Journal of Dairy Research

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Intramammary treatment of clinical mastitis quarters with ceftiofur does not cause anbiotic residues in adjacent untreated quarters

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Research Article

Cite this article: Carvalho ALMA, Nascimento Neto JdaP, Carvalho TBT, Lima Neto HR, Victali RM and Chalfun LHL (2024). Intramammary treatment of clinical mastitis quarters with ceftiofur does not cause anbiotic residues in adjacent untreated quarters. *Journal of Dairy Research* **91**, 73–75. https://doi.org/10.1017/S0022029924000025

Received: 21 November 2022
Revised: 31 October 2023
Accepted: 7 November 2023
First published online: 16 February 2024

Keywords:
Antibiotic therapy; dairy cattle; discard; milk

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Abstract

The study was carried out in dairy cows to elucidate whether treatment of clinical mastitis quarters with Spectramast® LC (ceftiofur hydrochloride, 125 mg, Zoetis) created a reason for discarding milk from adjacent untreated healthy quarters. The antibiotic was infused once daily in the affected mammary quarter for four days. Forty-nine cows were evaluated after diagnosis of clinical mastitis in three or fewer udder quarters. In all cases, quarters that did not receive treatment had milk samples collected one day after the end of treatment. All milk samples from untreated quarters were below the maximum permissible limit for the presence of antibiotic residues after analysis with the BetaStar S Combo test. Pharmacokinetic and pharmacodynamic characteristics may explain this finding. We conclude that it is feasible to use milk from untreated quarters of animals that have been treated with Spectramast® LC. We also reiterate the need to carry out tests with other pharmacological bases, and that the results found in this experiment cannot be extrapolated to other drugs.

Dairy cattle have considerable importance in the development of the Brazilian economy, being directly linked to economic and social progress. In the first half of 2020, 12.1 billion liters of milk were produced in Brazil and in 2019, there was a new record of 25.01 billion liters produced (IBGE, 2020). This production comes from a wide variety of production systems, coming from smallholder farmers as well as from large companies that use the latest technologies available on the market. Dairy production is a complex activity. For one to obtain economical success, several aspects must be monitored. Maintaining the health of animals is a top priority, and the literature suggests that various diseases are a common challenge for dairy producers. Mastitis is the main disease that affects dairy cows, responsible for considerable economic loss and significant zootechnical and productive challenges (Ruegg, 2017). It is considered the second leading cause of cow culling in dairy herds, behind reproductive problems. Mastitis is characterized by infection of the mammary gland and may or may not occur with inflammation, generating changes in the mammary tissue and properties of the milk. It is classifield into clinical or subclinical mastitis, according to presence or absence of clinical signs, and into contagious or environmental based on the causative agent (Correa *et al.*, 2001).

Understanding the anatomical and physiological characteristics of the mammary gland is important in decision making. Araújo *et al.* (2012) mentioned that the bovine udder consists of four individual mammary glands (quarters) which are independent, each being responsible for milk production and secretion. Several characteristics of the animal and management strategies influence the occurrence of this disease in dairy cows. Cardozo *et al.* (2015) explained that older animals with a low udder and therefore greater dirt accumulation, with hyperkeratosis of sphincter and with more days in milk (DIM), are more prone to inflammation of the mammary gland. They also mentioned that not maintaining the milking line properly and lack of maintenance of milking equipment also has a direct relationship with the occurrence of mastitis.

There is a wide variety of species of microorganisms that can cause mastitis. Bacteria are the main pathogen and in the past, researchers would recommend starting treatment with intramammary antibiotics along with or without systemic antibiotics as soon as clinical signs of mastitis were observed (Correa *et al.*, 2001). However, this concept has changed now, and isolating and identifying the causative agent *via* microbiological culture before treatment is initiated is becoming more common. This identification of bacteria or other microorganisms enables more specific decision-making and allows treatments to be more effective, reducing the indiscriminate use of antimicrobials (Ganda *et al.*, 2016). In 2010, approximately 63 000 tons of antimicrobials were used in the livestock industry in Brazil and the consumer is obviously

concerned about the situation. The trend is that until 2030, this number will rise around 67%, reaching 105 000 tons (van Boeckel *et al.*, 2015). Also in a 2010 survey, Brazil was the third country globally in the quantity of antibiotics used in animal production. New projections show that the speed of growth in the use of these substances has been reduced, indicating that the country has begun to adopt more efficient and controlled systems regarding the use of antimicrobials (van Boeckel *et al.*, 2015).

Amongst all the situations where antibiotic treatment is needed in animal production, the treatment of mastitis is one of the most common. Leitner *et al.* (2018) reported that when these drugs are used, the disposal of milk during and after treatment (due to the presence of antibiotic residues) can cost up to 50% of the total amount of mastitis expenses. The authors also mentioned that this factor is the main reason for producers to opt for non-antibiotic treatment of subclinical mastitis of lactating cows, raising the question of the need for alternatives. This issue of antibiotic residues is even more important because it is directly linked to public health. Indirect consumption of antibiotic residues could result in the evolution of antibiotic resistant bacteria that might adversely affect human health. This may manifest directly within the intestinal microbiota of individuals who consume products containing antibiotic residues, or it can result from the ingestion of bacteria already resistant that are present in milk with residues (Brown *et al.*, 2020). In order to reduce the risks of consumption of antibiotic residues in milk, Anika *et al.* (2019) evaluated whether heat treatment at 100°C for 20 min would be able to change the residual status of samples previously identified with antibacterials. There were no positive results, verifying that this procedure is not effective.

Intramammary antibiotic use as mastitis therapy or prophylactically requires discard of milk at cow level (ie both from treated and untreated quarters). This results in substantial economic loss to the dairy producers. This could potentially be reduced if it could be shown that untreatd quarters do not contain antibiotic residues. So, this study was carried out in order to measure whether milk samples from untreated udder quarter of cows treated for mastitis with an intramammary drug based on ceftiofur hydrochloride would be positive or negative for antibiotic residuals. We hypothesized that residues would not be present, based on the known anatomical independence of the four quarters.

Materials and methods

There was prior authorization from the owner for the use of the animals for experimental purposes and all ethical standards and good practices aimed at animal welfare were approved by the Ethics Committee on the Use of Animals (CEUA), from UNILAVRAS. Research protocol no. identified as 004/2019, 24 April 2019.

Animals and management

Forty-nine lactating dairy cows maintained in an intensive compost barn system were evaluated. They were diagnosed with clinical mastitis in at least one quarter. Milk production from the cows ranged from 0.8 to 40 kg and days in milk (DIM) between 43–248 d.

Experimental design

The diagnosis of clinical mastitis was based on changes in the physical appearance of the milk (such as the presence of clots) in addition to other changes such as redness and an increase in temperature, indicative of inflammation in the mammary gland. In some cases, microbiological culture was performed to identify the causative agent. After the disease was diagnosed, regardless of the degree of clinical mastitis, all cows received the same treatment that was performed exclusively with intramammary administration of Spectramast® LC, a drug composed of ceftiofur hydrochloride 125 mg (Zoetis). The treatment protocol lasted four days and the antibiotic was infused once a day in each affected mammary quarter.

Milk samples were collected from the untreated quarters only, 24 h after the last intramammary administration of ceftiofur. Sampling occurred during milking time using aseptic technique as was standard on the property due to the microbiological culture testing conducted in some cases of mastitis. Teats were cleaned with a 70% alcohol-based cotton swab, avoiding possible contaminants, and the milk from each quarter was homogenized and stored in a single container for analysis.

The commercially available screening test BetaStar® S Combo (©Neogen Corporation; see online Supplementary Fig. S1) was used to examine presence of ceftiofur hydrochloride residues in milk samples. This is a qualitative test that was performed with milk samples heated in a 220v heating block and is indicated for rapid analysis of the presence of residues of beta-lactams, tetracycline and metabolites of ceftiofur in milk samples. It has a reagent zone composed of antibodies and receptors that will bind to residues when present, forming a drug-antibody-particle complex. The visual interpretation of the strip is based on a standard reader (online Supplementary Fig. S2), lasting approximately 5 min and it is also approved by the Ministry of Agriculture and Animal Production. A mammary quarter is considered positive to antibiotic residue when the intensity of the identification lines is less than the control line. Negative cases are indicated when the identification lines are equal to or greater than the control line. The maximum residue limits (MRL) for ceftiofur and its metabolite regulated by the European Union (EU) are 100 µg/kg, and the test has the capacity to identify samples with 30 µg/kg of ceftiofur and 35 µg/kg of desfuroylceftiofur.

All cows that entered the experiment underwent a clinical examination, evaluating their health status, except for mastitis involvement. Proving that the animals did not have other concomitant conditions was important so that no other treatments were needed. When there was, the animals were excluded from the research group. All data were stored through individual scanned clinical sheets created in an Excel spreadsheet. The descriptive statistics analyses were performed after data were compiled. Due to the type of response found in the present study, where there were no variations in the results, so only this descriptive analysis of the data was sufficient to express and answer the proposed hypothesis. Variables such as milk production, age and parity did not interfere and did not cause any change in the animals evaluated in this research. The lack of these evaluations does not impair the reliability of the data considering that there were no external or intrinsic factors of the animals that could affect the results.

Results

Fifty-three quarters affected with clinical mastitis from 49 cows were treated with Spectramast® LC. These quarters consisted of 15 right-back, 9 left-back, 14 right-front and 15 left-front quarters (online Supplementary Table S1). Out of 49 cows, 4 had more than one quarters affected with clinical mastitis (online Supplementary

https://doi.org/10.1017/S0022029924000025 Published online by Cambridge University Press

Table S2). Irrespective of the number of quarters affected per cow, none of the milk sample collected untreated quarters ($n = 143$) was found positive by the test for ceftiofur residuals. The results showed that when using the drug Spectramast® LC, milk from untreated quarters can be used for consumption, and manufacturing of dairy products, without offering risks to human health, since the samples had concentration below the MRL.

Discussion

Products of animal origin are submitted to various sanitary inspection processes so that they do not represent risks to human health. The European Union determines that food can only be marketed if there is no drug residue or if this concentration is below the MRL. These limits are defined through information and research on the mechanism of action of each drug used on farms (Durel *et al*., 2019). In the specific case of the drug ceftiofur, the maximum permissible residual limits of ceftiofur in milk is 100 µg/kg (Nisha, 2008).

The present study showed that when Spectramast® LC was administrated by the intramammary route for the treatment of clinical mastitis, milk collected from adjacent untreated quarters did not show drug residuals above the detectable limits of the test. Brown *et al*. (1991) reported that ceftiofur is rapidly metabolized into desfuroylceftiofur after parenteral application and eliminated in feces and urine. This metabolite then binds in plasma proteins to become inactive. In healthy adult cows, 90% of its concentration is bound to proteins. We did not find similar pharmacokinetics studies in cows treated by intramammary ceftiofur. It could be probably assumed that the action and metabolization of the drug would be similar when it is used *via* different routes.

Studies related to this subject are scarce in the literature. Varón *et al*. (2016) obtained similar results in an experiment where intravenous, subcutaneous and subcutaneous-LA applications of ceftiofur were used in goats with pneumonia. The authors did not observe residues of this antibiotic being excreted in milk, but they did not examine the intramammary administration route. Durel *et al*. (2019) evaluated milk samples from six cows treated with ceftiofur hydrochloride intramuscularly. The animals received 1 ml/50 kg of body weight once a day for five days. The samples were collected immediately before the last application and at 12, 24, 36, 48, 60, 72, 84, and 96 h after the last application. Three tests for the identification of residues were used: Delvotest SP NT (Delvotest), SNAP Beta-Lactam ST Plus and ROSA MRL Beta-Lactam Test. In the first test, all samples were negative, which disagreed with the results found in the other two tests, where at least at one milk sample from all the six cows tested positive. The authors discuss that this is due to the capability of each test and emphasize that even though the first test is capable of identifying amounts below the MRL, the other two tests have a lower limit and higher sensitivity, identifying minimal residual doses of antibiotic.

Pharmacokinetic studies are commonly conducted in healthy animals, and recommendations regarding withdrawal period, dosage, and routes of administration are based on these results. Gorden *et al*. (2016) conducted an experiment to compare whether the pharmacokinetics of ceftiofur hydrochloride would demonstrate similar action in healthy and sick cows. Due to there being several differences found between the groups evaluated, the authors highlighted the hypothesis that the infectious process affecting the animal alters various factors related to the drug's action, absorption and excretion. Therefore, they discuss that the therapeutic approach used to test drugs in veterinary medicine should be redesigned to reach more reliable results that can support the correct approach of professionals. These findings reinforce the significance of the results obtained in the current study and emphasize the necessity to consider this important issue in the food industry.

In conclusion, the commercial screening test (BetaStar® S Combo) yielded negative antibiotic residue results, suggesting that in an individual cow, milk from untreated healthy quarters is unlikely to contain ceftiofur residues above the permissible limits of 100 µg/kg when other quarter(s) with clinical mastitis are treated with intramammary ceftiofur. So, it is feasible to consume milk from untreated healthy quarters without any public health concern. This study is important in enriching the scientific literature to assist in the decision-making process of veterinarians.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029924000025

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