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# Climate conditions associated with the occurrence of pyrethroid residues in bulk milk tank

[*Condições climáticas associadas à ocorrência de resíduos de piretroides em amostras de leite de tanque*]

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## ABSTRACT

The present study attempted to identify climate conditions that are associated with the occurrence of pyrethroid residues using a multivariate principal component analysis (PCA). A total of 132 raw milk samples were collected from dairy farms in Minas Gerais State - Brazil and analyzed for seven analytes using gas chromatography with electron capture detection. Of the 132 milk samples, pyrethroid residues were identified in 14 (10.6%) milk samples, of which 12 (9.1%) and two (1.5%) milk samples had the identification of cypermethrin and deltamethrin, respectively. From those samples, nine (6.8%) milk samples were regarded as non-compliant for cypermethrin with this analyte concentration above the maximum residue limits set by Brazilian legislation. A PCA assessing pyrethroid residues in bulk tank milk demonstrated that the average temperature and the Temperature-Humidity Index were associated with pyrethroids residues in bulk milk tank, although the relative humidity was inversely correlated. Thus, the data analysis indicated that the pyrethrid residues associated with some climate conditions can predict the moments with higher risk of occurrence of pyrethroid residues in bulk tank milk.

Keywords: veterinary drug, pesticide, raw milk, seasonality, dairy cow

## RESUMO

*O presente trabalho objetivou identificar fatores climáticos associados à ocorrência de resíduos de piretroides em amostras de leite de tanque por análise multivariada de componentes principais (ACP). Para o presente trabalho, 132 amostras de leite cru foram coletadas em fazendas leiteiras localizadas no estado de Minas Gerais (Brasil) e analisadas por sete analitos por cromatografia gasosa com detector por captura de eletróns para detecção de resíduos de piretroides. Das 132 amostras de leite analisadas, a presença de resíduos de piretroides foi detectada em 14 amostras (10,6%), e em 12 (9,1%) e duas (1,5%) foram detectados cipermetrina e deltametrina, respectivamente. Destas amostras, em nove (6,8%) a concentração de cipermetrina encontrada foi maior que a permitida pela legislação brasileira. A ACP demonstrou que a presença de resíduos de piretroides no leite de tanque ocorreu menos frequentemente nos dias com maior umidade relativa, embora a presença desses resíduos estivesse associada a maior temperatura média e a maior índice de temperatura e umidade. Dessa forma, conclui-se que alguns índices climáticos podem predizer períodos com maior risco de ocorrência de resíduos de piretroides no leite de tanque.*

*Palavras-chaves: medicamento veterinário, pesticida, leite cru, sazonalidade, bovino leiteiro*

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## INTRODUCTION

Approximately one billion cattle, most of which are in the tropical and subtropical regions of the world, are at risk from various ectoparasites species causing significant production and economic losses. The control of ectoparasites found on animals (i.e. ticks and flies) is largely based on the use of chemicals, which include the synthetic pyrethroids. Therefore, the extensive and improper use of these active compounds can lead to the presence of their residues in milk causing human health risks (Fagnani *et al.*, 2011; Raynal *et al.*, 2013). Notably, pyrethroid compounds are toxic and can accumulate in an organism causing severe neurological (Heck *et al.*, 2007; Roberts *et al.*, 2012; Burns *et al.*, 2013) and respiratory symptoms (Hudson *et al.*, 2014), as well as adversely affecting endocrine and immune systems (Du *et al.*, 2010; Costa *et al.*, 2013).

As milk is an essential food, the maximum residue levels (MRLs) of veterinary drugs are markedly lower than in other food matrices, due in part to the recommended greater consumption of milk by young children and older individuals (Bilandžić *et al.*, 2011; Roberts *et al.*, 2012). Thus, in attempt to protect human health and ensure a high product quality, several governmental authorities (Brasil, 1999) have set maximum residue limits (MRLs) for various veterinary drug residues.

Thus, considering that climate conditions influence infestation by insects and ticks, such as horn flies (*Haematobia irritans*) and *Rhipicephalus* (*Boophilus*) *microplus* (Souza *et al.*, 1988; Barros, 2001; Estrada-Peña *et al.*, 2006; Paim *et al.*, 2011), and the fact that the use of pyrethroids is mainly based on the subjective observation of infestation levels (Rocha *et al.*, 2012), it was hypothesized that some climate condition parameters were associated with the occurrence of pyrethroid residues in bulk milk tank. For this reason, data on climate influences in pyrethroid residues in raw milk are of great relevance, particularly in tropical climate countries as Brazil, where different conditions of rainfall, relative humidity and temperature are perceived on unequable basis (Picinin *et al.*, 2013). Thus, the present study aimed to identify the climate condition parameters that were associated with the occurrence of pyrethroids residues in bulk milk tank using a multivariate principal component analysis.

## MATERIALS AND METHODS

Raw milk samples were collected from August 2009 to February 2010 from 45 dairy farms in Minas Gerais State, Brazil. A total of 132 samples were collected from bulk milk tanks after milk homogenization. Given the extensive sampling that was required and the costs of the analyses, dairy herds were randomly selected based on the list of farms from the most important dairy plant of the region by considering their levels of daily milk production. Twenty-seven (60%) of the dairy farms had production levels of $\leq$500L/day, five (11.11%) had production levels between 501 and 1,000L/day and 13 (28.89%) had levels of > 1,000L/day. Thus, the milk samples that were obtained were representative of the dairy farms belonging to the region of study. Regarding the time period that was selected, this study was initiated in the dry period and finished in the rainy period, which resulted in great variability in climate conditions. This is representative of typical annual climate conditions that have been previously recorded based on official records of the Meteorological Center of the Minas Gerais State (Base…, 2011; Picinin *et al.*, 2013). In the present study, a milk sample was assumed to be positive if any analytical residue was detected, regardless of the drug concentration. Furthermore, the sample was considered to be non-compliant if a drug was present at a concentration above the Brazilian MRL value (Brasil, 1999).

The milk samples were stored frozen at -18°C until the quantitative analysis of pyrethroid residues was carried out.

The analytical standards γ-cyhalothrin (γCYH), λ-cyhalothrin (λCYH), cyfluthrin (CYF), cypermethrin (CYP), deltamethrin (DEL), fenvalerate (FEV), and permethrin (PER), which all possessed levels of 95% certified purity, were obtained from Sigma-Aldrich (St. Louis, MO, USA). For the pyrethroids, standard stock solutions of 1mg $mL^{-1}$ were prepared individually in acetonitrile (ACN) and stored in a refrigerator at 8°C. Working standard solutions containing all of the pyrethroids were diluted with ACN to give calibration solutions ranging

from 5.0ng $mL^{-1}$ to 80.0ng $mL^{-1}$. All of the standard solutions were stored at - 20°C.

The analytical system used for this study was a Trace GC Ultra gas chromatograph (Thermo Fisher Scientific, Milan, Italy) equipped with a splitless injection system, AI 3000 autosampler and $^{63}Ni$ electron-capture detector fitted with either an OV-5 fused silica capillary column (15.0m × 0.25mm × 0.1μm film thickness) or an OV-35 fused silica capillary column (15.0m × 0.25mm × 0.25μm film thickness). The programmed temperature cycle for the OV-5 column was as follows: 100ºC (1min) to 250ºC at a rate of 20ºC $min^{-1}$, followed by an increase to 260ºC (3min) at 5ºC $min^{-1}$ and finally, to 330ºC (5min) at 20ºC $min^{-1}$. The conditions for pyrethroid separation using the OV-35 column were similar to those used for the OV-5 column. The injector and detector were programmed at 240°C and 340°C, respectively. Nitrogen at 1.0mL $min^{-1}$ was used as the carrier gas. One microliter of each sample was injected in splitless mode. The identification of the peaks was based on comparisons of the retention times of compounds in the standard solutions.

The milk samples (5.0mL) were extracted with 10mL of ACN in 50mL polypropylene tubes, shaken for 20min and then incubated in a freezer at - 20ºC for approximately 12 hours. After this period, the organic phase, which contained an organic solvent with the extracted compounds, remained as a liquid, whereas the aqueous phase and lipidic fraction of the milk froze. The supernatants were passed through previously cooled glass wool containing anhydrous sodium sulfate (2.0g). The extracts were concentrated until dry in a water bath (38-40°C) under gentle nitrogen flow. The residues were dissolved in 1.0mL of ACN and analyzed by GC-ECD.

All of the methods have been fully validated and are currently in routine use in the laboratory network of the Ministério da Agricultura, Pecuária e Abastecimento (MAPA) for the National Residue Control Plan (Lins *et al.*, 2012). The results were corrected for recovery. All of the applied methods met the internal criteria for residue analysis.

The relative humidity, the average temperature, and the maximum temperature were recorded at the day of milk sample collection. The rainfall was also recorded monthly. Furthermore, the Temperature-Humidity Index (THI) was calculated using the following equation: THI = 1.8 × Ta - (1 - RH) × (Ta -14.3) + 32, as described by Bouraoui *et al.* (2002) where Ta is the average temperature in °C and RH is the average relative humidity as a fraction of unit.

The statistical analyses were performed using the multidimensional principal component analysis (PCA) with the STATA statistical software version 12 (Stata Corp., College Station, Texas, USA). To test the associations among all of the variables that were surveyed together, a PCA was performed to assess inferences regarding the possible biological meanings underlying the associations among the variables without the pre-establishment of cause/effect. Thus, this statistical technique allows us to graphically visualize the variables in the same dimensional plane and establish any relationships among them. The minimum percentage of inertia of the system for the multidimensional PCA was 65.0% in relation to the percentage of variance that was explained by the first three axes (components).

## RESULTS AND DISCUSSION

The pyrethroid residues in raw milk were identified in 14 (10.6%) milk samples, of which 12 (9.1%) and two (1.5%) milk samples had the detection of CYP and DEL, respectively. From those samples, nine (6.8%) milk samples are regarded as non-compliant for CYP with concentrations above the maximum residue limits (20μg $kg^{-1}$) set by Brazilian legislation (Brasil, 1999). The presence of pyrethroid residues determined by GC-ECD in bulk milk tank samples here indicated that withdraw periods of pyrethroids drugs has not been fully respected by all dairy farmers. Another fact that can explain the pyrethroid residues in raw milk is the use of pesticides in products destined to animal feed that also leads to pyrethroid residues in raw milk (Fagnani *et al.*, 2011). The presence of pyrethroid residues in raw milk should be emphasized considering their deleterious effect on human health (Heck *et al.*, 2007; Du *et al.*, 2010; Roberts *et al.*, 2012; Burns *et al.*, 2013, Costa *et al.*, 2013; Hudson *et al.*, 2014), and the fact that the raw milk is essential for the production of dairy products. In addition, it should be remembered that pyrethroid residues may be found in greater concentration in some

milk products than milk from which these were manufactured (Li *et al.*, 1970). Altogether, these data highlight the crucial importance of the dairy industry and government regulatory agencies in monitoring pyrethroid residues in milk combined with incentive, education, and training programs in an attempt to reduce the prevalence of excessive pyrethroid residue levels in raw milk.

The multidimensional PCA demonstrated that the average temperature and the THI were associated with pyrethroid residues in bulk milk tank, although the relative humidity was inversely correlated (Figure 1; Table 1). These facts strengthen our hypothesis that climate conditions influence the detection of pyrethroid residues in bulk milk tank probably as a result of the subjective use of pyrethroids mainly based on observation of infestation levels (Rocha *et al.*, 2012) and the influence of climate conditions on infestation by ectoparasites (Estrada-Peña *et al.*, 2006; Paim *et al.*, 2011). With this in mind, Souza *et al.*, (1988) described that lower infestation levels of *Rhipicephaus (Boophilus) microplus*, the major ectoparasite in cattle (Raynal *et al.*, 2013), occurred during months (i.e. August and September) with low temperatures and high relative humidity, in contrast with higher infestation levels of *Rhipicephaus (Boophilus) microplus* during months with high temperature and low relative humidity (i.e. January and February) (Souza *et al.*, 1988; Paim *et al.*, 2011).

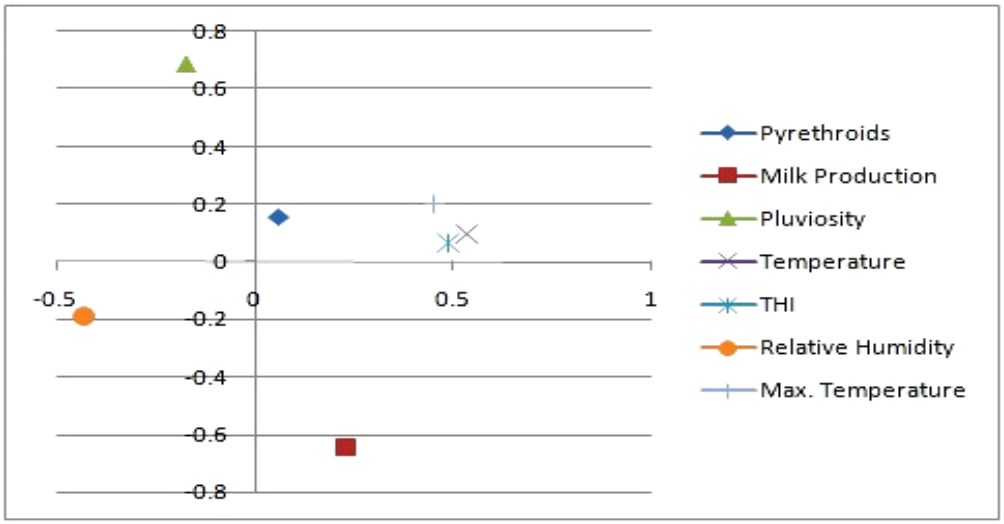


Figure 1. Plot of the principal multidimensional component analysis (PCA) of pyrethroid residues by confirmatory methods and their correlations with climate conditions - relative humidity, average temperature (temperature), maximum temperature (Max. temperature), pluviosity and the Temperature-Humidity Index (THI) for components 1 (x axis) and 2 (y axis) of the PCA. The variables in the same quadrant were closely associated, and those in opposite quadrants had opposite effects (components 1 and 2). The variables in other quadrants were regarded as independent variables.

Table 1. The relationship among milk production, climate conditions and pyrethroid residues by confirmatory tests expressed as loadings in a principal components analysis

| | Principal Component Analysis | | |
|---|---|---|---|
| Variables | Component 1 | Component 2 | Component 3 |
| Pyrethroids | 0.0595 | 0.1561 | 0.9634 |
| Milk Production | 0.2288 | - 0.6444 | - 0.0626 |
| Pluviosity | - 0.1724 | 0.6857 | - 0.2371 |
| Temperature | 0.5341 | 0.0959 | - 0.0272 |
| THI | 0.4878 | 0.0655 | - 0.0348 |
| Relative Humidity | - 0.4309 | - 0.1895 | 0.0201 |
| Maximum Temperature | 0.4533 | 0.2021 | - 0.0964 |

The screen test displayed 3 components. Temperature: average temperature. THI: temperature-humidity index.

Although the present study evaluated the effect of climate conditions on the antimicrobial and macrocyclic lactone residues in raw milk in a short period, their importance should at least be regarded in terms of world climate change. The changes in temperature and rainfall patterns may lead to alterations on the dynamics of pathogens of veterinary importance (Gale *et al.*, 2009; Van Dijk *et al.*, 2010), and consequently on the veterinary drug usage and the presence of their residues in food of animal origin with important human health implications. Thus, further studies are required considering a longer term to better determine the effect of these changes on animal health, veterinary drug usage, and their residues in food of animal origin.

## CONCLUSIONS

The results of the present study support the fact that some climate parameters can be associated with the residues in bulk tank milk. The data analyses indicated that some climate conditions can predict the moments with higher risk of occurrence of pyrethroid residues detected by confirmatory tests in bulk tank milk, and can be used to monitor pyrethroid residues in raw milk.

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