JOURNAL OF
Veterinary Pharmacology and Therapeutics

J. vet. Pharmacol. Therap. doi: 10.1111/jvp.12135

Closantel plasma and milk disposition in dairy goats: assessment of drug residues in cheese and ricotta

S. IEZZI*
A. LIFSCHITZ*
J. SALLOVITZ*
P. NEJAMKIN*
M. LLOBERAS†
J. MANAZZA†
C. LANUSSE* &
F. IMPERIALE*

*Laboratorio de Farmacología, Centro de Investigación Veterinaria de Tandil (CIVETAN), CONICET/CICPBA, Facultad de Ciencias Veterinarias, UNCPBA, Tandil, Argentina; †Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental Balcarce, Balcarce, Argentina

Iezzi, S., Lifschitz, A., Sallovitz, J., Nejamkin, P., Lloberas, M., Manazza, J., Lanusse, C., Imperiale, F. Closantel plasma and milk disposition in dairy goats: assessment of drug residues in cheese and ricotta. *J. vet. Pharmacol. Therap.* doi: 10.1111/jvp.12135.

Closantel (CLS) is currently used in programs for the strategic control of gastrointestinal nematodes. CLS is extralabel used in different dairy goat production systems. From available data in dairy cows, it can be concluded that residues of CLS persist in milk. The current work evaluated the concentration profiles of CLS in plasma and milk from lactating orally treated dairy goats to assess the residues pattern in dairy products such as cheese and ricotta. Six (6) female Saanen dairy goats were treated orally with CLS administered at 10 mg/kg. Blood and milk samples were collected between 0 and 36 days post-treatment. The whole milk production was collected at 1, 4, 7, and 10 days post-treatment to produce soft cheese and ricotta. CLS concentrations in plasma, milk, cheese, whey, and ricotta were determined by HPLC. The concentrations of CLS measured in plasma were higher than those measured in milk at all sampling times. However, the calculated withdrawal time for CLS in milk was between 39 and 43 days postadministration to dairy goats. CLS residual concentrations in cheese (between 0.93 and 1.8 μg/g) were higher than those measured in the milk used for its production. CLS concentrations in ricotta were sixfold higher than those in the milk and 20-fold higher than those in the whey used for its production. The persistent and high residual concentrations of CLS in the milk and in the cheese and ricotta should be seriously considered before issuing any recommendation on the extralabel use of CLS in dairy goat farms.

(Paper received 12 February 2014; accepted for publication 2 May 2014)

Dr Lifschitz Adrian Luis, Laboratorio de Farmacología, Centro de Investigación Veterinaria de Tandil (CIVETAN), CONICET/CICPBA, Facultad de Ciencias Veterinarias, UNCPBA, Campus Universitario, 7000 Tandil, Argentina.
E-mail: adrianl@vet.unicen.edu.ar

INTRODUCTION

Closantel (CLS) (N-{5-chloro-4-[(4-chlorophenyl) cyanomethyl]-2-methylphenyl}-2-hydroxy 3, 5-diiodobenzamide) is a member of the salicylanilide class of antiparasitic compounds. CLS is used primarily for treating infections by mature liver flukes (8 weeks old and adult *Fasciola hepatica*), bloodsucking nematodes, and larval stages of some arthropods which feed on blood and plasma (Courtney & Roberson, 1995). It can be administered as an oral drench, a subcutaneous injection, or a topical formulation (EMA, 2012). The salicylanilides uncouple the oxidative phosphorylation in mammals (Williamson & Metcalf, 1967; van Miert & Groeneveld, 1969) and in the liver fluke (Cornish & Bryant, 1976; Prichard, 1978).

Closantel is a poorly metabolized compound being 80% of the dose excreted by feces and <0.5% by urine. The primary route of metabolism is reductive deiodination leading to monoiodoclosantel metabolites. However, 90% of the compounds excreted correspond to the parent drug (Michiels *et al.*, 1987). Similarly, to other salicylanilides, 99% of CLS is extensively bound to plasma proteins (mainly albumin), which prolongs drug levels in plasma and limits the distribution to tissues (Michiels *et al.*, 1987; McKellar & Kinabo, 1991). After the administration of CLS to lactating dairy cows, a parallel decline of CLS concentrations in plasma and milk has been shown with a plasma/milk concentrations ratio in the order of 50/1. From available data in dairy cows, it can be concluded that residues of CLS persist in milk and that the parent compound is the main residue in this food commodity (EMA, 2012).

Currently, few flukicides are registered to be used in animals whose milk is destined for human consumption. For these products, a withdrawal time after treatment is required to avoid residual concentrations above of the allowed maximum residue limit (MRL). Recently, the European Medicines Agency (EMA) recommended a provisional MRL for CLS (45 μg/kg) in milk of bovine and ovine species, valid throughout the European Union (EMA, 2012).

Closantel is currently used in the strategic control programs of haemonchosis and also as an alternative treatment for benzimidazole- and levamisole-resistant *Haemonchus contortus* strain (Dorny *et al.*, 1994). In this context, CLS is extralabel used in different dairy goat production systems. However, goat milk MRL for CLS has not been established yet, although it seems to be highly necessary due to the worldwide development of the goat dairy industry. The work reported here was designed to evaluate the concentration profiles of CLS in plasma and milk from orally treated lactating dairy goats to assess the pattern of residues in dairy products such as cheese and ricotta.

MATERIAL AND METHODS

Experimental animals, treatment, and sampling

Six (6) Saanen dairy goats weighing between 46 and 65 kg were included in this trial. The experimental animals were clinically normal in the mid-late lactation period. They were kept under field conditions, grazing on pasture and with free access to drinking water during the whole experimental period. The animals' health was closely monitored prior to and throughout the trial. Dairy goats were milked twice a day (every 12 h) with a milking machine, and the whole milk production was measured prior to and throughout the trial. The average milk production during the trial was 1300 mL/day per animal.

Each animal was orally treated with a single dose of CLS (Galgosantel®, CLS 15%; Biogénesis-Bagó, Garín, Buenos Aires, Argentina) at a dosage of 10 mg/kg of body weight. Blood samples were taken from the jugular vein into heparinized vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA) prior to and at 4, 8, and 12 h and at 1, 2, 3, 4, 7, 10, 15, 22, 29, and 36 days post-treatment. Milk samples were collected prior to treatment and at 8 and 12 h and at 1, 2, 3, 4, 7, 10, 15, 22, 29, and 36 days post-treatment. At each sampling time, a composite milk sample (50, 25 mL from each udder) was collected by hand-milking after discarding 30–50 mL and before the complete mechanical milking of each goat. The blood samples were centrifuged at 2000 ***g*** for 20 min, and the recovered plasma was transferred to vials. Milk and plasma samples were frozen at −18 °C until being analyzed.

On days 1, 4, 7, and 10 post-treatment, the whole milk production of each treated animal ($n = 6$) was collected into a pool and processed to replicate local methods of cheese and ricotta production. After cheese making, solid (cheese) and liquid by-product (whey) were sampled. All the whey recovered was used for the ricotta production, and a ricotta sample was collected in an appropriate vial. All samples were frozen at −18 °C until being analyzed.

Analytical procedures

Sample clean-up/extraction. Pure reference standards (99% purity) of CLS and demethylated CLS (as internal standard, IS) were provided by Janssen Pharmaceutica (Beerse, Belgium), and they were used to validate the HPLC method. The standard solutions of CLS and IS were prepared by successive dilutions in methanol from stock solutions (1 mg/mL) and stored at −18 °C.

The fortified and experimental samples (plasma, milk, cheese, whey, and ricotta) were added with 0.2 μg of demethylated CLS as IS. The chromatographic conditions to quantify CLS and IS in fortified and experimental samples were carried out following modifications of a previously described method by Stoev *et al.* (1999). Detailed information on the chromatographic and extraction procedures of CLS from these biological matrices is given below.

Drug extraction. Liquid samples (plasma, milk, and whey) were extracted using the following procedure: acetonitrile (0.5 mL) and deionized water (125 μL) were added to each tube containing 0.5 mL of liquid sample and mixed for 15 min. After thorough mixing, the batch of tubes containing the mixtures was placed in an ultrasonic bath for 8 min and centrifuged at 2000 ***g*** for 15 min. The precipitates obtained from the samples were extracted again with 0.5 mL of acetonitrile as described above.

Solid samples (cheese and ricotta) were extracted using the following procedure: 0.5 mL of concentrated aqueous sodium chloride solution (26% w/v) and 1.5 mL of acetonitrile were added to each tube containing 0.5 g of solid sample. After thorough mixing for 20 min, the batch of tubes was centrifuged at 2000 ***g*** for 15 min. Supernatants were collected. The precipitates obtained from the ricotta samples were extracted twice as described above, and supernatants were collected and mixed to the previous ones.

Water (a volume equal to that of acetonitrile) was added to the supernatants. The supernatants collected from solid and liquid extractions were then passed through to a conditioned C18 cartridge (Strata; Phenomenex, Torrance, CA, USA). After washing with 1 mL of water followed by 1 mL of water/methanol (4:1, v/v), the cartridges were dried for 5 min and the samples were eluted with 1.5 mL (plasma, milk, and whey) or 5 mL (cheese and ricotta) of methanol and collected. The elutes were evaporated to dryness under a gentle stream of nitrogen at 46 °C in a water bath. The dry residues of the elution were dissolved with 200 μL of ACN, and an aliquot (50 μL) of this solution was injected directly into the chromatographic system.

Chromatographic conditions: drug analysis samples. A total of 50 μL of each extracted sample was injected by an autosampler (Shimadzu SIL 10AF Automatic Sample Injector) into a Shimadzu LC-20A HPLC system (Shimadzu Corporation, Kyoto, Japan) fitted with a Kromasil C18 (5 mm, 250 × 4.60 mm)

reverse-phase column (Eka Chemicals, Brewster, NY, USA) at 30 °C and a fluorescence detector (Shimadzu; RF 10A XL detector) reading at 335 nm excitation and 510 nm emission. The mobile phase consisted of acetonitrile-water (15:85 v/v) containing 0.05% diethylamine at pH 2.5, adjusted with phosphoric acid with a flow rate set at 1.5 mL/min. The total run time for the method was 25 min. The analytes were identified with the retention times of 99% pure reference standards. Chromatographic peak areas of each molecule were measured using the integrator software (Class LC 10; Shimadzu Corporation) of the HPLC system.

Method validation. A complete validation of the analytical procedures for the extraction and quantification of CLS in each matrix was performed before the analysis of experimental samples. Calibration lines in the ranges of 0.1–10 μg/mL (milk and whey) or μg/g (cheese and ricotta) and 0.5–60 μg/mL (plasma) were plotted using the peak-area ratios between CLS and the IS. The data were analyzed for linearity using a least-squares linear regression analysis and using the Run Test and ANOVA to determine whether the data differed from a straight line. The absolute recovery of CLS was measured by comparison of the peak-area ratios from spiked samples with the peak-area ratios resulting from direct injections of standards in methanol. The CLS recovery percentages for each matrix were obtained using three replicates for each drug concentration. The interday precision of the extraction and chromatographic procedures were evaluated by processing four replicate aliquots of pooled liquid and solid samples containing known amounts of CLS (1 and 5 μg/mL for milk and whey or μg/g for cheese and ricotta and 10 and 30 μg/mL for plasma) on different working days. The accuracy of the analytical method was estimated by the differences between observed and calculated concentrations, and it is expressed as the percentage of relative error (%RE). The accuracy was estimated for all matrices under study at the CLS concentrations of 0.25 and 5 μg/mL (milk, whey) or μg/mL (cheese and ricotta) or 10 and 60 μg/mL (plasma) with three determinations for each concentration value. The coefficients of variation (CV) for recovery and interday precision of the method were calculated (Bolton, 1984). The limit of quantification (LOQ) was defined as the lowest concentration that can be measured with acceptable precision (CV < 20%) and accuracy (20%; Snyder *et al.*, 1997).

The linearity of the analytical method to measure CLS was confirmed by the estimated values of r^2 values, which ranged between 0.991 and 0.999 for the different matrices. The inter-assay precision of the analytical method showed a CV between 4.7% and 8.6%. The absolute extraction recovery for plasma, milk, and derived products in different concentrations analyzed ranged between 67.5% and 99%. The LOQ and the limit of detection were 0.05 and 0.025 μg/mL, respectively.

Drug quantitation, pharmacokinetic and statistical analyses of the data. Drug concentrations in experimental samples were determined by HPLC calculating the ratio between the areas under the peaks of CLS and the IS using the CR10 software and interpolating these areas on the calibration lines prepared for each matrix. The statistical program (Instat 3.0; Graph Pad Software Inc., San Diego, CA, USA) was used for linear regression analyses and linearity tests. The milk and plasma concentration vs. time curves obtained after treatment in each individual animal were analyzed with the PK Solution 2.0 (Summit Research Services, Ashland, OH, USA) computer program and the PKSolver 2-0 computer program (Zhang *et al.*, 2010).

Pharmacokinetic variables were determined using a noncompartmental method. The peak concentration (C_{max}) and time to peak concentration (T_{max}) were read from the plotted concentration–time curves in each individual animal. The terminal half-life ($T_{1/2el}$) was calculated as ln $2/\lambda_z$, where λ_z is the elimination rate constant. The λ_z was determined performing regression analysis using at least five points of the terminal phase of the concentration–time plot. The areas under the concentration–time curves (AUC) were calculated by the trapezoidal rule (Gibaldi & Perrier, 1982) without extrapolation to infinity. The volumes of distribution based on the terminal slope and clearance, both referred to bioavailability (F), were calculated for plasma.

The $AUC_{milk/plasma}$ ratios were estimated for CLS treatment using the AUC values. The percentage of total dose excreted in milk for each individual animal was estimated using the values of drug concentration at each sampling time interval and the volume of milk production during the experiment. The withdrawal time for CLS in milk was calculated with the WTM 1.4 software (EMA). The method for the determination of withdrawal periods for milk was the Time to Safe Concentration Method (TTSC; EMA, 1998). Also withdrawal time for CLS in milk was calculated using the following formula:

Withdrawal time: 1.44 ln (C0/tolerance; Elimination half-life; Riviere & Sundlof, 2009).

The CLS kinetic parameters in plasma and milk were reported as mean ± standard deviation (SD). The Student's t-test and Mann–Whitney U-test were used to estimate the differences between kinetic parameters obtained in milk and plasma. Statistical differences were considered at $P < 0.05$.

RESULTS

Closantel was recovered in plasma between 4 h and 36 days and in milk between 8 h and 29 days post-treatment. The concentrations of CLS measured in plasma were significantly higher than those obtained in milk at all sampling times. The plasma and milk concentration profiles measured after the oral administration of CLS in dairy goats are compared in Fig. 1. The observed differences between plasma and milk concentrations of CLS were reflected on the main pharmacokinetic parameters. Plasma concentrations increased progressively to reach a C_{max} of 34.8 μg/mL on day 1 compared with 0.8 μg/mL in milk. CLS plasma AUC values after oral administration were 69-fold higher than those in milk ($P < 0.01$). As previously reported, CLS showed a long persistence in the body of goats with the mean plasma elimination half-life of 8.9 days.

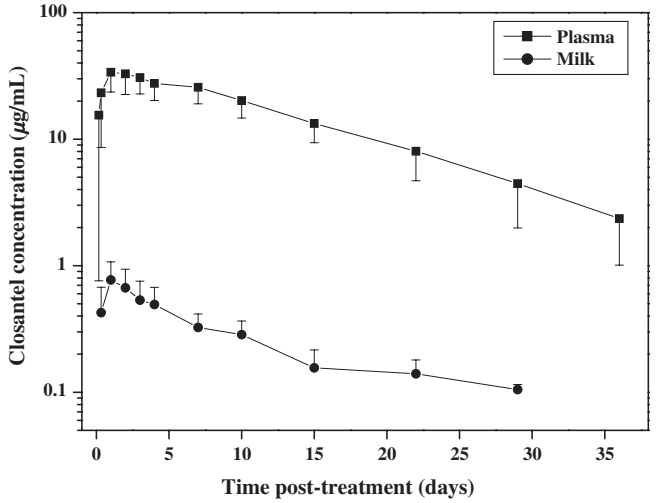

Fig. 1. Comparative plasma and milk concentration profiles (mean ± SD) of closantel measured after oral administration (10 mg/kg) to dairy goats (n = 6).

Table 1. Mean (±SD) of the kinetic parameters describing the disposition of closantel (CLS) in plasma and milk of dairy goats after oral administration (10 mg/kg; n = 6)

Kinetic parameters	Plasma	Milk
C_{max} (μg/mL)	34.8 ± 10.8	0.8 ± 0.30 (**)
T_{max} (day)	1.00 ± 0.00	1.00 ± 0.00
$T_{½el}$ (day)	8.90 ± 2.51	9.90 ± 3.05
AUC_{0-t} (μg·day/mL)	493 ± 133	7.5 ± 2.63 (**)
MRT (day)	13.1 ± 3.13	–
Ratio $AUC_{milk/plasma}$		0.015 ± 0.003
Vz/F (mL/kg)	221 ± 44.0	–
Cl/F (mL·day/kg)	20.9 ± 7.55	–
Percentage dose recovered		1.65 ± 0.556

Mean kinetic variables obtained for CLS in plasma are statistically different at $P < 0.01$ (**) from those obtained in milk after oral administration. C_{max}, peak plasma or milk concentration; T_{max}, time to peak concentration; $T_{1/2el}$, elimination half-life; AUC, area under the concentration vs. time curve; ratio AUC milk/plasma, the ratio between AUC values obtained in milk and plasma; MRT, mean residence time; Vz/F, volume of distribution based on the terminal slope referred to bioavailability (F); Cl/F, clearance referred to bioavailability (F).

The plasma clearance (in relation to the bioavailability; Cl/F) was 20.9 mL·day/kg. The pharmacokinetic parameters summarizing the disposition of CLS in plasma and milk are shown in Table 1.

In the current work, the milk collected from treated goats at 1, 4, 7, and 10 days post-treatment was used for cheese making and the drained whey was used for making ricotta. Once the cheese-making process was completed, cheese samples were collected. CLS residual concentrations in cheese (between 0.93 and 1.8 μg/g) were higher than those measured in the milk used for its production. The mean ratio between CLS residual concentration values in the cheese and in the milk used for its production was threefold (Fig. 2). CLS concentrations in whey were between 0.1 and 0.7 μg/mL. CLS concentrations found in ricotta were

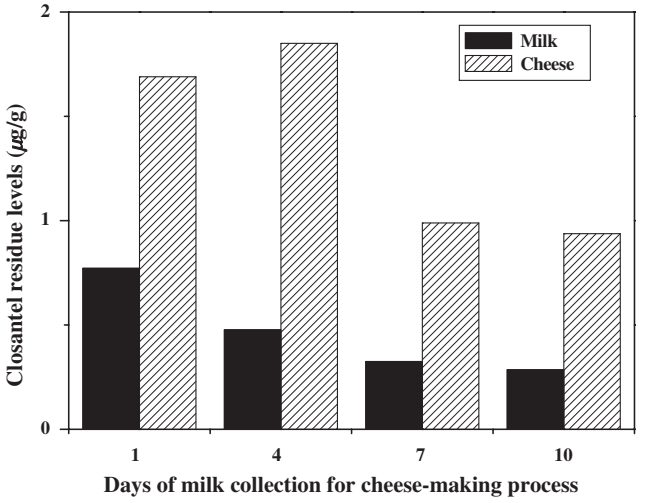

Fig. 2. Comparison of closantel residue levels measured in pooled milk and cheese elaborated with milk collected from treated goats (n = 6) at different time post-treatment.

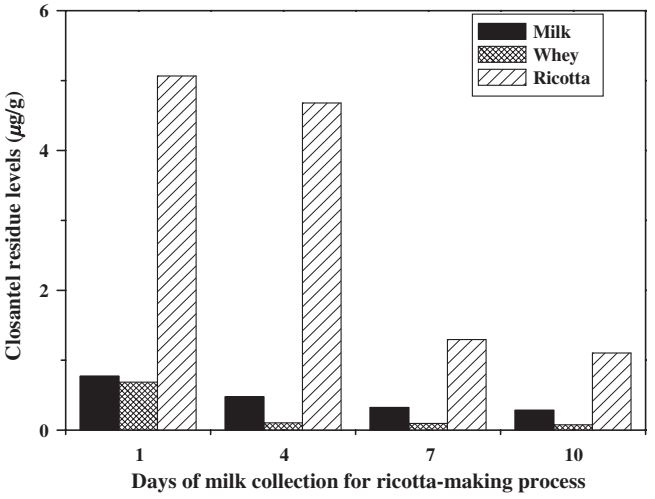

Fig. 3. Comparison of closantel residue levels measured in pooled milk, whey and ricotta elaborated with whey collected during the cheese-making process done with milk collected from treated goats (n = 6) at different time post-treatment.

sixfold higher than those in milk used for cheese making and 20-fold higher than those in whey used for its production (Fig. 3). The calculated withdrawal time for CLS in milk was between 39 and 43 days postadministration to dairy goats.

DISCUSSION

Scarce available data on the pharmacokinetics behavior of CLS in goats have been published. Hennessy *et al.* (1993a) characterized the plasma disposition of CLS in goats and sheep. Higher and more persistent concentrations of CLS were measured in the current work compared to those reported by Hennessy *et al.* (1993a). A different goat breed and a more sensitive analytical method (fluorescent detection) used in the

current trial may explain these differences. The long persistence of CLS plasma concentrations in the current trial was reflected on the half-life elimination value (8.9 days) and the low plasma clearance (20.9 mL·day/kg). However, the persistence of CLS plasma concentrations in goats was shorter than that reported after its administration to sheep (Hennessy *et al.*, 1993a). CLS is highly associated with plasma proteins almost exclusively to serum albumin, which may explain the long plasma persistence. It is unlikely that the shorter elimination half-life of CLS in dairy goats compared with other animal species (Mohammed-Ali & Bogan, 1987; Hennessy *et al.*, 1993a) should be attributed to the differences in the turnover of albumin. It is suggested that goats possess a faster hepatic metabolism than sheep resulting in more rapid elimination of different drugs. Some works have demonstrated the more rapid metabolism and clearance of other antiparasitic drugs such as oxfendazole (Sangster *et al.*, 1991; Hennessy *et al.*, 1993b) and albendazole (Hennessy *et al.*, 1993c) in goats due to a high hepatic metabolism in this ruminant species. Similarly, the shorter elimination half-life of CLS in dairy goats in the current work compared to those reported in sheep by Hennessy *et al.* (1993a) may be based on the higher rate of hepatic activity because the liver is the main site of reductive deiodination of CLS (Michiels *et al.*, 1987).

After the CLS oral administration to dairy goats, milk concentrations were persistent but significantly lower compared to those observed in plasma. Results reported here show that even though CLS distributes from the blood to the milk, although in a limited amount, most of the drug remaining in the bloodstream. This is clearly depicted by the low volume of distribution during the elimination phase (221.2 mL/kg), the AUCmilk/plasma ratios, and the low percentage of the dose recovered in the milk (1.65 ± 0.56%; Table 1).

Different works have recently evaluated the secretion mechanism of antiparasitic drugs into the milk. The involvement of breast cancer resistance protein (BCRP) has been described in the secretion mechanism of moxidectin and triclabendazole in sheep (Barrera *et al.*, 2012). The interaction of CLS as inhibitor of *P*-glycoprotein and BCRP has been observed in *in vitro* and *ex vivo* methods (Dupuy *et al.*, 2010; Ballent *et al.*, 2012). The low levels of CLS in milk may indicate that BCRP does not participate in its secretion into the milk. Although this mechanism should be evaluated in the target species, it seems that CLS may modulate the BCRP-mediated milk secretion of other antiparasitic drugs co-administered in combined pharmaceutical formulations.

The relevance of the presence of drug residues in milk is depicted by to the MRLs established by regulatory authorities. Recently, the European Medicines Agency adopted the provisional CLS MRL for cattle and sheep milk of 45 μg/kg (EMA, 2012). In the current trial, the mean concentration of CLS in milk on day 1 postadministration was 0.77 μg/mL, that is, 17-fold above the established MRL for milk. CLS milk residues were above the established MRL at all sampling times up to 29 days post-treatment (0.1 μg/mL). In agreement with our results, CLS milk residues were above the established MRL for 52 days after its subcutaneous administration to dairy cattle (Power *et al.*, 2013).

Closantel concentrated in milk-derived products elaborated with the milk collected on days 1, 4, 7, and 10 after its oral administration to dairy goats. CLS residual concentrations were detected in pooled milk at all days of cheese-making process, and they were above the provisional MRL established for sheep milk (EMA, 2012). CLS concentrations in cheese and ricotta were significantly higher than those measured in milk. On the other hand, CLS whey concentrations were lower than those observed in milk. Considering the CLS residues found in cheese prepared with milk collected on day 4 post-treatment in the current trial, the ADI value for CLS (EMA, 2012) and the maximum estimated daily cheese intake (59 g·person/day) determined by the EU Scientific Co-operation Programme (SCOOP), and the consumption of CLS in cheese could reach the 12% of the established ADI.

Similarly, Power *et al.* (2013) reported a transfer of CLS residues from milk of treated dairy cattle to derived products such as cheese, cream, and butter, being CLS concentrations between six- and 10-fold higher in these products compared with the milk obtained on days 2 and 23 after its subcutaneous administration to dairy cattle. Using different methods, a milk withdrawal interval following CLS administration in goats was determined. Based on the elimination half-life of CLS in milk, a period of discard time of 39 days was established. The use of the elimination half-life in milk should be useful to determine the discard times when approved withdrawal times do not exist (Riviere & Sundlof, 2009), as is the case of CLS. Interestingly, the calculation of the withdrawal time of CLS in goat milk with the TTSC method (EMA, 1998) resulted in a discard time of 43 days. As the drug is concentrated in cheese during the cheese-making process, more research would be necessary to determine an appropriate withdrawal interval before milk from treated goats can be used in the manufacture of cheeses. However, the long withdrawal time of CLS would result in an impractical use of this compound in the different dairy production systems.

A different behavior was observed for other member of the group of salicylanilides. Whereas oxyclozanide was also eliminated by milk after its oral administration to dairy cattle, its residual levels found in soft and hard cheeses were lower compared to those detected in the milk used for their elaboration. The highest concentrations of oxyclozanide were found in the whey obtained during the cheese making (Whelan *et al.*, 2010). Another important issue is related to the thermal stability of drug residues during the milk-derived products elaboration. Oxyclozanide residues were shown to be stable enough to endure the milk heat treatment and the fermentation process and to persist in cheese (Whelan *et al.*, 2010). During a previous experimental work in our laboratory, the residual concentrations of CLS were stable during thermal processing of goat milk (Iezzi *et al.*, 2011). Similarly, Power *et al.* (2013) also showed the heat stability of CLS in cattle milk to pasteurization and spray-drying temperatures, which had no influence on the CLS concentration in the milk-derived products.

The anthelmintic drugs are extensively used in different livestock production systems. Although many antiparasitic drugs

are licensed for treating parasitic diseases in food-producing animals, only a small number of formulations are registered to be used in goats.

The widespread resistance of nematodes to different anthelmintic groups, especially in small ruminants, stimulated farmers to an extralabel use of them. Therefore, CLS is considered by farmers as an alternative to control highly resistant *Haemonchus contortus* to macrocyclic lactones and benzimidazoles in dairy goat farms located in many countries. Thus, a concern has risen to the potential presence of detectable drug residues in milk and derived products. Based on the data reported in the current trial, the persistent concentrations of CLS in the milk of goats and the large residues concentrations recovered in cheese and ricotta should be seriously considered before issuing any recommendation on the extralabel use of CLS in dairy goat farms.

ACKNOWLEDGMENTS

The technical assistance of Med. Vet. Daniel Colombo, owner of goat dairy farm 'La Piedra', is greatly acknowledged. Authors thank to Dr. Esteban Turic (Biogénesis-Bagó, Argentina) for supplying the commercial oral formulation of closantel used in the present study. The current work was supported by CONICET, Universidad Nacional del Centro, and Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), all of them from Argentina.

REFERENCES

Ballent, G.M., Lifschitz, A., Virkel, G., Mate, L., Sallovitz, J.M. & Lanusse, C. (2012) Comparative ex vivo interaction of ivermectin, moxidectin and closantel with intestinal ABC transporters. *Journal of Veterinary Pharmacology and Therapeutics*, **35** (Suppl. 3), 127–128.

Barrera, B., Otero, J.A., Egido, E., Prieto, J.G., Seelig, A., Álvarez, A.I. & Merino, G. (2012) The anthelmintic triclabendazole and its metabolites inhibit the membrane transporter ABCG2/BCRP. *Antimicrobial Agents and Chemotherapy*, **56**, 3535–3543.

Bolton, S. (1984) Basic definitions and concepts. In *Pharmaceutical Statistics. Practical and Clinical Applications*. Ed. Swarbrick, J., pp. 19–22. Dekker, New York, NY, USA.

Cornish, R.A. & Bryant, C. (1976) Changes in energy metabolism due to anthelmintics in *Fasciola hepatica* maintained *in vitro*. *International Journal for Parasitology*, **6**, 393–398.

Courtney, C. & Roberson, E. (1995). Antinematodal drugs. In *Veterinary Pharmacology and Therapeutics*, 7th edn. Ed. Adams, H.R., pp. 916–922. Iowa State University Press, Ames. IA, USA.

Dorny, P., Vercruysse, J., Jalilla, A., Sani, R. & Symoens, C. (1994) Control of haemonchosis in Malaysian goats with closantel. *Veterinary Parasitology*, **53**, 233–241.

Dupuy, J., Alvinerie, M. & Lespine, A. (2010) Interaction of anthelmintic drugs with P-glycoprotein in recombinant LLC-PK1-mdr1a cells. *Chemico-Biological Interactions*, **186**, 280–286.

EMA (1998). *The European Medicines Agency. Committee for Medicinal Products for Veterinary Use, CVMP*. Note for guidance for the determination of withdrawal periods for milk, EMA/CVMP/473/98-FINAL.

EMA (2012) *The European Medicines Agency. Committee for Medicinal Products for Veterinary Use, CVMP*. Closantel, EMA/CVMP/813350/2011.

Gibaldi, M. & Perrier, D. (1982) Pharmacokinetics. In *Drugs and the Pharmaceutical Sciences*, 2nd edn. Ed. Gibaldi, M., pp. 45–109. Marcel Dekker, Inc., New York, NY, USA.

Hennessy, D.R., Sangster, N.C., Steel, J.W. & Collins, G.H. (1993a) Comparative pharmacokinetic disposition of closantel in sheep and goats. *Journal of Veterinary Pharmacology and Therapeutics*, **16**, 254–260.

Hennessy, D.R., Sangster, N.C., Steel, J.W. & Collins, G.H. (1993b) Comparative kinetic disposition of oxfendazole in sheep and goats before and during infection with *Haemonchus contortus* and *Trichostrongylus colubriformis*. *Journal of Veterinary Pharmacology and Therapeutics*, **16**, 245–253.

Hennessy, D.R., Sangster, N.C., Steel, J.W. & Collins, G.H. (1993c) Comparative pharmacokinetic behaviour of albendazole in sheep and goats. *International Journal for Parasitology*, **23**, 321–325.

Iezzi, S., Nejamkin, P., Sallovitz, J., Farias, C., Lifschitz, A., Imperiale, F. & Lanusse, C. (2011) *Evaluation of Thermal Stability of Closantel During the Cheese Elaboration with Goat Milk*. XLIII Reunión anual de la Asociación Argentina de Farmacología Experimental (SAFE). ISSN 2250-4079. Abstract B8-75, pp. 50.

McKellar, Q.A. & Kinabo, L.D.B. (1991) The pharmacology of flukicidal drugs. *British Veterinary Journal*, **147**, 306–321.

Michiels, M., Meuldermans, W. & Heykants, J. (1987) The metabolism and fate of closantel (Flukiver) in sheep and cattle. *Drug Metabolism and Reviews*, **18**, 235–251.

van Miert, A.S.J.P.A.M. & Groeneveld, H.S. (1969) Anthelmintics, used for the treatment of fascioliasis as uncouplers of oxidative phosphorylation in warm blooded animals. *European Journal of Pharmacology*, **8**, 385–388.

Mohammed-Ali, N.A.K. & Bogan, J.A. (1987) The pharmacodynamics of the flukicidal salicylanilides, rafoxanide, closantel and oxyclosanide. *Journal of Veterinary Pharmacology and Therapeutics*, **10**, 127–133.

Power, C., Sayers, R., O'Brien, B., Clancy, C., Furey, A., Jordan, K. & Danaher, M. (2013) Investigation of the persistence of closantel residues in bovine milk following lactating-cow and dry-cow treatments and its migration into dairy products. *Journal of Agricultural and Food Chemistry*, **61**, 8703–8710.

Prichard, R.K. (1978) The metabolic profile of adult Fasciola *hepatica* obtained from rafoxanide treated sheep. *Parasitology*, **76**, 277–278.

Riviere, J.E. & Sundlof, S.F. (2009) Chemical residues in tissues of food animals. In *Veterinary Pharmacology and Therapeutics*, 9th edn. Eds Riviere, J.E. & Papich, M., pp. 1453–1462. Wiley-Blackwell, Ames, IA, USA.

Sangster, N.C., Rickard, J.M., Hennessy, D.R., Steel, J.W. & Collins, G.H. (1991) Disposition of oxfendazole in goats and efficacy compared with sheep. *Research in Veterinary Science*, **51**, 258–263.

Snyder, L., Kirkland, J. & Glajch, J. (1997) Completing the method: validation and transfer. In *Practical HPLC Method Development*. 2nd edn. Ed. Wiley, J., pp. 685–713. Wiley and Sons Inc., New York, NY, USA.

Stoev, G., Dakova, T. & Michailova, A.L. (1999) Quantitative determination of Closantel residues in milk by high-performance liquid chromatography with fluorescence detection. *Journal of Chromatography A*, **846**, 383–386.

Whelan, M., Chirollo, C., Furey, A., Cortesi, M.L., Anastasio, A. & Danaher, M. (2010) Investigation of the persistence of levamisol and oxyclozanide in milk and fate in cheese. *Journal of Agricultural and Food Chemistry*, **58**, 12204–12209.

Williamson, R.L. & Metcalf, R.L. (1967) Salicylanilides: a new group of active uncouplers of oxidative phosphorylation. *Science*, **158**, 1694–1695.

Zhang, Y., Huo, M., Zhou, J. & Xie, S. (2010) PKSolver: an add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel. *Computer Methods and Programs in Biomedicine*, **99**, 306–314.