RATIONAL DECISION ON THE USE OF ANTIBIOTICS DURING THE DRY PERIOD IN DAIRY COWS

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ABSTRACT

The aim of this study was to evaluate the use of antibiotics in cows during the dry period. The survey was performed on 148 teats during the dry period, with sample collection in the period D-70 (70 days before delivery) and D14 (14 days after delivery). The milk samples were collected for the Strip Cup Test (SCT), California Mastitis Test (CMT), Microbiological Culture, Somatic Cell Count (SCC), Somatic Cell Score (SCS) and Hyperkeratosis (HK). The groups in which there were no microorganisms grow were divided into two groups, in the first group only the internal sealant in the teat was used (Group 1) and there was another group with the intramammary antibiotic use associated with the internal sealant (Group 2). Teats which were considered positive, with microbiological growth, were treated with the intramammary antibiotic associated with the internal sealant (Group 3). In the comparison of the results of the CMT test between D-70 and D14, a statistical difference was observed in Groups 2 and 3. Group 3, which comprises the positive teats in D-70 presented a reduction of 83.87% and 32.26% in the CMT test between D-70 and D14. Regarding HK, group 1 and 2 had a statistical difference in relation to group 3 in D-70 and D14. As for the numbers of bacteria isolated in D-70 and D14, there was no difference comparing Group 1 and Group 2, unlike Group 3, which had a difference. Group 1 and Group 2 were all negative teats in D-70, showing that the intramammary antibiotic did not influence the outcome of D14. In group 3 there was a reduction of isolates from 62 to 15 in D14. The most prevalent microorganism was Streptococcus agalactiae with 43.37% of the total isolates, followed by Staphylococcus aureus (16.87%) and Corynebacterium spp. (13.25%) and Coagulase negative Staphylococcus (SCN) (10.84%). The selective treatment of teats in dry dairy cows has advantages over Blanket Dry Cow Therapy by reducing the indiscriminate use of antibiotics, avoiding bacterial resistance, ensuring better milk quality and greater food safety. Antibiotics should only be used for teats with subclinical mastitis, with the microbiological culture at the end of lactation performed by fourth individual mammary.

Introduction

Mastitis is the most frequent disease which affects dairy herds causing prejudice worldwide [(1)], either due to reduced milk production, total or partial loss of the mammary gland, risk of transmission to other animals or even the animal's death [(2)]. The cause of mastitis is multifactorial, it can be influenced by the environment in which the animal is found, the affected microorganism and the response of each individual to the causative agent [(3-5)].

There is a trend towards the rational use of antibiotics, both for reducing residues in animal products for food safety and also for decrease of the microorganisms' resistance to antibiotics used in animals [(6)]. The reduction in the use of antibiotics is important for the efficiency of treatments in cases of diseases [(7)].

In dairy cows, besides the use of antibiotics in the treatment of clinical and subclinical mastitis during lactation, the use of antibiotics for intramammary use at the time of drying among lactations is also used, but without the proper diagnosis of mastitis, with application of antibiotics in all teats and generally with association with internal sealant, acting as mechanical barrier against environmental agents in the pre-labour [(8,9)].

Mastitis is a complex and widespread disease with multiple microorganisms' etiology and conditioned by several factors, added to the specificity of treatment to each of the agents, it becomes even more difficult to treat, requiring studies that evaluate the best treatment in the period of drying, to perform the selective treatment in dairy cows (STDC) [(10,11)].

Legislation on the use of antibiotics in disease prevention is increasingly restricted in the area of animal production, including the treatment of antibiotics in the drying period [(12,13)]. There is a concern of decreasing the use of antibiotics in the drying of dairy cows, treating only the teats with intramammary infections with the previous diagnosis.

During the drying period in dairy cows, there is no technical standard in decision making, some professionals choose to treat all teats with antibiotics regardless of historical and mammary gland health assessment (BDCT), other professionals and producers use internal sealants or antibiotics in teats according to the record of SCC and clinical mastitis of the animal, justifying studies to clarify the best management of cows at the end of lactation at each farm.

Many studies on the selective treatment in the drying period consider the cow with a history of SCC during lactation (8,9,14). It can be noticed that there is a need for further studies with individual evaluation of mammary quarters for an even greater reduction in the use of antibiotics (15).

The objective of this research was to evaluate the use of antibiotics in cows during the drying period.

Materials and methods

Herd characterization

The study was carried out on a dairy farm, with a latitude of 17º 50 '07 "S and a longitude of 46º 30' 52" W, with a daily average production of 9,000 liters of milk, two milking per day, with approximately 450 dairy cows, the average per cow of 30 kg per day in 3 milking, with blood level ranging from 1/2 to 31/32 Dutch/Gir, with semi-intensive breeding system, throughout the year. All animals within the same farm were submitted to the same management, with silage-based feed and concentrate throughout the year, varying proportions according to their milk production, lactation days and days in gestation (NRC, 2001).

The primiparous cows, the ones with antibiotic application or case of clinical mastitis were discarded in the last 30 days before drying, presence of lumps on the D-70 Strip Cup Test

(70 days before delivery), presence of conditions in the hull, drying period less than 50 days and greater than 90 days before delivery.

Trial design

The research was performed in 37 cows (148 teats) in the drying period, for six months, with sample collection on D70 period (70 days before delivery) and D14 (14 days after delivery), with sampling by teat.

Drying of the dairy cows was performed on average 60 ± 7 days before the expected date of delivery, with the type of treatment in each group according to the results of the tests in D-70, 10 days before drying.

The milk samples were collected for the Strip Cup Test (SCT), CMT (California Mastitis Test), microbiological culture, SCC (somatic cell count), SCS (Somatic Cell Score), hyperkeratosis (HK) in each animal on D-70 and D14, according to Figure 1.

Negative teats were considered as those whose milk samples did not show growth of microorganisms, and the positive teats were the milk samples that showed growth of microorganisms from the microbiological culture test performed on D-70.

The 148 teats were divided into three groups after the results of the examinations on D-70. The teats considered negative had two different treatments, in the first one only the internal sealant was used in the teat (Group 1), and another group with the intramammary antibiotic associated with the internal sealant (Group 2). The teats considered positive were treated with the intramammary associated with the internal sealant (Group 3).

The intramammary antibiotic used was based on Cloxacillin Benzathine 600 mg, using one tube per teat (3.6 g), in groups 2 and 3. In all teats, in the 3 treatment groups, one tube was used per internal sealant teat based on bismuth subnitrate (4 g) to reduce the influence of the environment on the health of the mammary gland during the dry period.

The use of the sealant in all the teats was to avoid external contamination of the teats during the dry period, to better evaluate the dynamics of the results in the established groups.

In the application of the products at the time of drying (D-60), the teats were disinfected with 10% topical polyvidone iodine solution and paper towel drying after 30 seconds, performing another antisepsis of the teat sphincter using alcohol-wetted cotton to 70%. After the disinfection of the teats, the cannula of the antibiotic tubes was introduced, depending on the treatment group, massaging the teat to propel the substance upwards and soon after the internal sealant tube was applied via cannula inside the canal of the teat.

CMT Test

For the CMT, 2 mL of milk from each mammary quarter were removed on the CMT plate, to which was added, in the same proportion as the CMT solution, an anionic detergent (alkyl lauryl sodium sulfate), homogenized with circular movements for 10 seconds, prior to reading, which is determined by the sample gelation, if the test is positive.

The interpretation of the test is usually classified as negative, one cross, two crosses or three crosses, according to absence or increase of the viscosity from the mixture (17,18). The mammary quarters that presented viscosity from the score of a cross were considered positive on this research.

The animals were evaluated by performing the CMT Test, on D-70 and D14, always performed by the same person to avoid subjectivity to the test.

Hyperkeratosis score in the teats (HK)

The severity of hyperkeratosis, an excessive growth of the teat skin, was classified visually through an evaluation in scores, ranging from 0 (normal) to 3 [(19)]. This tool has as the main objective the aid of problem identification, which among the main causes is the proper functioning of the milking equipment and correct management of milking in dairy herds.

Somatic Cell Count

In order to perform the Somatic Cell Count (SCC), milk samples were collected in plastic bottles containing bronopol preservative on D-70 and D14 and sent on the same day of collection to the laboratory.

Bronopol is a tablet-form preservative which in contact with milk results in a light pink-colored blend. For the SCC determination of the milk samples, the flow cytometry technique was applied using the SOMACOUNT 500 device, with a result expressed the number of cells multiplied by 10^3 / mL of milk (20). In the SCC results, it is usually considered an animal with subclinical mastitis presenting values > 200,000 cells / mL of milk (ECS>4) (21).

In order to linearize the data, SCC was transformed into Somatic Cell Score (SCS), with SCS = [log2 (SCC / 100)] +3 (22,23).

Microbiological Culture

On D-70 and D14, after the Strip Cup and CMT tests, milk samples collections were carried out for individual microbiological diagnosis in all teats, aiming at the identification of pathogens causing mastitis. The milk samples were obtained immediately prior to milking, after discarding the first three milk jets.

It was disinfection of the teats with 10% topical polyvidone iodine solution and drying with paper towels after 30 seconds was performed. At the time of collection, the antisepsis of teat sphincter was held using cotton wool moistened with 70% alcohol. The milk samples were collected from individual teats and placed in sterile bottles previously identified with the number and teat of the animal. The sampled material was frozen and then sent in an isothermal container with recyclable ice to the laboratory for microbiological diagnosis with the isolation and characterization of the microorganisms.

Samples of milk were cultured in 5% (v / v) blood agar plates, incubated in aerobiosis in a bacteriological heating chamber at 37ºC and analyzed after 24 and 48 hours. Following the incubation, the growth characteristics of the colonies on blood agar were recorded, such as hemolysis production, pigment, type of development and pigmentation of the colonies, observing hereinafter the morpho-tinctorial characters using the Gram staining technique.

Colonies which demonstrated to be Gram-positive cocci were submitted to catalase and slow coagulase tests with rabbit plasma. The readings for the verification of coagulase production were performed one, two, three, four and 24 hours after incubation of the samples at 37 ° C [24]. Biochemical tests were performed to identify isolated microorganisms [25].

The catalase and coagulase positive strains were tested for acetoin production with the Methyl Red and Voges-Proskauer test (MRVP broth) for the differentiation of Staphylococcus aureus and another coagulase-positive Staphylococcus. The acetoin-producing strains were tested for whether or not maltose and trehalose could be used [26]. Strains that presented positive results for these tests were classified as *Staphylococcus aureus* [27,28]

Statistical analysis

For the comparison among the three groups with HK, the Tukey's test was used. By comparing the results of D-70 and D14 analyses with the CMT test, the Chi-Square and T-Test were performed on the HK results.

As for the numbers of isolates, the Chi-Square test was used to compare the results of the D-70 and D14 analyses among each group.

In all statistical tests, a significance level of 5% was applied, using ACTION 3.0 software [29].

Economic analysis

For the analysis of the cost regarding the drying treatment of the cows, the real value of the examinations from this study was used, adding to the values of the medicines medicine. The values of microbiological culture tests were $2.05 per sample, SCC test $ 0.70 per animal, CMT test $0.03 per teat. The cost of the antibiotic was $3.07 and the sealant $2.25 per teat.

Results and discussion

Comparing the results of the CMT test between D-70 and D14, a statistical difference was observed in Groups 2 and 3, according to Table 1. Group 3, which are positive teats on D-70, had a reduction of 83.87% and 32.26% in the CMT test between D-70 and D14, with statistical difference.

Table 1. Mean and Standard Deviation of the CMT and HK test in each treatment group on D-70 and D14.

GROUP	n	CMT +[1]	CMT +[2]	HQ[1]		HQ[2]	
1	43	$34,88\%^{a}$	$25,58\%^{a}$	$0,81^{Aa}$	±0,79	$0,44^{Ab}$	±0,70
2	43	$48,84\%^{a}$	$13,95\%^{b}$	$0,91^{ABa}$	±0,68	$0,70^{ABa}$	±0,80
3	62	$83,87\%^{a}$	$32,26\%^{b}$	$1,26^{Ba}$	±1,02	$0,95^{Bb}$	±1,12

Note: Numbers followed by different lowercase letters on the same line or different capital letters in the same column are statistically different ($p < 0.05$). 1: data collected on the D-70. 2: data collected on day D14.

Intramammary antibiotic treatment during the dry period is recommended for treatment of subclinical mastitis, in association with internal sealant, as used in this research (11). The bismuth subnitrate used in this study is the only sealant base found in the market, with proven results in other studies (11,30).

The positivity of the CMT test in Groups 1 and 2 on D-70 in teats that did not show bacterial growth demonstrates the need for caution when using the test at the time of drying. The presence of microorganisms in negative teats may occur in the CMT Test, with low specificity (31,32).

The mean SCS values of the three treatment groups, before and after the dry period, were above 4 (greater than 200,000 cels / mL), even in groups 1 and 2 that did not have microbiological growth. For the selective treatment in drying, research has reported that SCS and CMT, may have errors in interpretations and interfere with the segregation of treatment in the dry period (31,33-36).

In the individual SCC, values below 200.000 cels / mL of milk refer to the animals without intramammary infection, but research shows that intramammary infection can occur even in values within the reference standards (37-39) and that the CMT Test, even with a negative result, may present microorganisms in the mammary quarter (31).

In Table 1, regarding HK, group 1 and 2 obtained a statistical difference in relation to group 3 on D-70 and D14. There was a difference in group 1 and 3 and no difference in group 2 comparing the results of each group on D-70 with D14, which shows that the dry period can decrease the degree of HK, but in some cases are irreversible, according to the injury level in the teat (19). The higher the HK score, the lower the mechanical barrier and the higher the ease of teat contamination, consequent incidence of subclinical mastitis [(19,40,41)].

As for the numbers of isolated bacteria on D-70 and D14, there was no difference comparing Group 1 and Group 2, but with a difference in group 3. Groups 1 and 2 were all negative teats on D-70, showing that the intramammary antibiotic did not influence the result of D14, according to Table 2. In group 3 there was a reduction of isolates from 62 to 15 on D14. Research has reported a reduction of 70.27% in infection in treated teats, comparing before and after delivery [(15)].

The most prevalent microorganism, according to Table 2, on D-70 and D14 was *Streptococcus agalactiae* with 43.37% from the total isolates, followed by *Staphylococcus aureus* (16.87%) and *Corynebacterium spp.* (13.25%) and *Staphylococcus coagulase negative (SCN)* (10.84%), with a significant difference in the number of isolates, found on D-70 and D14. Surveys obtained a frequency of *Streptococcus spp*. (24.52%), *Staphylococcus aureus* (13.69%), *Corynebacterium spp.* (6.87%) and SCN (75.97%) [(15)]. *Streptococcus spp.* (2.79%) was less frequent, there was no growth of *Staphylococcus aureus*. On the other hand, CNS

(54.31%) were more frequent, followed by *Corynebacterium spp*. (12.69%), in the results of microbiological culture examinations per mammary quarter before and after delivery [42].

Table 2. The number of microorganisms isolated in milk samples in relation to the treatment groups on D-70 and D14.

	Groups							
	1		2		3		Total	
	D-70	D14	D-70	D14	D-70	D14	n	%
Corynebacterium sp.	0	1	0	0	10	0	11	13,25
Escherichia coli	0	0	0	0	2	2	4	4,82
Klebsiella pneumoniae	0	0	0	0	0	1	1	1,20
Staphylococcus aureus	0	0	0	1	5	8	14	16,87
Staphylococcus coagulase negative	0	2	0	2	5	0	9	10,84
Streptococcus agalactiae	0	0	0	0	32	4	36	43,37
Streptococcus bovis	0	0	0	0	3	0	3	3,61
Streptococcus uberis	0	0	0	0	5	0	5	6,02
Total:	0^{a}	3^{a}	0^{a}	3^{a}	62^{a}	15^{b}	83	100

Note: Numbers followed by different lowercase letters on the same line are statistically different ($p < 0.05$).

The bacteria *Corynebacterium spp., Staphylococcus coagulase negative, Streptococcus bovis* and *Streptococcus uberis* were bacteriologically cured in the treatment of group 3 between D-70 and D14, according to table 2. The frequency of *Streptococcus agalactiae* decreased from 32 to 4 (87.5%), and *Staphylococcus aureus* increased from 5 to 8 (60%) in the treatment of Group 3 on D-70 compared to D14. The result in the treatment of subclinical mastitis in positive teats in the dry period is directly related to the type of agent most prevalent in the herd [43].

Groups 1 and 2, comparing the number of isolated microorganisms on D-70 and D14, were not statistically different. The difference in the treatment in Groups 1 and 2 was the use of the antibiotic, reinforcing the possibility of the STDC in teats considered negative. In another study, they compared the same treatment with and without antibiotic, associated with the sealant, but in low-risk cows with a history of SCC below 200 x 103 cells / mL, and obtained the same result of negative teats with and without antibiotics, with prior segregation by SCC analysis history (44).

The economic viability of STDC is related to the incidence of mastitis in each herd, that is, the decrease in the use of antibiotics is directly related to the incidence of subclinical mastitis at the end of lactation within each herd (9).

Table 3 shows the estimated cost of treatment with the microbiological culture test, comparing with the hypotheses of segregation using the CMT, SCC and BDCT (Blanket Dry Cow Therapy) tests which use antibiotic in all teats regardless of the previous examination.

Table 3. The estimated cost of the selective treatment in the drying of dairy cows according to each diagnostic method in positive and negative teats.

	Positivos			Negativos			
Método	n	CP ($)	CTP ($)	n	CN ($)	CTN ($)	CF ($)
Cultura	83	7,37	611,71	65	4,30	279,50	891,21
CMT	88	5,35	470,80	60	2,28	136,80	607,60
BDCT	148	5,32	787,36	0	5,32	-	787,36

Note: CP: Cost per positive teat (exams + antibiotic + sealant), TCP: total cost in positive teats, CN: Cost per negative teat (exams + sealant), TCN: total cost in negative teats and FC: Final cost (positive and negative teats).

Treatment at the end of lactation using SCC history during lactation has been the most used in other studies (9,14,21,45), but microbiological growth may occur in cows with values below 200 x 10^3 cells / mL (45). With the use of the microbiological culture as a parameter, the FC was $891.21, with the SCC test of $792.72 and with the use of the BDCT method was $787.36. The cost using culture test is variable, which depends on the contamination degree of the mammary gland, the antibiotic use varies, with the possibility of being more financially viable than the BDCT.

The estimated cost of treatment in positive teats, using the previous culture examination (CP), was $7.37, test of CMT $5.35 and BDCT $5.32. In negative teats (CN), the estimated cost of the culture test was $4.30, CMT test $2.28 and BDCT $5.32. When the CMT test was used to segregate positive and negative teats, the final cost (FC) was lower at the time of drying with $607.60, but there is a possibility of microbiological growth in teats considered negative (31,46), with fake positive occurrences in the test, in negative teats, as it occurred in this study.

Using the culture test at the end of the D-70 lactation, it has a greater number of negative teats diagnosed, using fewer antibiotics and reducing the cost of treatment in the dry period. Selective treatment at the time of drying using the culture method may be economically more viable than BDCT if the number of intramammary infections is low with lower intramammary antibiotic expense [9, 42].

There are several guidelines from international organizations recommending caution in the use of antibiotics in production animals in order to reduce residues in food and bacterial resistance in animals and also humans [7,13,47-50].

Both economically and in relation to the sanity of the fourth mammary during the dry period, the selective treatment of teats with the microbiological culture is recommended. The SCC test should not be used in the STDC at the end of lactation as the test results judge the animal rather than the individual teat, limiting the result in the antibiotic reduction protocol established in the drying of cows. It is important to provide a safety diagnosis on drying using the STDC to ensure a reduction in the use of antibiotics and sanity of the mammary gland during the dry period.

Conclusion

The selective treatment of teats in the drying of dairy cows offer advantages over Blanket Dry Cow Therapy, reducing the indiscriminate use of antibiotics, avoiding bacterial resistance, as well as ensuring better milk quality and greater food safety. Antibiotics should only be used for teats with subclinical mastitis, with the microbiological culture at the end of lactation performed by individual mammary quarter.

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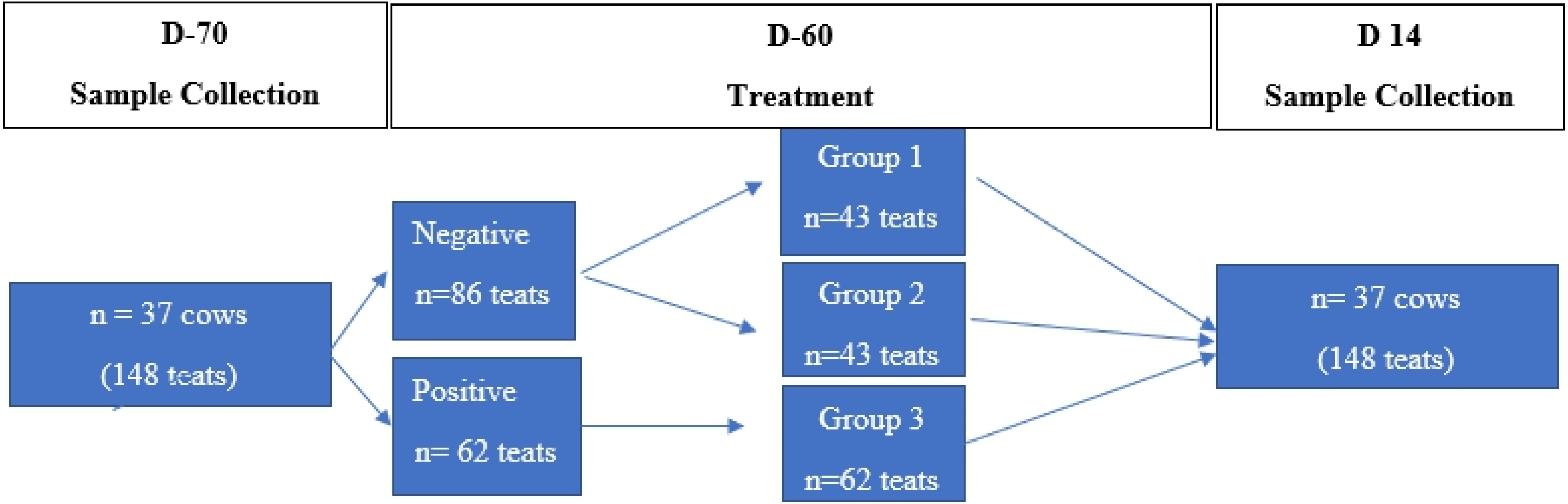
Figure 1. Description of procedures performed before (D-70 D-60) and after delivery (D14).



Figure