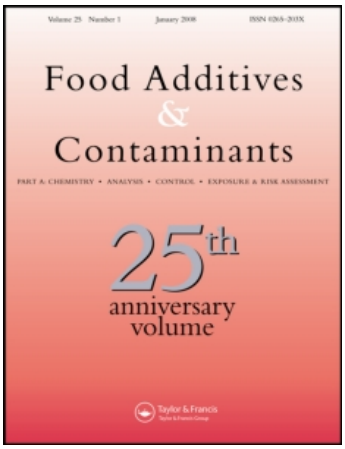

Food Additives & Contaminants: Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713599661

Thermal stability of antiparasitic macrocyclic lactones milk residues during industrial processing

F. A. Imperiale [ab]; C. Farias [ab]; A. Pis [a]; J. M. Sallovitz [ac]; A. Lifschitz [ab]; C. Lanusse [ab]
[a] Laboratorio de Farmacología, Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires, Tandil, Argentina [b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina [c] Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CICPBA), Argentina

First Published:January2009

To cite this Article Imperiale, F. A., Farias, C., Pis, A., Sallovitz, J. M., Lifschitz, A. and Lanusse, C.(2009)'Thermal stability of antiparasitic macrocyclic lactones milk residues during industrial processing',Food Additives & Contaminants: Part A,26:1,57 — 62
To link to this Article: DOI: 10.1080/02652030802322879
URL: http://dx.doi.org/10.1080/02652030802322879

Food Additives and Contaminants
Vol. 26, No. 1, January 2009, 57–62

Taylor & Francis
Taylor & Francis Group

Thermal stability of antiparasitic macrocyclic lactones milk residues during industrial processing

F.A. Imperiale[ab]*, C. Farias[ab], A. Pis[a], J.M. Sallovitz[ac], A. Lifschitz[ab] and C. Lanusse[ab]

[a]*Laboratorio de Farmacología, Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires, Tandil, Argentina;* [b]*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina;* [c]*Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CICPBA), Argentina*

(*Received 13 March 2008; final version received 2 July 2008*)

The chemical stability of residues of different antiparasitic macrocyclic lactone compounds in milk subjected to thermal treatment was assessed. Concentrations of ivermectin (IVM), moxidectin (MXD) and eprinomectin (EPM) in sheep milk, equivalent to those measured *in vivo* in milk excretion studies, were subjected to 65°C over 30 min or to 75°C for 15 s. Residue concentrations of IVM, MXD and EPM in milk were measured by high-performance liquid chromatography (HPLC) (fluorescence detection) before and after heat treatment of the drug-fortified milk samples. No evidence of chemical loss was obtained in either of the thermal treatments under evaluation. The stability of the parent compounds in milk was evidenced by the lack of bioconversion products (metabolites) after both thermal treatments. Only very minor changes on drug concentrations were observed at the end of the treatments, which fell within the limits of the variation of the validated analytical method. In conclusion, residue concentrations of macrocyclic lactones are unaffected by industrial-simulated milk thermal procedures. Based on the reported findings, it can be postulated that residue concentrations of IVM, MXD and EPM measured in raw sheep milk may be used to estimate consumer exposure and dietary intake for these veterinary drugs.

Keywords: ewe milk; antiparasitic drugs; milk residues; thermal stability; industrial processing

Introduction

Small ruminants' livestock production is increasing worldwide. This is indicative of a growing consumption in many countries of meat and, mainly, dairy products from these species. During the last 20 years, the increase of dairy goat milk production (69%) exceeded that of dairy cow milk (10%) worldwide, while dairy sheep production (2%) increased to a lesser extent. In some areas, particularly where a population is influenced by Hispanic culture, there are great opportunities for the development of dairy sheep and goat herding (Haenlein 2001; Dubeuf et al. 2004).

The administration of chemotherapeutic drugs to treat diseases of dairy sheep and goats constitutes a common cause of drug residues in milk. The presence of drug residues is a problem that has been focused mainly towards antimicrobial drugs, which can affect the bacteriological processes used in the manufacture of fermented milk products as yoghurt and cheese (Cogan 1972), determining economic losses for the industry. Other extensively used chemotherapeutics in animal practice are the antiparasitic drugs for parasite control (McKellar and Benchaoui 1996). The useful effects of treating dairy cows (Ploeger et al. 1989; Nødtvedt et al. 2002) and dairy sheep (Juste Jordán and García Perez 1991; Fthenakis et al. 2000, 2005) with anthelmintics on milk yield have been largely documented. As antiparasitic drugs, the endectocide compounds (macrocyclic lactones) present the advantage of being active against endo- and ectoparasites making them attractive drugs for parasite control in animal husbandry (Reinemeyer and Courtney 2001). Recently, some beneficial effects of anthelmintic treatment on birth weight of lambs and sheep milk yield have been reported (Fthenakis et al. 2005).

After treating food animals with veterinary drugs, the risks associated with chemical residues may be present, bearing in mind that the primary control of residues is by adherence to label withholding periods (WHP) statements by the farmer. The patterns of residues in milk for different endectocide compounds in lactating dairy cows (Toutain et al. 1988; Alvinerie et al. 1999) and, more recently, in dairy sheep (Cerkvenik et al. 2002; Imperiale et al. 2004, 2006) have been determined. Most of the information on antiparasitic residues in food (meat, milk products, eggs, etc) is related with concentrations in raw the product. However, most of the foodstuffs are cooked or pasteurised before consumption.

*Corresponding author. Email: fernanda@vet.unicen.edu.ar

ISSN 0265–203X print/ISSN 1464–5122 online

DOI: 10.1080/02652030802322879
http://www.informaworld.com

Although there is much information about the presence of drug residues in milk, particularly antimicrobial drugs, little is known about the effect of technological food processing on the fate of these residues and even less of antiparasitic drugs in milk or other edible matrices. The Codex Alimentarius Commission of the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) of the United Nations stated (Codex Alimentarius Commission 2001) that the scientific literature on effects of processing on drug residues in milk is insufficient to clearly assess the effect, if any, that processing may have on the level of most drug residues that could happen in milk and that additional studies are needed in this area.

Few studies on the effect of heating on residues of antiparasitic drugs such as levamisole, oxfendazole and ivermectin have been reported (Slanina et al. 1989; Rose et al. 1995, 1997, 1998). Therefore, information regarding to the effect of milk heating on residues is required for determining more accurate estimations of consumer exposure to residual veterinary medicines.

Considering the indiscriminate use of chemotherapeutic drugs in parasite control programmes, the widespread development of antihelmintic resistance has provided an impetus for the research of alternative parasite control methods (Waller and Thamsborg 2004). However, the determination of antiparasitic drug residues present in raw milk is still very important, since the composition of sheep milk is ideal for making cheese and yoghurt production (Haenlein 2001). Therefore, the fate of residues is particularly important when raw milk is subjected to different industrial processes (heating, cooling, clotting, etc.). The information about the effects of heating milk on drug residues is very important because the pasteurization is the most common processing technique employed to eliminate the risk of pathogens in milk.

Objective

The goal of the current experimental work was to evaluate the stability of residual concentrations of antiparasitic drugs; ivermectin, moxidectin and eprinomectin (IVM, MXD and EPM, respectively) in raw sheep milk under typical conditions of heat treatment during processing.

Material and methods

Chemicals and equipment

Anhydride trifluoroacetic acid, *N*-methyl-imidazole and triethylamine of analytical grade were purchased from Aldrich (Sigma-Aldrich, St Louis, MO, USA). Acetonitrile and methanol (HPLC grade) were obtained from Baker (Baker, Phillipsburg, NJ, USA). Acetic acid (99.1% purity) was obtained from Biopack (Buenos Aires, Argentina). Water was deionised (Simplicity Water Purification System, Millipore Corporation, Sao Paulo, Brazil) before use. Sample extraction was performed manually using a Lichrolut vacuum manifold (Merck, Nogent-Sur-Marne Cedex, France) and Strata C18-T cartridge (Phenomenex, Torrance, CA, USA). Milk samples were thermally treated in a water bath (Vicking S.R.L, Buenos Aires, Argentina). IVM (97.5% purity), MXD (92% purity), EPM (97% purity) and ABM (97% purity) were used as standard substances.

A Shimadzu LC-10AT$_{VP}$ HPLC system (Shimadzu Corporation, Kyoto, Japan) fitted with a Selectosil C_{18} (Phenomenex) for IVM and MXD and a BDS C_{18} (Thermo Quest, Hypersil Division, MA, USA) for EPM reverse phase columns (5 µm, 250 × 4.60 mm) kept in an oven at 30°C and a fluorescence detector were used. The area under the peak was calculated using the integrator software (Class LC 10 Software 1.2, Shimadzu) of the HPLC system.

Analytical and experimental procedures

Experimental design

Drug-free milk samples (1 ml) collected from untreated lactating ewes were fortified with either IVM (0.25–10 ng ml^{-1}), MXD (10–200 ng ml^{-1}) or EPM (0.1–5 ng ml^{-1}). The concentrations spiked were similar to those obtained *in vivo* in previous experimental work on milk residues excretion in sheep (Imperiale et al. 2004, 2006) carried out in our laboratory. Reference standards of IVM, MXD and EPM were used to fortify the milk samples and to validate the HPLC method. Standard solutions of IVM, MXD and EPM were prepared by successive dilutions in methanol or acetonitrile from the parent stock solution (1 mg ml^{-1}). Fortified samples were supplemented with 100 µl of abamectin (ABM) solution as internal standard (100 ng ml^{-1}).

Heat treatments

Drug-fortified milk samples were thermally treated at 65°C during 30 min (pasteurization) or at 75°C for 15 s (high-temperature short-time pasteurization) in a water bath. In order to measure the real temperature of the milk sample, the thermometer was introduced into the sample during the time of heating. After heating, the samples were immersed in an iced water bath for fast cooling. Thermally treated and untreated milk fortified samples were supplemented with abamectin (ABM, used as an internal standard) extracted and drug concentrations determined. Moreover, milk samples collected from untreated lactating ewes (blank samples) were thermally treated and HPLC

analysed to ruling out any possible interference at the retention time of the endectocide drugs by endogenous substances.

Analysis of drug concentrations

The extraction procedures and chromatographic conditions to quantity IVM, MXD and EPM in spiked milk samples were carried out following the techniques previously described by Imperiale et al. (2004, 2006).

Method validation

A complete validation of analytical procedures for extraction and quantification of IVM, EPM and MXD in sheep milk samples was performed before analysing the experimental samples following the techniques previously described elsewhere (Imperiale et al. 2004, 2006). The coefficient of variation (CV) for recovery and inter-day precision of the method was calculated (Swarbrick 1984). The limit of quantification (LOQ) was defined as the lowest concentration that can be measurable with acceptable precision ($CV < 20\%$) (Snyder et al. 1997).

Drug quantification 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 IVM, MXD or EPM and ABM using the Class LC 10 Software, version 1.2, and interpolating these areas on the calibration lines prepared for sheep milk. The statistical program Instat 3.0 (Graph Pad Software Inc., San Diego, CA, USA) was used for linear regression analyses and linearity tests.

The Student's *t*-test was used to estimate the differences between drug concentrations obtained in untreated and thermally treated milk samples. A $p < 0.05$ value was considered as being statistically significant.

Results and discussion

The stability of IVM, MXD and EPM standards in milk subjected to different heat treatments was examined. The concentrations spiked in milk were those measured in previous studies (*in vivo*) (Imperiale et al. 2004, 2006) and the thermal processes studied were those frequently used in dairy sheep industry for cheese elaboration, i.e., 65°C during 30 min (pasteurization) or 75°C for 15 s (high-temperature short-time pasteurization).

Analytical methods for detection of IVM, MXD and EPM residues in ewes milk were validated in our laboratory. The average recoveries were greater than 85% for the macrocyclic lactone compounds studied with relative standard deviations lower than 5%. The analytical methods are available from the published literature (Imperiale et al. 2004, 2006). Typical chromatograms of drug free and IVM, MXD and EPM fortified and thermally untreated milk samples are shown in Figure 1a and b. No interferences were observed at retention times of IVM, MXD and EPM in chromatograms of thermally untreated blank milk samples from the control sheep. Moreover, after heating at 65°C for 30 min or 75°C for 15 s, no metabolic products of degradation of the antiparasitic compounds were observed in chromatograms of spiked and blank milk samples, ruling out any possible endogenous interference at their retention times.

The internal temperature of heated milk samples for each heating method was monitored during the assay. The results for each heating methods are given in Table 1. These are expressed as percentage of the changes observed between the milk samples drug concentrations obtained before and after heating treatments. The percentages obtained from these differences fell in the range −5.0% to 2.9% (IVM), −2.3% to 3.6% (MXD), and −5.6% to 0.9% (EPM), as shown in Table 1.

No evidence of instability was obtained with any of the heating methods investigated. No significant changes in the IVM, MXD and EPM residue profiles were observed after either thermal treatment (65°C for 30 min or 75°C for 15 s). The observed changes in drug concentrations were within the limits of variation of the validated analytical method. Similarly, Cerkvenik et al. (2001) showed that no one of the milk heating processes affects IVM concentration in sheep milk. Moreover, the IVM residues in different animal's tissues such as muscle and liver were stable under cooking conditions (microwave, fried, boiled), hence Rose et al. (1998) concluded that IVM concentrations obtained in raw tissues could be applicable for cooked tissue, since neither increase nor loss was observed.

All the available data indicate that milk excretion is an important route of elimination for lipophilic drugs such as IVM and particularly for MXD (Cerkvenik et al. 2004; Imperiale et al. 2004) in ruminant species. Milk–plasma concentration ratios for these antiparasitic drugs were >1, the high disposition of these compounds in milk and milk fat content is positively related (Cerkvenik et al. 2004). Moreover, residual concentrations of IVM (Cerkvenik et al. 2004), MXD (Imperiale et al. 2004) and EPM (Imperiale et al. 2006) detected in cheese during the ripening period were positively correlated with the percentages of water loss, total solids and fat contents ($r < 0.90$).

The macrocyclic lactone of all avermectins has at C13 an α-L oleandrosyl-α-L oleandrosyloxy

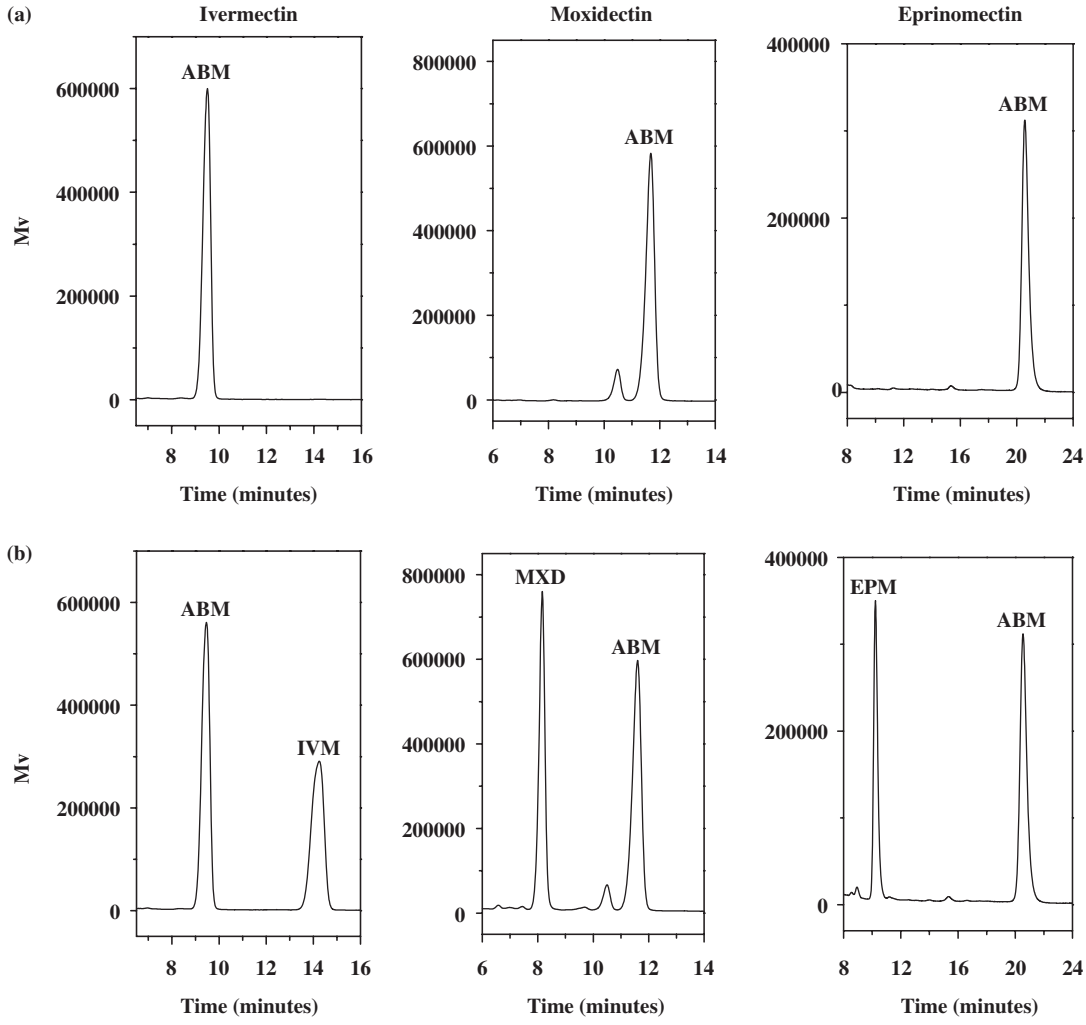

Figure 1. HPLC – chromatograms of blank (a) and ivermectin (IVM), moxidectin (MXD) and eprinomectin (EPM) fortified (10 ng ml^{-1}) sheep milk samples (b) thermally untreated Abamectin (ABM) was used as internal standard (10 ng ml^{-1}).

Table 1. Effect of heat treatment on the stability of macrocyclic lactone residues in sheep milk. Concentration differences (increment or decrease) are expressed as percentage of the initial concentration in the raw milk.

		Percentage of change on drug residual concentrations*		
Heating treatment	Sample ($n = 3$)	Ivermectin	Moxidectin	Eprinomectin
65°C for 30 min	1	−1.2	−2.3	0.7
	2	−2.4	0.7	−5.1
	3	−2.8	−0.5	−5.6
	4	2.1	1.3	−1.8
	5	−3.2	0.7	−1.8
	6	–	−0.3	–
	7	–	1.3	–
	Mean	−1.5	0.1	−2.7
75°C for 15 s	1	−1.2	2.1	0.7
	2	2.9	−1.4	0.9
	3	−2.3	3.0	−0.1
	4	0.2	3.6	−4.6
	5	−5.0	−2.2	−3.2
	6	–	−1.9	–
	7	–	−1.3	–
	Mean	−1.1	0.3	−1.3

*Values were obtained as the difference between drug concentration before and after heat treatment.

substituent which is a 2-deoxy sugar glycoside and as such it is relatively sensitive to acid hydrolysis or alcoholysis yielding mainly the aglycones and/or monosaccharides. Although the sensitivity of IVM to acidic conditions has been reported (Fisher and Mrozik 1989), the residues of IVM (Cerkvenik et al. 2004) and MXD (Imperiale et al. 2002) in milk during lactic acid fermentation in yoghurt elaboration were stable even when the pH reached values around 4.0–4.6. Complementarily, as it is reported in the current work, IVM, MXD and EPM residues in milk are stable during thermal milk processing. Nevertheless, if conditions of pH and temperature are appropriate for time enough, avermectins can transform into their respective aglycone molecules (Fisher and Mrozik 1989), rendering them undetectable with the chromatographic conditions commonly used for detecting endectocides. The aglycone molecules present a different retention time, appearing early in the chromatogram and can be mistaken with other molecules.

The results obtained on thermal stability of the milk residues for the three assayed antiparasitic compounds are in agreement with those reported when IVM residues in meat products (Rose et al. 1998) and milk (Cerkvenik et al. 2001) were subjected to different heating processes. However, residual concentration profiles of different molecules including penicillin and other antimicrobials were reduced between 10–25% during yoghurt production. Some factors identified as contributing to this decrease of residual penicillin concentrations were heat treatment of milk, fermentation temperature and time of exposure (Grunwald and Petz 2003).

In conclusion, IVM, MXD and EPM residues in sheep milk were stable to conventional milk heating processes extensively used in the dairy industry. Therefore, the levels of drug residues detected in sheep raw milk could be directly applicable to estimate consumer exposure and dietary intake calculations when consuming heat-processed fluid milk.

Acknowledgement

The financial support of the Agencia Nacional de Promoción Científica y Tecnológica (PICT 08-13763) is gratefully acknowledged.

References

Reinemeyer C, Courtney C. 2001. Antinematodal drugs. In: Adams HR, editor. Veterinary pharmacology and therapeutics. Ames (IA): Iowa State University Press. p. 947–979.

Alvinerie M, Sutra JF, Galtier P, Mage C. 1999. Pharmacokinetics of eprinomectin in plasma and milk following topical administration to lactating dairy cattle. Res Vet Sci. 67:229–232.

Fisher MH, Mrozik H. 1989. Chemistry. In: Campbell W, editor. Ivermectin and abamectin. New York (NY): Springer. p. 1–23.

Cerkvenik V, Bogdan Perko B, Rogelj I, Doganoc D, Skubic V, Beek W, Keukens H. 2004. Fate of ivermectin residues in ewes milk and derived products. J Dairy Res. 71:39–45.

Cerkvenik V, Doganoc DZ, Skubic V, Beek WMJ, Keukens HJ. 2001. Thermal and long-term freezing stability of ivermectin residues in sheep milk. Eur Food Res Technol. 213:72–76.

Cerkvenik V, Grabnar I, Skubic V, Doganoc DZ, Beek WMJ, Keukens HJ, Drobnic Kosorok M, Pogacnik M. 2002. Ivermectin pharmacokinetics in lactating sheep. Vet Parasitol. 104:175–185.

Codex Alimentarius Commission. 2001. Committee on Residues of Veterinary Drugs in Foods, Document: Control of Veterinary Drug Residues in Milk and Milk Products, CX/RVDF 01/8.

Cogan T. 1972. Susceptibility of cheese and yoghurt starter bacteria to antibiotics. J Appl Microbiol. 23:960–965.

Dubeuf JP, Morand-Fehr P, Rubino R. 2004. Situation, changes and future of goat industry around the world. Small Rumin Res. 51:165–173.

Fthenakis GC, Papadopoulos E, Himonas C. 2005. Effects of three anthelmintic regimes on milk yield of ewes and growth of lambs. J Vet Med A 52:78–82.

Fthenakis GC, Papadopoulos E, Himonas C, Leontides L, Kritas S, Papatsas J. 2000. Efficacy of moxidectin against sarcoptic mange and effects on milk yield of ewes and growth of lambs. Veterinary Parasitol. 87:207–216.

Grunwald L, Petz M. 2003. Food processing effects on residues; penicillins in milk and yoghurt. Anal Chim Acta. 483:73–79.

Haenlein G. 2001. Past, present, and future perspectives of small ruminant dairy research. J Dairy Sci. 84: 2097–2115.

Imperiale F, Busetti M, Suárez V, Lanusse C. 2004. Milk excretion of ivermectin and moxidectin in dairy sheep: assessment of drug residues during cheese elaboration and ripening period. J Agric Food Chem. 52:6205–6211.

Imperiale F, Pis A, Sallovitz J, Lifschitz A, Busetti M, Suárez V, Lanusse C. 2006. Pattern of eprinomectin milk excretion in dairy sheep unaffected by lactation stage: comparative residual profiles in dairy products. J Food Prot. 69:2424–2429.

Imperiale F, Sallovitz J, Lifschitz A, Lanusse C. 2002. Determination of ivermectin and moxidectin residues in bovine milk and examination of the effects of these residues on acid fermentation of milk. Food Addit Contam. 9:810–818.

Juste Jordán R, García Pérez A. 1991. Effect of treatment with netobimin on milk production of sheep. Vet Parasitol. 38:173–183.

Mckellar QA, Benchaoui HA. 1996. Avermectins and milbemycins. J Vet Pharmacol Therap. 19:331–351.

Nødtvedt A, Dohoo I, Sanchez J, Conboy G, Des Côteaux L, Keefe G. 2002. Increase in milk yield following eprinomectin treatment at calving in pastured dairy cattle. Vet Parasitol. 105:191–206.

Ploeger HW, Schoenmaker GJW, Kloosterman A, Borgsteede FH. 1989. Effect of anthelmintic treatment of dairy cattle on milk production related to some parameters estimating nematodes infection. Vet Parasitol. 34:239–253.

Rose MD, Argent LC, Shearer G, Farrington WH. 1995. The effect of cooking on veterinary drug residues in food: 2. Levamisole. Food Addit Contam. 12:185–194.

Rose MD, Farrington WH, Shearer G. 1998. The effect of cooking on veterinary drug residues in food: 7. Ivermectin. Food Addit Contam. 15:157–161.

Rose MD, Shearer G, Farrington WH. 1997. The effect of cooking on veterinary drug residues in food; 5. Oxfendazole. Food Addit Contam. 14:15–26.

Slanina P, Kuivinen J, Ohlsen C, Ekstrom LG. 1989. Ivermectin residues in the edible tissues of swine and cattle: effect of cooking and toxicological evaluation. Food Addit Contam. 6:475–481.

Snyder L, Kirkland J, Glajch J. 1997. Practical HPLC method development. New York (NY): Wiley, Chapter 15, Completing the method: validation and transfer, p. 685–713.

Swarbrick J. 1984. Basic definitions and concepts. In: Bolton S, editor. Pharmaceutical statistics. Practical and clinical applications. New York (NY): Marcel Dekker. p. 1–31.

Toutain PL, Campan M, Galtier P, Alvinerie M. 1988. Kinetic and insecticidal properties of ivermectin residues in milk of dairy cows. J Vet Pharmacol Therap. 11:288–291.

Waller PJ, Thamsborg SM. 2004. Nematode control in 'green' ruminant production systems. Trends Parasitol. 20:493–497.