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DETERMINATION OF RESIDUES OF ANTIBIOTICS IN RAW COW MILK AFTER THE WITHDRAWAL PERIOD - CAUSES AND AFTEREFFECTS IN THE MILK INDUSTRY

Ljubica Trajkoska^{1*}, Marija Runchevska¹, Filip Davkov¹, Natela Kordzaia²

***Undergraduate student (third year) Mentors:**

Biljana Trajkovska, Ljupche Kochoski

¹University "St. Kliment Ohridski", Faculty of Biotechnical Sciences–North Macedonia

²Odessa National Academy of Food Technologies – Ukraine

e-mail:ljubicaatrajkoska@gmail.com

Abstract: With the increase of the human population, the production of milk and dairy products is increasing in parallel. On the other hand, dairy animals are at a higher risk of infections every day, and the need for antibiotics to treat them is increased. Therefore, the purpose of this study was to determine the presence of residues from the most commonly used antibiotics on the farm before and after a withdrawal period in raw milk, in cows with a different clinical appearance of mastitis. From the results obtained, it can be concluded that tetracycline and macrolide are secreted up to 24h after the withdrawal period, while the penicillin excretion ends 12h before the withdrawal period. It is therefore inevitable to control the antibiotic residues even after the expiry of the withdrawal period, because in the case where antibiotic residues are present in raw milk after the withdrawal period, they can survive pasteurization and be present in pasteurized milk, even at high levels.

Keywords: raw cow milk, residues from antibiotics, withdrawal period

Introduction: At the farm level, antibiotics are still the primary tools for the treatment of infectious diseases (Priyanka., et al., 2017), especially for mastitis, the main consequence of which is the reduction in milk yield. The group of commonly used antibiotics includes β - lactam, fluoroquinolones, tetracycline, and macrolides (Serranino A., et al., 2013), whose use is permitted in accordance with the marketing authorization (Official Gazette of the Republic of Macedonia No. 22 / 2017). In order to eliminate the emergence of antibiotic residues in milk, milk producers need to respect the withdrawal period, i.e., the required period for the animal to metabolize the administered drug. During that period the level of the drug in the tissues and primary products would be reduced to a safe or acceptable level (Siljanoski A., et al., 2018). Accordingly, each antibiotic has a prescribed withdrawal period, which, if respected, would have an impact in obtaining the safer product, and in addition, technical problems in the dairy industry will be eliminated (TrajkoskaLj., et al., 2018).

Materials and Methods: For the purpose of this study 20 samples of raw cow milk were taken individually from cows previously treated with tetracycline, macrolides (administered

intramammary) and a combination of penicillin with fluoroquinolones (administered intramuscularly), depending on the clinical appearance of mastitis in individual cows, during the winter period, from farms in the Pelagonia region. The sampling dynamics for analysis included collecting 4 samples of raw cow's milk from each cow individually. The samples were collected at a different time interval, 12 hours before the expiration of the withdrawal period, on the day of the withdrawal period, 12 and 24 hours after the expiration of the withdrawal period. The analyses were done in duplicate, following the rapid test methods for antibiotic residues Beta Star (B.S.), Beta Star Combo (B.S.C) and the microbiological inhibitory test BRT Inhibition Test (B.R.T) (Chr. Hansen's., 2016).

Results and discussion: Beta Star and Beta Star Combo are the most commonly used fast and explicit tests (Trajkovska B. 2017), but the inability to detect antibiotic residues below the maximum tolerable limit (Fejzich N., et al., 2014) limits their application. Compared to them, although the BRT test produces qualitatively more accurate results, the longer analysis time leads to the failure of this test by milk producers (Kroll S., et al., 2000).

Table 1 - Results from samples of milk cleared by a cow treated with a combination of penicillin and fluoroquinolones

Number of sample	A sample of cow milk treated with	Sampling	B.S. (normal)	B.R.T
1	25 ml penicillin + 20 ml fluoroquinolones (i.muscular)	12h before the expiration of withdrawal period	+	+
2		On the day of the withdrawal period	/	-
3		12h after the expiration of the withdrawal period	/	/
4		24h after the expiration of the withdrawal period	/	/

"+" - positive, "-" - negative test

According to the results obtained in Table 1, it can be noted that on the day of the withdrawal period (72 hours after the intramuscular application of the combination of penicillin and fluoroquinolones) there are no residues from the applied therapy of penicillin. Our results are in accordance with the results from other studies where they showed that the 72-h withdrawal period is sufficient for elimination of penicillin to the acceptable level (Burnanczuk A., et al., 2017; TrajkoskaLj., et al., 2018). From Table 2 it can be noticed that tetracycline is excreted in milk even after the withdrawal period (eight milkings), which can further result in public health problems (Aalipour F., et al., 2015). In order to "prevent" their appearance, it is best and most effective to combine with other drugs (Siljanoski A., et al., 2018).

Table 2 - Results from samples of milk cleared by a cow treated with tetracycline

Number of sample	A sample of cow milk treated with	Sampling	B.S.C.	B.R.T
1	10 ml of tetracycline intramammary	12h before the expiration of withdrawal period	+	+
2		On the day of the withdrawal period	/	-
3		12h after the expiration of withdrawal period	/	-
4		24h after the expiration of withdrawal period	/	/
5	10 ml of tetracycline intramammary	12h before the expiration of withdrawal period	+	+
6		On the day of the withdrawal period	+	+
7		12h after the expiration of withdrawal period	+	+

8		24h after the expiration of withdrawal period	/	-
9	10 ml of tetracycline intramammary	12h before the expiration of withdrawal period	+	/
10		On the day of the withdrawal period	+	/
11		12h after the expiration of withdrawal period	+	/
12		24h after the expiration of withdrawal period	+	+

"+" - positive, "-" - negative test

The presence of tetracycline residues may also result from the way of administration. According to Oliver P.S., et al. (1990), antibiotics that are applied intramuscularly are excreted for a maximum of 72 hours after treatment, while those that are applied intramammary may appear in milk even after 120 hours after the treatment, which fully coincides with our results.

The results shown in Table 3 indicate the long half-life of macrolides. Such results are in correlation with the findings from Avci T. and Elmas M., (2014). In correlation with this, the concentration of macrolides in milk is very often higher than in plasma (Boothe M. D), especially in cows treated for mastitis.

Table 3 - Results from samples of milk cleared by a cow treated with macrolides

Numb er of sample	A sample of cow milk treated with	Sampling	B.R.T
1	macrolides (i.muscular)	12h before the expiration of withdrawal period	+
2		On the day of the withdrawal period	+
3		12h after the expiration of withdrawal period	+
4		24h after the expiration of withdrawal period	+

Results obtained in this study clearly shows that recommendations from the producers, for the withdrawal period, should be strictly followed because according to Moghadam M. M, et al., (2016) and Schlemper V. and Sachet P.A, (2017) in cases where antibiotic residues are present in raw milk after the withdrawal period, they can survive pasteurization and could be present in pasteurized milk even at high level. Precisely because of this, it is necessary to check the antibiotic residues after the expiration of the withdrawal period, because it can be prolonged as a result of the route of administration of the veterinary drug, multiple treatments of the animals and other factors (Siljanoski A., et al., 2018).

Conclusion: In the context of the above results, it can be concluded that even after the withdrawal period, the applied antibiotics in cows with a different clinical appearance of mastitis can be eliminated and it is advisable to test the milk for antibiotic residues even after the expiration of the withdrawal period. Tetracycline and macrolide residues had prolonged excretion as a result of the independent route of administration, while penicillin residues were eliminated before the withdrawal period.

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