

DETECTION OF TETRACYCLINE RESIDUES IN BOVINE MILK FOLLOWING MASTITIS TREATMENT

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The purpose of this study was to check tetracycline residues in milk from treated mastitic quarters. Milk from 35 cows, which were intramammary treated with a preparation containing tetracycline, was collected before and after the withholding period. Twinsenor, a competitive receptor-based test was used for quick detection of tetracycline residues. The positive samples were analyzed with LC-MS/MS. In none of the untreated quarters tetracycline residues were detected. In 40% of the milk from treated quarters tetracycline residues were above Maximum Residue Limit after the withdrawal period. In 11.4% the concentration of tetracycline was above the 700 µg/kg. The withholding period stated in the label of the medicinal product is determined in healthy animals and only for that particular medicinal product. During mastitis the conditions in the quarter are different, the production of milk usually decreases, and thus the excretion time can differ compared with a healthy animal. From the treatment records, we could see that sometimes the treatment was extended, always more than one antimicrobial product were used, and the withholding time was prolonged.

Key words: Tetracycline, milk, residues, mastitis

Introduction

The tetracycline group of antibiotics counts around 10 members of which oxytetracycline, chlortetracycline, and demethylchlortetracycline occur naturally. Chlortetracycline and oxytetracycline were the first members of the tetracycline group discovered in the 1940s as products of *Streptomyces aureofaciens* and *S. rimosus*. They exhibit broad-spectrum of activity against gram-negative and gram-positive bacteria, as well as some other microorganisms such as chlamydia, mycoplasmas, rickettsia, and protozoan parasites. Tetracyclines inhibit protein synthesis in bacteria by reversible binding to 30S ribosomal subunit, and specifically at the aminoacyl-tRNA acceptor ("A") site on the mRNA ribosomal complex preventing ribosomal translation. In veterinary medicine tetracyclines are used in cattle for treating general, respiratory, urinary, local infections and infectious mastitis. Intramammary route of application of tetracyclines is a frequent source of milk contamination. Tetracyclines are not recommended for children up to the age of 6-8 years and by pregnant women because of the risk of developing secondary tooth discoloration (Chopra I et al, 2001).

To prevent harmful health effects Maximum Residual Limits (MRLs) are established. According to Commission Regulation (EU) No 37/2010 on pharmacologically active substances the MRL for oxytetracycline (OTC), chlortetracycline (CTC), and tetracycline (TTC) in milk is set to 100 µg/kg.

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Material and methods

Milk samples from 35 treated mastitic cows in lactation were collected from various farms in the following manner:

- one milk sample before the beginning of the treatment for determination of the type of bacteria,
- after the treatment samples were taken two milkings before and two milkings after the withdrawal period set by the attending veterinarian,
- from each milking one sample was collected from the treated mastitic quarter and one from an untreated healthy quarter.

Total of 9 samples were collected per cow. The samples for antibiotic analysis were kept at -20°C until analysis. The sample for determining the agent was kept at 4 – 8°C and analyzed within 24 hours.

The attending veterinarians provided us with information about the treatment of the animal.

The samples for determination of the cause of mastitis were plated on blood agar and incubated at 37°C. Determination of the agent was done the next day with Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF).

All milk samples were analyzed first with Twinsensor kit, a competitive receptor-based test, for rapid detection of tetracycline residues. The sensitivity limit of the test was 80-100 µg/kg for tetracycline. All milk positive samples after withdrawal period, together with the samples before the withdrawal period, were analyzed with Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS) for tetracycline identification and quantification.

Results

In the milk samples from the untreated quarters tetracycline were not found. In 18 out of 35 cases (51,4%) milk samples from treated mastitic quarters contained tetracycline after the withdrawal period. But in only 14 cases (40,0%) MRL was exceeded.

Discussion

Mastijet Fort was the only medicinal product used by the veterinarians that contained tetracycline. It is composed of: tetracycline (200 mg), neomycin (250 mg), bacitracin (2000 IU) and prednisolone (10 mg). Its recommended withdrawal period is 96 hours or 8 milkings and its recommended dosage is one injector per quarter every 12 hours, with a maximum of four treatments. From the treatment records that we received from the attending veterinarians we could see that Mastijet Fort was sometimes used 6 treatments in a row. However, Mastijet Fort was never applied alone, it was usually administered in a combination with other products. According to the European Medicine Agency guidelines, "Time To Safe Concentration" is used for the determination of withdrawal periods. Nonetheless, prolonged usage of preparations or increased doses during mastitis is not taken into consideration (EMEA/CMVP/473/98-final).

In our cases different, longer withdrawal periods ranging from 4 to 7 days (9 – 14 milkings) were prescribed. In 14 cases the MRL was exceeded in the milk from treated quarters after the withdrawal period of 10, 12, 13, and 14 milkings.

If we take into consideration the concentration of tetracycline in the total milk volume from one animal, the concentration would decline due to the dilution effect with the milk from the other untreated quarters. Thus, it is expected the final concentration of tetracycline to reach probably level below the MRL. Hypothetically, if one quarter is treated, the final concentration of the drug in the total milk volume from a cow would be 25% regarding the milk from the untreated quarter. Considering this calculation, only in 4 cases MRL was exceeded. The concentrations of tetracycline in milk from the treated quarters after the withdrawal period were 701, 718, 749, 750 µg/kg, and the corresponding quantities in the milk from all four quarters were 175, 179, 187, and 187 µg/kg, respectively. The prescribed withdrawal periods were 12, 13, 12, and 10 milkings, respectively. In three of these cases milk production dropped. The presence of tetracyclines in milk in Czech Republic was reported by Navratilova. Tetracycline has been detected in all bul tanks and tanker trailers. However, the concentration of tetracycline in all samples was below the MRL (Navratilova P et al, 2009).

There are few articles that state prolonged excretion of antibiotics in milk from treated cows with mastitis. During mastitis physical and chemical changes in the milk and in the mammary gland occur, which have the potential to alter the distribution of intramammary drugs. The milk-duct system may also be compressed in an extremely swollen udder or obstructed by inflammatory product, thus resulting in poor or uneven drug distribution (Gehring R et al, 2006).

References

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