









## Determining anthelmintic residues in goat milk in Brazil

### *Determinação de resíduos anti-helmínticos no leite caprino no Brasil*

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### SUMMARY

Anthelmintics are used to combat nematodes. The misuse of anthelmintics can raise the cost of milk production. The objective of this research was to determine the presence of anthelmintics in goat milk. Twenty goats were used, divided into four groups of five animals: I- animals treated with an ivermectin-based anthelmintic; II- animals treated with moxidectin; III- animals treated with levamisole hydrochloride; and IV: animals treated with albendazole. Milk samples were collected individually: before, and 1, 2, 3, 15 and 21 days after administration of the anthelmintics. Determination of anthelmintic residues was performed by liquid chromatography-mass spectrometry (LC-MS). According to the results, there was an exponential effect ( $P < 0.05$ ) for ivermectin and moxidectin. Moxidectin was the anthelmintic that left a residue in the milk for the longest time, up to 21 days. However, with all the anthelmintics researched, residues were below the maximum limit recommended by the inspecting agencies.

**Keywords:** Goats, mass spectrometry, maximum residue limit, moxidectin, ivermectin.

### RESUMO

Anthelmintics são usados para combater nematodes. O mau uso de anti-helmínticos pode aumentar o custo da produção de leite. O objetivo desta pesquisa foi determinar a presença de anti-helmínticos no leite de cabra. Foram utilizados vinte cabras, divididos em quatro grupos de cinco animais: I - animais tratados com um antihelmíntico à base de ivermectina; II - animais tratados com moxidectina; III- animais tratados com cloridrato de levamisol; E IV: animais tratados com albendazole. Amostras de leite foram coletadas individualmente: antes, e 1, 2, 3, 15 e 21 dias após a administração dos anti-helmínticos. A determinação dos resíduos antihelmínticos foi realizada por cromatografia líquida-espectrometria de massa (LC-MS). De acordo com os resultados, houve um efeito exponencial ( $P < 0.05$ ) para ivermectina e moxidectina. A moxidectina foi o anti-helmíntico que deixou um resíduo no leite por mais tempo, até 21 dias. Contudo, com todos os anthelmintics pesquisados, os resíduos estavam abaixo do limite máximo recomendado pelas agências de inspeção.

**Palavras-chave:** cabras, espectrometria de massa, limite máximo de resíduos, moxidectina, ivermectina



## INTRODUCTION

Gastrointestinal parasites are very relevant in small ruminants because they result in the loss of production and productivity, reduced utilization of nutrients, low milk production, and growth retardation (YOSHIHARA et al., 2013). Costs of anti-parasite drugs in this activity are high. Among the principles used for helminths, often without treatment, are benzimidazoles, avermectins, imidazothiazoles, and salicylanilides. The indiscriminate use of medicines against gastrointestinal parasites results in resistance to the drugs existing in the market (MELO et al., 2015).

According to Guimarães et al. (2011), pharmacological groups of the most used anthelmintics on goat farms in Brazil are first, macrocyclic lactones, and second, benzimidazoles and imidazothiazoles. Anthelmintic resistance has already been described in the three pharmacological groups (THOMAZ-SOCCOL et al., 2004). Since anthelmintics are used in food-producing animals, residues in animal tissues and their products are of great concern, due to the safety of consumers (JEDZINIAK et al., 2009). Furthermore, residues from chemical compounds, eliminated in the excreta of animals also cause serious effects on the environment (BISWAS et al., 2012).

In order to protect consumers, some substances in foods are monitored by inspection agencies to establish a Maximum Residue Limit (MRL), which is the maximum concentration of residue allowed from the use of a veterinary medicinal product. Codex Alimentarius (2012) does not establish a MRL for moxidectin and levamisole in milk, while the values for albendazole and ivermectin are defined as  $10\mu\text{g/L}^{-1}$

and  $100\mu\text{g/L}^{-1}$ , respectively. The European Union advocates for the MRL in milk for albendazole and moxidectin to be  $100\mu\text{g/L}^{-1}$  and  $40\mu\text{g/L}^{-1}$ , respectively. Brazil establishes the MRL for veterinary medicinal products recommended by Mercosul, Codex Alimentarius, European Union and/or United States (ANVISA, 2009).

Research on anthelmintics in the milk and meat of small ruminants is still very scarce. It is geared more toward cow's milk, which is more consumed in various regions of the world. However, concern with anthelmintic residue is more related to economic factors than to food safety or harm caused in the production of dairy products like cheese and yogurt. Therefore, studies on anthelmintic residue control, the pharmacokinetics of drugs in lactating animals, and the determination of residue levels in goat milk are necessary to ensure food safety to consumers. It is essential to provide information to producers in relation to the drugs released, the recommended doses, the waiting period, and the consequences of their misuse. Hence, the objective of this research was to determine the existence and amount of residue of different anthelmintics in goat milk in Brazil.

## MATERIAL AND METHODS

The experiment was conducted at the Goat and Sheep Farming department of the Federal University of Paraíba (UFPB), located in the city of Bananeiras ( $6^{\circ}45'4''$  S latitude and  $35^{\circ}38'0''$  W longitude) in the state of Paraíba, Brazil. This study was approved by the Animal Ethics Committee of the Federal University of Paraíba (UFPB), Brazil (protocol no. 88/15).



Milk was used from 20 crossbred Saneen goats, at an average weight of 40 kg. The goats were allocated in a completely randomized design and divided into four groups of five animals each. The animals were randomly distributed during the treatments: *Group I*: treated with an ivermectin-based anthelmintic at 1% in a subcutaneous dose of 0.2 mg/kg; *Group II*: treated with a moxidectin-based anthelmintic at 0.2% in an oral dose of 0.5 mg/kg; *Group III*: treated with a levamisole hydrochloride-based anthelmintic at 5% in an oral dose of 1.0 mL/10 kg; *Group IV*: treated with an albendazole-based anthelmintic at an oral dose of 2.0 ml/10 kg. The drug doses used were those recommended by the manufacturers for goats and sheep.

Milk samples were collected in polyethylene containers, then hermetically sealed and individually identified. Samples were collected before the administration of the anthelmintics, and then 1, 2, 3, 15, and 21 days after deworming, totaling six samples. Subsequently, the milk samples were packed at -20 °C and sent to the Technology Institute of Pernambuco (ITEP), to the Pesticides and Contaminants in Foods and

Alcoholic Beverages Laboratory (LabTox), for residue analysis.

The residues from each anthelmintic were extracted by using the QUECHERS method (ANASTASSIADES et al., 2003). The anthelmintic residues were analyzed by liquid chromatography-mass spectrometry (LC-MS/MS), chromatograph Waters Acquity UPLC System, composed by the following modules: Binary Solvent System (BSM), Sample Manager (SM), and Sample Organize (SO). Mass spectrometry Waters, model Quattro Premie.

The results were analyzed by using the *Statistical Analysis System* program, Version 10 (2011), using the procedure PRO REG to estimate the decrease in the variable residue according to time (days), with a level of 5% probability.

## RESULTS AND DISCUSSION

There was a significant exponential effect ( $P < 0.05$ ) for ivermectin and moxidectin, which belong to the group of macrocyclic lactones, whose permanence of residues in goat milk was longer compared to levamisole and albendazole (Table 1).

**Table 1.** Effect of the period of collection on the concentration of anthelmintic residues ( $\mu\text{g/L}$ ) in goat milk

Treatment	Collect days					
	0	1	2	3	15	21
Ivermectin	0.0000	0.0164b	0.0180b	0.0045b	0.0000b	0.0000b
Moxidectin	0.0000	0.0880a	0.0520a	0.0352a	0.0120a	0.0036a
Levamisole	0.0000	0.0038b	0.0002b	0.0000b	0.0000b	0.0000b
Albendazole	0.0000	0.0000b	0.0000b	0.0000b	0.0000b	0.0000b

Means in rows followed by the same letters are statistically different by Tukey test ( $P < 0.05$ )

Macrocyclic lactones, (ivermectin and moxidectin) are lipophilic, and the fat in the milk facilitates connection to the

drug. Thus, in studies related to the ivermectin residues in animal products, milk is more important than meat

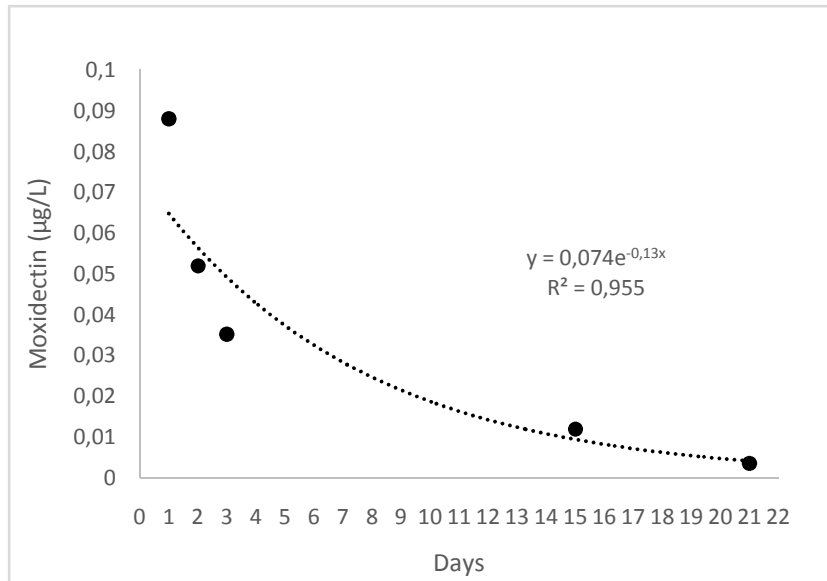


(FLAJS et al., 2005). Radiometric studies reported by Codex Alimentarius (2012) to determine the quantity of residues of two molecules (H2B1a and H2B1b) of ivermectin treated with lithium in sheep found residues in fat, liver, muscle and kidneys at 3, 5, and 7 days after intraruminal administration. Fat tissue proved to have the longest residue time.

Alvinerie et al. (1993) found that ivermectin residue levels reached a maximum concentration of 7.26  $\mu\text{g}/\text{kg}$  in goat milk at 2.8 days after treatment. They estimated that 5% of the ivermectin dose was secreted in the milk. Cerkvenik et al. (2002) showed that ivermectin residue levels reached a maximum concentration of 23  $\mu\text{g}/\text{kg}$  in sheep milk 1.3 days after treatment, and residues were still detected 23 days after treatment. Cerkvenik et al. (2004) investigated the destination of ivermectin residues in sheep milk and

dairy products (raw milk, raw milk yogurt, pasteurized milk yogurt, cheese, and whey) of ewes submitted to a residue depletion test. The highest levels of ivermectin residue were found at 2 days of milking in raw milk (22  $\mu\text{g}/\text{kg}$ ), yogurt (23  $\mu\text{g}/\text{kg}$ ), and cheese (96  $\mu\text{g}/\text{kg}$ ).

Moxidectin was the antiparasitic drug whose residue permanence was detected (0.0036  $\mu\text{g}/\text{L}$ ) up to 21 days after treatment (Table 1), corroborating the results of Imperiale et al. (2004) that moxidectin is considered to be more lipophilic than ivermectin. This reinforces the data in the present study (Figure 1). Moxidectin is more persistent in the body and milk of the animal, as has been demonstrated in cows (ALVINERIE et al., 1999), in sheep (ALVINERIE et al., 1998), in goats (CARCELES et al., 2001), and in horses (PEREZ et al., 1999).



**Figure1.** Regression equation for concentration of Moxidectin (µg/L) in goat milk

Although there is a ban by CODEX on the use of moxidectin in dairy animals for human consumption (CODEX ALIMENTARIUS, 2012) and in Brazil it is not licensed for use in goats, several studies show the use of moxidectin, and the occurrence of nematode resistance to this antiparasitic drug in goats (TERRILL et al., 2001; MAVROGIANNI, et al., 2004; AHID, et al., 2007; LIMA et al., 2010).

Residues of the antiparasitic levamisole were detected in goat milk at low levels on the first and second days, but were not detected at 3, 15 and 21 days after administration. This anthelmintic drug is licensed for administration in all food producing species, but severe grace periods were defined and its use was banned in animals producing milk for human consumption. A maximum residue limit (MRL) was set at 10 µg/kg in muscle, fat, and kidneys, and 100 µg/kg in liver (EC, 1998). However several studies have been published on the use of levamisole in dairy goats (LIMA et al., 2010) and on the detection of levamisole residue in

animal tissues and milk (CANNAVAN et al., 1995; SCHENCK et al., 1998; RUYCK, et al., 2000).

There was no detectable residue in the milk of goats treated with the anthelmintic albendazole in up to 21 days after administration. The albendazole residue is a result of its sum with its metabolites (CR, 1992). The pharmacokinetics of albendazole was analyzed in different animal species. Studies involving sheep (BATZIAS & DELIS, 2004), calves (FORMENTINI et al., 2001) and humans (KITZMAN et al., 2002) demonstrated that albendazole is rapidly and extensively metabolized into albendazole sulfoxide (ABZ-SO), albendazole sulfone (ABZ-SO<sub>2</sub>), and albendazol-2-amino-sulfona (ABZ-SO<sub>2</sub>-NH<sub>2</sub>). Probably, the method used for detection of albendazole residue in this study was not effective to detect residues of its metabolites.

The determination of benzimidazole residues in biological matrices is a challenge for analysts due to specific chemical properties (JEDZINIAK et al, 2009). Despite the similarities in the structures of molecules and course of



action, these drugs differ in lipophilicity and pKa values. Moreover, the definition of residue indicators requires the simultaneous determination of a parent compound and its metabolites. Among the anthelmintics studied, moxidectin leaves residue in milk for the longest time, up to 21 days. However, in all anthelmintics researched, residues are below the maximum residue limit recommended by the inspecting agencies.

## REFERENCES

- ALVINERIE M.; SUTRA. J. F.; GALTIER P. Ivermectin in goat plasma and milk after subcutaneous injection. **Veterinary Research**, v. 24, p. 417-421, 1993.
- ALVINERIE, M.; ESCUDERO, E.; SUTRA, J. F.; EECKHOUTTE, C.; GALTIER, P. The pharmacokinetics of moxidectin after oral and subcutaneous administration to sheep. **Veterinary Research**, v. 29, p. 113-118, 1998.
- ALVINERIE, M.; SUTRA, J. F.; GALTIER, P.; MAGE, C. Pharmacokinetics of eprinomectin in plasma and milk following topical administration to lactating dairy cattle. **Research in Veterinary Science**, v. 67, p. 229-232, 1999.
- ANVISA-Agência Nacional de Vigilância Sanitária, Relatório PAMVet. (2009). Disponível em: <http://portal.anvisa.gov.br/>. Accessed may 22, 2016
- ANASTASSIADES, M.; LEHOTAY, S. J.; STAJNBAHER, D.; SCHENCK, F. J. Fast and easy multi residue method employing acetonitrile extraction/partitioning and “Dispersive Solid-Phase Extraction” for the determination of pesticide residues in produce. **Journal of AOAC International**, v. 86, p. 412-431, 2003.
- AHID, S. M. M.; CAVALCANTE, M. D. A.; BEZERRA, A. C. D. S.; SOARES, H. S.; PEREIRA, R. H. Anthelmintic effectiveness in goats from Alagoas State, Brazil. **Acta Veterinária Brasileira**, v.1, p. 56-59, 2007.
- BATZIAS, G. C.; DELIS, G. A. Reversed-phase liquid chromatographic method with fluorescence detection for the simultaneous determination of albendazole sulphoxide, albendazole sulphone and albendazole 2-aminosulphone in sheep plasma. **Journal of Chromatography B**, v. 805, p. 267-274, 2004.
- BISWAS, A.K., KONDAIAH, N., ANJANEYULU, A.S.R., MANDAL, P.K. Food safety concerns of pesticides, veterinary drug residues and mycotoxins in meat and meat products. **Asian Journal of Animal Sciences**, v. 4, p. 46-55, 2010.
- CANNAVAN, A.; BLANCHFLOWER, W. J.; KENNEDY, D. G. Determination of levamisole in animal-tissues using liquid chromatography thermospray mass-spectrometry. **Analyst**, v. 120, p. 331-333, 1995.
- CARCELES, C. M.; DIAZ, M. S.; VICENTE, M. S.; SUTRA, J. F.; ALVINERIE, M.; ESCUDERO, E. Milk kinetics of moxidectin and doramectin in goats. **Research Veterinary Science**, v. 70, p.227-231, 2001.



CERKVENIK, V.; GRABNAR, I.; SKUBIC, V.; DOGANOC, D. Z.; BEEK, W. M. J.; KEUKENS, H. J.; DROBNIC-KOSOROK, M.; POGACNIK, M. Ivermectin pharmacokinetics in lactating sheep. **Veterinary Parasitology**, v. 104, p. 175-185, 2002.

CERKVENIK, V.; PERKO, B.; ROGELJ, I.; DOGANOC, D. Z.; SKUBIC, V.; BEEK, W. M.; KEUKENS, H. J. Fate of ivermectin residues in ewes' milk and derived products. **Journal Dairy Research**, v. 71, p. 39-45, 2004

CODEX ALIMENTARIUS. Maximum Residue Limits for Veterinary Drugs in Foods, In: Updated as at the 35th Session of the Codex Alimentarius Commission in, CAC/MRL 2-2012. Disponível em: [http://www.agricultura.gov.br/arq\\_edit\\_or/file/CRC/LMRsCodexAlimentarius-Atualizaçãode2012.pdf](http://www.agricultura.gov.br/arq_edit_or/file/CRC/LMRsCodexAlimentarius-Atualizaçãode2012.pdf). Accessed september 15, 2015.

COMMISSION REGULATION (CR) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. Disponível em: [http://ec.europa.eu/health/files/eudralex/vol-5/reg\\_2010\\_37/reg\\_2010\\_37\\_pt.pdf](http://ec.europa.eu/health/files/eudralex/vol-5/reg_2010_37/reg_2010_37_pt.pdf). Accessed may 15, 2016.

FLAJS, V. C.; GRABNAR, I.; ERZEN, N. K.; MARC, I.; POZGAN, U.; GOMBAC, M.; KOLAR, L.; POGACNIK, M. Pharmacokinetics of doramectin in lactating dairy sheep and suckling lambs. **Analytical Chemical Acta**, v. 529, p. 353-359, 2005.

FORMENTINI, E. A.; MESTORINO, O. N.; MARINO, E. L.; ERRECALDE, J. O Pharmacokinetics of ricobendazole in calves. **Journal of Veterinary Pharmacology and Therapeutics**, v. 24, p.199-202, 2001.

GUIMARÃES, A. S.; GOUVEIA, A. M. G.; CARMO, F. B.; GOUVEIA, G. C.; SILVA, M. X.; VIEIRA, L. S.; MOLENTO, M. B. Management practices to control gastrointestinal parasites in dairy and beef goats in Minas Gerais; Brazil. **Veterinary Parasitology**, v.176, p. 265–269, 2011.

IMPERIALE, F. A., BUSETTI, M. R., SUÁREZ, V. H.; LANUSSE, C. E. Milk excretion of ivermectin and moxidectin in dairy sheep: assessment of drug residues during cheese elaboration and ripening period. **Journal of Agricultural and Food Chemistry**, v. 52, p. 6205-6211, 2004.

JEDZINIAK, P.; SZPRENGIER-JUSZKIEWICZ, T.; OLEJNIK, M. Determination of benzimidazoles and levamisole residues in milk by liquid chromatography–mass spectrometry: Screening method development and validation. **Journal of Chromatography A**, v. 1216, p. 8165–8172, 2009.

KITZMAN, D.; CHENG, K.; FLECKENSTEIN, L. HPLC assay for albendazole and matabolites in human plasma for clinical pharmacokinetic studies. **Journal of Pharmaceutical and Biomedical Analysis**, v. 30, p. 801-813, 2002.

LIMA, M. M.; FARIAS, M. P. O.; ROMEIRO, E. T.; FERREIRA, D. R. A.; ALVES L. C.; FAUSTINO, M. A. G. Efficacy of moxidectin, ivermectin



and albendazole against gastrointestinal helminths in goat and sheep farms from Pernambuco state, Brazil. **Ciência Animal Brasileira**, v. 11, p. 94-100, 2010.

MAVROGIANNI, V. S.; FTHENAKIS, G. C.; PAPADOPOULOS, E.; SKOUFOS, J.; CHRISTODOULOPOULOS, G.; TZORA, A. Safety and reproductive safety of moxidectin in goats. **Small Ruminant Research**, v.54, p. 33-41, 2004.

MELO, V.F.P.; PINHEIRO, R.S.B.; HOMEM JUNIOR, A.C.; AMÉRICO, J.H.P.; SANTOS, V.C.; ROSESTOLATO, L.L.R. Management of anthelmintics to the control of gastrointestinal infections in goats. **Revista Brasileira de Saúde e Produção Animal**, v.16, p. 916-924, 2015.

PEREZ, R., CABEZAS, I., GARCIA, M.; RUBILAR, L.; SUTRA, J. F.; GALTIER, P.; ALVINERIE, M. Comparison of the pharmacokinetics of moxidectin (Equest) and ivermectin (Eqvalan) in horses. **Journal of Veterinary Pharmacology and Therapeutics**, v. 22, p. 174-180, 1999.

RUYCK, H.; VAN RENTERGHEM, R.; DE RIDDER, H.; DE BRABANDER, D. Determination of anthelmintic residues in milk by high performance liquid chromatography. **Food Control**, v. 11, p. 165-173, 2000.

SCHENCK, F. J.; PODHORNIAK, L. V.; WAGNER, R. A highly gas chromatographic determination of levamisole in milk. **Food Additive and Contaminants**, v. 15, p. 411-414, 1998.

TERRILL, T. H.; KAPLAN, R. M.; LARSEN, M.; SAMPLES, O. M.; MILLER, J. E.; GELAYE, S. Anthelmintic resistance on goat farms in Georgia: efficacy of anthelmintics against gastrointestinal nematodes in two selected goat herds. **Veterinary Parasitology**. v.97, p. 261-268, 2001.

THOMAZ-SOCCOL, V. T.; SOUZA, F. P.; SOTOMAIOR, C.; CASTRO, E. A.; MILCZEWSKI, V.; MOCELIN, G.; SILVA, M. C. E. Resistance of gastrointestinal nematodes to anthelmintics in sheep (*Ovis aries*). **Brazilian Archives of Biology and Technology**, v. 47, p. 41-47, 2004.

YOSHIHARA, E.; MINHO, A.P.; YAMAMURA, M.H.; Anthelmintic effect of condensed tannins in gastrointestinal nematodes of sheep (*Ovis aries*). **Semina: Ciências Agrárias**, v.34, p.3935-3950, 2013.