INVESTIGATIONS ON THE DURATION OF ELIMINATION OF CERTAIN MEDICATIONS IN THE MILK OF COWS TREATED WITH MEDICATIONS

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Abstract

The highest frequency is on day 1 (after treatment) after which it gradually decreases. It should be noted that no antibiotic residues were detected in the collected milk samples prior to treatment. Antibiotic residues may be detected in the milk of recently calved animals due to intramammary treatments during the dry period (chronic mastitis). If the period after treatment is less than 15 days until calving, the frequency of detecting residues in milk after calving is high. As noted, there were two (10%) positive results, after the expiration of the waiting period, one in intramammary treated animals and one in intramuscular treated animals on the fourth day, the first day after the waiting period. The somatic cell count in the positive sample from intramammary treated animals was 2850000 cells/ml and 337000 cells/ml in the sample from intramuscular treated animals. The antibiotic residue test was influenced by the components of the milk with mastitis. From the analysis of the treatment register, it was found that the intramuscularly treated cow received an overdose of penicillin. This overdose resulted in a massive accumulation of penicillin in the animal's body resulting in an extension of the elimination period by one more day.

***Key words**: calving, milk, intramammar, antibiotic*.

INTRODUCTION

Common purslane, Although maximum milk residue limits (MRL) have been established, some situations note that in some countries contamination with antibiotic residues is still a problem (e.g. Brazil, Mastius-Junior et al., 2007; Bonda et al., 2009; China: Bai et al., 2005; Bai and Huang, 2006; Kenya).

Many studies of antibiotic residue testing are focused on liquid milk, with little attention paid to milk powder. The need to monitor imports of milk powder for a variety of potentially harmful substances is becoming very important.

Biotechnology presents an extremely rapid development and responds in the most natural way to major, fundamental both human and animal needs (Bonciu, 2020). In this context, antibiotics are biotechnological products that inhibit bacterial growth. But the residues of antibiotics and pesticides in animal feed may represent a risk to human health; this risk results from inappropriate use, as pathogenic-resistant organisms can proliferate in food products. However, regarding the retention of some toxic substances, not all side effects are known precisely yet (Bonciu, 2018, 2019).

In this respect Kneebone et al., 2010 tested the effectiveness of IDEXX tests (IDEXX Laboratories Inc) for detecting antibiotic residues in 5 milk powder assortments. The results suggest that IDEXX tests (New Beta-Lactum and New Tetraidine IDEXX Snap test Kits) effectively detect residues in commercial milk powder samples (Nestle -3 samples, Campina one sample and Regilait one

sample) and can be used for monitoring antibiotic residues in reconstituted powdered milk products.

Also, the designated rapid tests for farm-level results were found to be very good for detecting antibiotic residues in mixed milk from several animal species (Contreras et al., 1997; Cola et al.,2020; Andrew, 2000).

The incidence of false positive results in raw milk has been correlated with several factors, including high levels of lactoferrin, lysozyme, milk fat, milk protein and the milk somatic cell count (Carlsson et al., 1989; Andrew, 2000).

Interestingly, the performance of antibiotic residue testing devices is different for dairy cattle breeds. Andrew, 2000, notes an increasing trend in false positives for tests used in the evaluation of milk from the Jersey breed compared to tests used in the evaluation of milk from the Holstein breed.

Immunological tests are methods that detect specific interactions between the antibody and the antigen. These tests are divided into two basic categories, direct or indirect; the measurement of the primary antibody-antigen reaction, or the secondary antibody-antigen reaction. Applications of these immunoassays for the analysis of antibiotic residues are made on different devices: LFD (lateral flow device), STICKs, ELISA, RIA, SPR (Campbell et al., 2007; Nuet et al., 2006; Hanghey and Baxter, 2006).

Rapid tests that monitor enzymatic activity for the detection of the β-lactum class of antibiotics are available and now constitute a well-established technology.

Enzyme tests are generally considered as qualitative techniques that detect the presence of a specific chemical residue or are based on the change of the color reaction by evaluating the end point of the test .

Decision 2002/657/EC defines that confirmatory methods are to be based on physico-chemical techniques.

The most popular ones are based on the extraction of samples, using liquid extraction or liquid-solid extraction, liquid chromatography and detection by UV-VIS spectrometry, fluorimetric detection or mass spectrometry.At present almost all antibiotic residues used in Veterinary Medicine can be quantified and confirmed at the levels of interest (MRL) in most EU national reference laboratories.

MATERIALS AND METHODS

20 cows from the Holstein Friesian breed were taken in experience, between August 2020 and January 2021, at SC Fenov SRL, Dolj..

The decision to treat and the selection of antibiotics was made by the management of the farm. Only intramammary, intramuscular and combined intramammary and intramuscular treatments were followed. The antibiotic used, the date of administration, the number of quarters treated and the waiting period were noted.

Milk samples were collected before treatment as well as on days 1, 2, 3, 4, 5 and 6 after treatment.

The milk samples were refrigerated and analysed after 14-24 hours (depending on the two milkings).

All samples both pre- and post-treatment were tested for antibiotic residues using the EKOTEST machine.

Machine used: burette, graduated pipette; hot water incubator (Ekotest); test tubes with stoppers; stand

Reagents: lyophilising active substance; reagents no. 1; reagents no. 2 .

Work method: The milk sample is heated to 90 ^{o}C for a few seconds then cooled; take two test tubes and put 10 ml of milk in each (one control tube and the other one for testing); add 1.5 ml of the active substance in each test tube; close the test tubes with stoppers and mix the contents by repeated inversions; place the test tube in the incubator after the water temperature has reached 44 ^{o}C and place the control tube in the stand; incubate for 10 minutes at 44 ^{o}C; add 2 drops of reagent no. 1 in the control tube; the contents are mixed by repeated inversions of the tube then titrated with the reagent no. 2 which is added by pipette or from a burette (not less than 2 ml) under continuous agitation until the pink colour appears, which must persist for 30 seconds; note the amount of reagent added and place the tube in the stand. Take the test tube from the incubator and add two drops of reagent no. 1 mixing the contents by repeated inversions; add the same amount of reagent no. 2 as was added to the control tube; after a few repeated inversions the colour of the test tube is compared with the colour of the control tube. In addition to this test in the laboratory a positive sample containing 0.01 IU penicillin/ml milk was prepared.

Interpretation of results

Negative result: the milk sample tested (incubated) is white; there are no inhibitory substances and the milk can be processed technologically.

Positive result: the milk sample tested (incubated) is pink; there are inhibitory substances in the milk. This sample can also be compared with the positive sample prepared in the laboratory.

For ease of collecting individual samples from cows, these samples are usually collected by milking the first of all four quarters, and not a sample of all the milk milked of all four quarters. Generally, the first milk has a high concentration of somatic cells. The high content of somatic cells influences tests for antibiotic residues in milk. We consider the concentration of antibiotic residues in all the milk milked to be much more representative of the milk added to the storage tank. A few constituents of milk with mastitis interfere with the antibiotic screening test in milk, and these are: lactoferrin, lysozyme, microorganisms, somatic cells and free fatty acids. Somatic cells in milk with mastitis were determined with the Somascope MK II apparatus. The milk found positive after the end of the waiting period was also analysed. They were considered positive samples when 11 tubes (91.66%) out of 12 tubes had a positive reaction.

RESULTS AND DISCUSSIONS

Antibiotics used to treat sick animals are described in table no. 1. This table also lists the waiting period necessary for the elimination of the antibiotic via milk (during which time the milk is not used for human consumption). Of the 20 cows, 5 cows (25%) were treated with penicillin G (400,000 u.i./ml), 3 cows (15%) with ampicillin, 2 cows (10%) with asomicin, 5 cows (25%) with penicillin-streptomycin and 5 cows with erythromycin (Table no. 1)

Table 1 Treated animals and antibiotics

Product or antibiotic	Number of cows treated	Waiting period (hours)
Penicillin G 400,000 IU/ml	5	72
Ampicillin	3	48
Ascomycin	2	48
Penicillin/Streptomycin (Penstrep)	5	72
Mastitiker E (Erythromycin)	5	72
Total	20	72

Table no. 2 shows the frequency of antibiotic residues detected with the EKOTEST apparatus in milk samples collected daily after the last treatment for 6 days. The sensitivity of the EKOTEST apparatus for detecting antibiotic residues is presented by table no. 3.

Table 2 Frequency of detection of residues of antibiotics in milk

Administration route	Before the taratament	Number of positive milk samples (quarter udder) after treatment on day:					
	0	1	2	3	4	5	6
Intramammary n = 15	0	15	14	14	1	0	0
Intramuscular n = 3	0	3	2	1	1	0	0
Combined n = 2	0	2	2	1	-	-	-

The highest frequency is on day 1 (after treatment) after which it gradually decreases (fig. 1). It should be noted that no antibiotic residues were detected in the collected milk samples prior to treatment. Antibiotic residues may be detected in the milk of recently calved animals due to intramammary treatments during the dry period (chronic mastitis). If the period after treatment is less than 15 days until calving, the frequency of detecting residues in milk after calving is high.

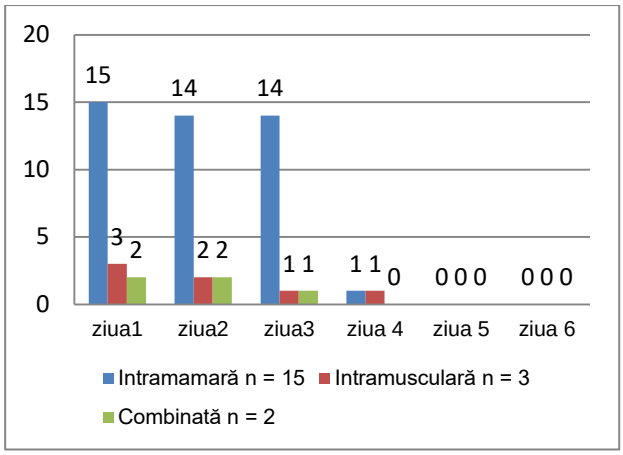

Figure 1 Frequency of detection of residues of antibiotics in milk

As shown in table no. 2, there were two (10%) positive results at the end of the waiting period, one in animals treated intramammaryally and one in animals treated intramuscularly on the fourth day, the first day after the waiting period.

The somatic cell count in the positive sample from the intramammary group was 2850000 cells/ml and 337000 cells/ml from the intramuscular group. Antibiotic residue screening was influenced by the components of the milk with mastitis.

From the analysis of the treatment register, it was found that the intramuscularly treated cow received an overdose of penicillin. This overdose resulted in a massive accumulation of penicillin in the animal's body resulting in an extension of the elimination period by one more day. Overdose treatments in food producing animals for human consumption are subject to wide-ranging discussion in all scientific journals and publications. Two major issues arise: the possibility of antibiotic residues being transferred to dairy products for human consumption (the objective of these

investigations) and the safety of animals as such.

Table 3 Sensitivity of the EKOTEST apparatus

Antibiotic	Ekotest * (ng/mL)	MRL ** (ng/mL)	Ekotest in correlation with MRL
Ampicillin	5	4	1.25 x MRL
Bacitracin	100	100	1 x MRL
Cephalexin	70	30	2.33 x MRL
Cephazolin	50	50	1 x MRL
Ceftiofur	70	100	0.7 x MRL
Erythromycin	400	40	10 x MRL
Gentamicin	300	100	3 x MRL
Penicillin G	2	4	0.5 x MRL
Streptomycin	250	200	1.5 x MRL
Sulfadimethoxine	100	100	1 x MRL
Sulfamethazine	100	100	1 x MRL
Sulfathiazole	100	100	1 x MRL
Tetracycline	100	100	1 x MRL

*after Baltagieva and Laboratory LB Lact;
**after EU Council Regulation No. 2377/ 90.

Screening tests should be included at farm level in the monitoring programmes for antibiotic residues.

Milk samples have a high compositional diversity, especially in terms of microbiological contamination, somatic cell content or natural antimicrobial content (lactoferrin, lysozyme).

These animal defence mechanisms may cause some tests to show inhibitory substances in milk after the waiting period has expired.

It is very important to assess whether the positive results of the milk samples after the waiting time are caused by antibiotic residues or by the natural inhibitors in the milk.

A first measure to reduce false-positive results is to boil milk samples before the tests are carried out. The action of natural milk inhibitors is reversed by boiling.

Screening tests based on inhibition of microbial growth are very sensitive tests, but because they are not specific for antibiotics, these methods are affected by the antimicrobial components present in milk.

From further investigations it was found that one of the two positive milk samples came from an animal with very many somatic cells (2850000). Not all antimicrobial components have been inactivated by boiling. Neutrophil diapedesis is known to cause components of the blood serum to pass through the breast epithelium. Among these components are listed some substances with lipolytic effect resulting in inhibitory free fatty acids or sodium that alter the electrical conductivity of the milk and, as a result, inhibit the development of the tissue microorganism.

In these situations, the microbial inhibition test used to identify positive milk samples should be accompanied by the use of the California Mastitis Test or the measurement of the electrical conductivity of the milk.

A rapid and massive influx of neutrophils occurs during the bacterial invasion. Neutrophils will be the predominant type of cells found in breast tissue and milk in the early stages of infection. Neutrophils have specific immune function and are important in reducing the number of

bacteria at the beginning of the disease through phagocytosis.

If the bacteria are able to maintain their numbers, the inflammatory process is maintained and there will be a dyspepsia containing leukocytes to the alveolar humen and in the milk.

The somatic cell count (SCC) in cow's milk is used to establish the presence of an infection in one or more breast quarters. A lot of studies have been done to establish a somatic cell count threshold to predict an infection. There is no fixed threshold as at any set threshold there will be quarters with infections below it and quarters without infections above it. The choice of a threshold is a compromise between sensitivity, i.e. the number of cows with infections that are infected from the total number of cows with infections, and specificity, i.e. the number of cows without infections that are identified from the total number of cows without infections.

The threshold of 200,000 somatic cells per ml of milk is usually used. At this threshold the sensitivity and specificity is about 80%. Around 80% of cows with infections have somatic cell count above 200,000/ml and 80% of cows without infections have somatic cell count equal to or below 200,000/ml. This threshold of 200,000 somatic cells per ml of milk can be for all the milk milked on the farm but also the threshold for each cow in the herd. In well managed herds a threshold below 200,000 somatic cells per ml of milk means that more than 90% of cows have SCC below 200,000 and less than 5% of cows have SCC above 200,000 at any control.

The California Mastitis Test (CMT) is used to detect mastitis during milking in cows. This test is based on the amount of somatic cell nuclear protein present in milk.

The test reflects the number of somatic cells as an indicator of the severity of mastitis infections.

The scale of assessment and its correlation with the number of somatic cells is presented in table no. 4.

Table 4 Correlations between the grading scale of the CMT and the somatic cell count

CMT grading scale	Symbol	Variation in somatic cell counts	Average number (approx)
Negative	N	0 to 200000	100000
Mild but precipitable precipitate (possible infection)	T	From 200000 to 500000	400000
Precipitate with a tendency to gelling (weakly positive)	1	From 500000 to 1500000	800000
Thick precipitate; gel with displacement towards the centre (distinctly positive))	2	From 800000 to 5000000	1600000-3200000
Gel everywhere with convex surface (strongly positive)	3	Exceeding 5000000	6400000

The second positive sample was due to the use of higher doses of penicillin than those specified in the product leaflet.

In this case, the elimination of the antibiotic was prolonged one day after the end of the waiting period. This is not surprising as the waiting period specified on the product is based on the doses specified in the package leaflet. Non-

compliance with these doses prolongs the elimination of residues in milk.

CONCLUSIONS

Residues of antibiotics have been detected in milk after the withdrawal period has expired when animals have been treated with drug overdoses.

Research is needed to see the true prevalence of overdose use in dairy animals.

When it is found necessary to use overdoses of a drug, replacing it with another antibiotic, used in the prescribed doses, could avoid the problem of drug residues in milk.

From a hygienic point of view, antibiotic residues and contaminants should have the lowest possible threshold. The maximum limit of milk residues guarantees the protection of the consumer including those at the end of the food chain (especially children) and offers protection from one link to another against accumulations that may occur in the human body.

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