Alvinerie 2002; Lespine et al. 2004).

Avermectins are highly lipophilic drugs that are extensively distributed from the bloodstream to different tissues. A large volume of distribution has been reported after intravenous administration of DRM in sheep (7.6 l/kg; Gottschall 1997). The high lipid solubility of DRM facilitates its deposition in adipose tissue. Gottschall (1997) demonstrated that the subcutaneous administration of DRM produces higher concentrations in fat than in other edible tissues, such as liver, kidney, and muscle. Therefore, high lipid solubility of avermectins and a large fat reservoir in sheep may contribute to the long persistence of DRM in plasma at low concentrations, probably because of the lower blood supply to fat tissues (Atta and Abo-Shihada 2000). Consequently, the low level of plasma availability of DRM in parasitized lambs could be attributed to a reduction in fat reserves in these animals in comparison with the nonparasitized lambs. These differences explain the greater quantity of drug that is available to be eliminated from the body of parasitized animals through the normal excretion pathways, which is in line with the differences observed in the clearance values between parasitized and nonparasitized animals (Pérez et al. 2007).

Drugs with a large molecular weight, such as endectocidal compounds, are eliminated in the bile rather than in urine Baggot (1992). As demonstrated in sheep, a significant portion of the systemically available DRM is secreted through the bile, reabsorbed, and enterohepatically recycled (Hennessy et al. 2000); extremely high biliary concentrations of radio-labeled DRM occurred within 4 h of intravenous administration, amounting to 132% of the dose in the 28-day study. This extended high concentration in bile is influenced by prolonged exchange from lipid reserves and the enterohepatic recycling of biliary compounds through the portal and biliary pools (Hennessy and Alvinerie 2002).

In addition to the effects on body fat reserves, pathophysiological changes in the host because of gastrointestinal parasitism may also influence drug fate. One of the key features of gastrointestinal nematode infection is an increased loss of endogenous protein into the gastrointestinal tract partially as a result of plasma protein leakage and partially because of increased mucoprotein production and sloughing of epithelial cells into the alimentary tract (Poppi et al. 1986; Coop and Kyriazakis 1999). The increased permeability of the digestive epithelium to plasma proteins in the gastrointestinal lumen reduces the half-life of plasma albumin and immunoglobulin up to 50% (Barriga 1996). Binding studies in humans and dogs showed that ivermectin bound extensively to plasma proteins (Okonkwo et al. 1993), including albumin and lipoproteins (Rohrer and Evans 1990). Consequently, plasma protein leakage into the gut might have been an important factor for increasing the fecal excretion of DRM and reducing the tissue distribution of the drug in the parasitized lambs in this study.

The WP is the necessary interval between a drug's last administration under normal conditions of use and the point when the treated animals can be slaughtered for the production of safe foodstuffs. The WP should provide a high degree of assurance both to the producer and the consumers that the concentration of residues in food derived from treated animals are below the maximum residue levels (Vranic et al. 2003). Considering the maximum residue limits established by the EMEA (1999) for DRM in liver (50 μg/kg) and fat (100 μg/kg) in sheep, the calculated WPs in the healthy group were 43 days (liver) and 32 days (fat). These values were significantly higher than those obtained for parasitized lambs, 26 and 22 days for liver and fat, respectively.

The current study's WP results for healthy nonparasitized lambs are similar to the recommended WP for the injectable formulation of DRM in sheep, which have the following ranges: 28–70 days in Europe, 35 or 50 days in Latin America, 35–42 days in the Middle East and Africa, and 35 days in Asia Conder and Baker (2002). Normally, pharmacokinetic and tissue residue depletion studies on anthelmintics are carried out in healthy nonparasitized animals. As has been demonstrated in the current work, however, subcutaneous administration of DRM to parasitized lambs produced lower, less-persistent tissue concentrations, resulting in a significant reduction in WP values, which are lower than those established by the national licensing authorities of various countries across the world.

In conclusion, because of the changes induced in the gastrointestinal tract and the nutritional condition of parasitized lambs, gastrointestinal parasitism significantly reduced the tissue distribution and persistence of DRM. Consequently, the calculated WPs of DRM are shorter in parasitized than in healthy lambs.

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References

Aerts MML, Hogenboom AC, Brinkman UAR (1995) Analytical strategies for screening of veterinary drugs and their residues in edible products. J Chromatogr B 667:1–40

Atta AH, Abo-Shihada MN (2000) Comparative pharmacokinetics of doramectin and ivermectin in sheep. J Vet Pharmacol Ther 23:49–52

Baggot JD (1992) Disposition and fate of drugs in the body. In: Booth NH, McDonald LE (eds) Veterinary pharmacology and therapeutics. 6th edn. Iowa State University Press, Ames, pp 38–71

Barriga O (1996) Veterinary parasitology. Burgess International Group, Inc., Minneapolis MN, USA, pp 16.1–16.12

Bogan JA, McKellar Q (1988) The pharmacodynamic of ivermectin in sheep and cattle. J Vet Pharmacol Ther 11:260–268

Conder GA, Baker WJ (2002) Chemistry, pharmacology and safety: doramectin and selamectin. In: Vercruysse J, Rew RS (eds) Macrocyclic lactones in antiparasitic therapy. CAB International, Wallingford, UK, pp 30–50

Coop RL, Kyriazakis I (1999) Nutrition–parasite interaction. Vet Parasitol 84:187–204

Coop RL, Barger IA, Jackson F (2002) The use of macrocyclic lactones to control parasites of sheep and goats. In: Vercruysse J, Rew RS (eds) Macrocyclic lactones in antiparasitic therapy. CAB International, Wallingford, UK, pp 303–321

Dynes RA, Poppi DP, Barrel GK, Sykes AR (1998) Elevation of feed intake in parasite-infected lambs by central administration of a cholecystokinin receptor antagonist. Br J Nutr 79:47–54

EMEA (1996) Note for guidance: approach towards harmonisation of withdrawal periods. EMEA/CVMP/036/95. Final

EMEA (1999) Doramectin (extension to deer including reindeer). Summary report (4). EMEA/MRL/637/99-Final

Fox MT (1997) Pathophysiology of infection with gastrointestinal nematode in domestic ruminants: recent developments. Vet Parasitol 72:285–308

Gottschall DW (1997) A comparison of the pharmacokinetics and tissue residues of doramectin after intravenous and intramuscular administration in sheep. In: Proceedings of 16th International Conference of the World Association for the Advancement of Veterinary Parasitology, Sun City, South Africa, 13 August, pp 9–19

Hennessy D, Page SW, Gottschall DW (2000) The behaviour of doramectin in the gastrointestinal tract, its secretion in bile and pharmacokinetic disposition in the peripheral circulation after oral and intravenous administration to sheep. J Vet Pharmacol Ther 23:203–213

Hennessy DR, Alvinerie MR (2002) Pharmacokinetics of the macrocyclic lactones: conventional wisdom and new paradigm. In: Vercruysse J, Rew RS (eds) Macrocyclic lactones in antiparasitic therapy. CAB International, Wallingford, UK, pp 97–123

Holmes PH (1987) Pathophysiology of nematode infection. Int J Parasitol 17:433–451

Lanusse C, Gascon LH, Prichard RK (1995) Comparative plasma disposition of albendazole, fenbendazole, oxfendazole and their metabolites in adult sheep. J Vet Pharmacol Ther 18:196–203

Lanusse C, Lifschitz A, Virkel G, Alvarez L, Sánchez S, Sutra JF, Galtier P, Alvinerie M (1997) Comparative plasma disposition kinetic of ivermectin, moxidectin and doramectin in cattle. J Vet Pharmacol Ther 20:91–99

Lespine A, Sutra JF, Dupuy J, Alvinerie M, Aumont G (2004) The influence of parasitism on the pharmacokinetics of moxidectin in lambs. Parasitol Res 93:121–126

Lifschitz A, Murno G, Sallovitz J, Virkel G, Lanusse C (1997) Malnutrition modifies ivermectin disposition kinetics in calves. J Vet Pharmacol Ther 20(Suppl. 1):102–103

Lifschitz A, Imperiale F, Virkel G, Muñoz-Cobeñas M, Scherling N, DeLay R, Lanusse C (2000) Depletion of moxidectin tissue residues in sheep. J Agric Food Chem 48:6011–6015

Meyer D, Coles E, Rich L (1992) Veterinary laboratory medicine: interpretation and diagnosis. Saunders, Philadelphia PA, USA, p 331

Nowakowski M, Lynch M, Smith D, Logan N, Mouzin D, Lukaszewicz J, Ryan N, Hunter R, Jones R (1995) Pharmacokinetics and bioequivalence of parenterally administered doramectin in cattle. J Vet Pharmacol Ther 18:290–298

Okonkwo PO, Ogbuokiri JE, Ofoegbu E, Klotz U (1993) Protein binding and ivermectin estimations in patients with onchocerciasis. Clin Pharmacol Ther 53:426–430

Pérez R, Palma C, Araneda M, Cabezas I, Rubilar L, Arboix M (2007) Gastrointestinal parasitism reduces the plasma availability of doramectin in lambs. Vet J 173:169–175

Pérez R, Palma C, Cabezas I, Araneda M, Rubilar L, Alvinerie M (2006) Effect of parasitism on the pharmacokinetic disposition of ivermectin in lambs. J Vet Med 53:43–48

Poppi DP, MacRae JC, Brewer A, Coop RL (1986) Nitrogen transactions in digestive tract of lambs exposed to the intestinal parasite *Trichostrongylus colubriformis*. B J Nutr 55:593–602

Rohrer SP, Evans DV (1990) Binding characteristics of ivermectin in plasma from collie dogs. Vet Res Commun 14:157–165

Sklavounos C, Dmers N, Lukaszewicz J, Nowakowski M (1994) Photoisomerization of aromatic doramectin derivatives. J Agric Food Chem 42:1228–1231

Steel JW (1974) Pathophysiology of gastrointestinal nematode infections in the ruminant. Proc Aust Soc Anim Prod 10:139–147

Stromberg B, Vatthauer R, Schotthauer J, Myers G, Haggard D, King V, Hanke H (1997) Production responses following strategic parasite control in a beef cow/calf herd. Vet Parasitol 68:315–322

Sykes AR (1978) The effect of subclinical parasitism in sheep. Vet Rec 102:32–34

Vranic ML, Marangunich L, Fernandez Courel H, Fernandez Suarez A (2003) Estimation the withdrawal period for veterinary drug used in food producing animals. Anal Chim Acta 483:251–257

Wells RJ (2000) Do validation requirements for chemical methods in quantitative analysis need to be flexible? In: Proceeding of the 8th International Congress of EAVPT, Jerusalem, Israel, 30 July–3 August

Wicks SR, Kaye B, Weatherley AJ, Lewis D, Davidson E, Gibson SP, Smith DG (1993) Effect of formulation on the pharmacokinetics and efficacy of doramectin. Vet Parasitol 49:17–26

Zajac AM (1994) Fecal examination in the diagnosis of parasitism. In: Sloss MW, Kemp RL, Zajac AM (eds) Veterinary clinical parasitology. 6th edn. Iowa State University Press, Ames IA, USA, pp 3–93