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Research Article

The Evaluation of Oxytetracycline Residues in Cow's Milk in Tetovo, Macedonia

Biotechnology and Food

Keywords: oxiteteracicline, cow, milk, Macedonia.

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Abstract

Oxiteraciclines are antibiotics widely used in treatment of cattle against many infections caused by deferent gram-positive and gram-negative bacteria.This research was focused on qualitative and quantitative detection of oxyteteracycline residues in cow's milk produced in different rural areas of Tetovo in Macedonia. 138 milk samples were collected from 60 milking cows' farms and are checked for oxitetracicline residues using qualitative ELISA test. From this control are found positive samples in 5, 8% (8/138) of milk samples. All positive samples are confirmed were by high performance liquid chromatography (HPLC) method in Institute of Food Safety and Veterinary in Tirana. Analytical control carried out using HPLC resulted that only 6 /138 or 4.3% were positive for oxytetracycline residues. Ths evaluation of oxiteteracixline residues with HPLC was carried out confirming following: 90ug/l, 180ug/l, 200ug/l, 4000 ug/l, 1600 ug/l and 1400ug/l (ppb) Al positive results are confirming treatment of milking cows with high doses of oxytetracycline so the level of oxiteteracicline in the cow's milk remain problematic.

Introduction

The group of oxitetracycline is commonly used in treatment of animal infection. As antibiotic with broad spectrum it is used for treatment of human and animal infections. Oxitetracycline are used in lactating and non-lactating dairy cattle for the treatment of bacterial infections. This antibiotic stops the spread of the infection and the remaining bacteria are killed by the immune system or eventually die (Bishop et al., 1994).

However, some strains of bacteria have developed resistance to this antibiotic, which has reduced its effectiveness for treating some types of infections (Grave et al., 1993).

Even thought the advantages of treatment the oxyteteracycline residues are having adverse effects on people allergic to antibiotics, potential buildup of antibiotic-resistant organism in humans and inhibition of starter cultures used to produce cultured milk products such as yogurt and cheeses (Alica et al., 2003). One of main cause for having residues in cow s' milk is treatment of metritis and other genital infections which have the incidence ranged from 10 to 15 % (Lewis et al, 1997).

Commonly observed disease conditions recorded in the dairy farms are mastitis in average incidence more the 48.2%. From some authors are reported that 10.3 % of infections in farms have as cause enteritis, even other types of diseases were recorded in 16.2% of farms (Alica et al., 2003). The other disease conditions such as dystocea, retained fetal membrane, metabolic problem and foot problem recorded in dairy farms in 18%. More than 50% of the farmers interviewed utilized oxytetracycline, pinstripe and multiject antibiotics respectively. Only 5.9% of the farmers were aware of dry cow therapy for controlling mastitis (Forst et al., 1991). On 16-70% of the dairy herds surveyed, veterinarians, assistants and owners themselves were used to administer antibiotics. Administration of antibiotics was accomplished using the routes of intramuscular, intramammary, intrauterine and perous on 52.9%, 30.9%, 8.8% and 14.7% of the farms respectively (Grave et al., 1999). For above reason oxitetraxicline in milking cows is commonly used and risk of residues of this group of antibiotics is higher. One of main cause of having residues in cow s' milk is treatment of metritis and other genital infections with incidence ranged from 7% to 15 % (Lewis, 1997).

For these reasons it is important effectively control of antibiotic residues in milk and therefore, regulatory authorities have enacted maximum residue limits (MRLs) for oxytetracycline in milk. Regulatory limits protect consumers from over-exposure to oxytetracycline above maximum residue limit (MRL) of 100ug/kg milk. Many countries have introduced in national residues monitoring programs the control for residues of oxytetracycline in cow s' milk used for human consumption. On this context are Republic of Macedonia is realizing each year national residues monitoring plan according to EU specific legislation for residues control. Detectable concentrations of antibiotic residues in milk supplies higher than the MRLs are not allowed to be used to by consumers. Regulatory limits protect consumers from over-exposure to oxytetracycline above maximum. Concerning to public health treatment with oxyteteracycline residues are causing adverse effects on people exposed to this kind of antibiotics.

Materials and method

Study was focused in 20 cattle farms located in Tetovo district in Macedonia.138 raw milk samples were collected in 2014 from different dairy farms in Tetovo. After collection of milk samples from different locations of cattle farms they were kept in the refrigerator (4°C) and analytical procedure was performed within two days.

Use of ELISA for qualitative detection

For detection of oxytetracycline residues in raw milk samples collected from milking cows in Tetova are used MaxSignal® Oxytetracycline ELISA Test Kit as competitive enzyme immunoassay for the qualitative and quantitative analysis of milk samples. The method based on a competitive colorimetric ELISA assay. During the analysis, sample is added along with the primary antibody specific for the target drug. If the target is present in the sample, it will compete for the antibody, thereby preventing the antibody from binding to the drug attached to the well. The secondary antibody, tagged with a peroxidase enzyme, targets the primary antibody that is complexed to the drug coated on the plate wells. The resulting color intensity, after addition of substrate, has an inverse relationship with the target concentration in the sample. Use of MaxSignal® Oxytetracycline ELISA Test is performed according to instruction closed to kit box.

HPLC analysis for qualitative detection of positive samples

All positive samples confirmed by MaxSignal® Oxytetracycline ELISA Test are tested with HPLC to quantify the residue of oxytetracycline.

To perform HPLC procedure are used chemicals and material: Acetonitrile and methanol were of HPLC grade; oxalic acid dihydrate Suprapur and Na2HPO4 heptahydrate; ethylene diamine tetraacetic acid (EDTA) disodium salt, citric acid monohydrate (Thermo Fischer Scientific) were of purity grade. Solid phase extraction (SPE) column Oasis HLB, 3 cc, 60 mg was purchased from Waters (Milford, USA). The vacuum unit for SPE was purchased from Supelco. The other hardware included an analytical balance (Kern, Balingen, Germany), a cooling centrifuge (Mechanika Precyzyjna, Poland), and a rotary vacuum evaporator (Bűchi, Flawil, Switzerland), (Petkovska et al., 2006). For the qualitative and quantitative evaluation, the external standard method was used. Each sample was analyzed in duplicates way at the least, every series containing a blank sample. Simultaneously, aliquots of the milk samples with the addition of standard solutions of known concentrations were measured. The detection and quantization limits were established based on the standard deviation of the blind test and the slopes of the calibration curves, repeatability was based on 20 parallel determinations and the recovery was based determinations of the milk sample with the addition of the solution of standards of known concentrations (50μg/l and 100μg/l). Basic statistical processing was done using the Unistat software, Version 5.1 (Unistat Ltd. 1998).

Results and discussion

Table 1. Milk samples collected in dairy farms and positive case of oxytetraxycline residues from 2011-2014 in Tetovo, Macedonia.

No. milk samples	Detection of oxitetraxycline residues by ELISA (%)	Detection of oxitetraxycline residues with HPLC (%)	Above MRL (100 μg/L) in %
138	5.8% (8/138)	4.3% (6/138)	3.6% (5/138)

In our study detection of of oxitetracycline residues in milk samples was achieved by use of ELISA test which is able to find out if the oxytetracycline residue is present or not. Even thought use of the MaxSignal® Oxytetracycline ELISA for quantitative evaluation of oxytetracycline residues, many of studies recommended it only for qualitative detection. All positive cases were performed with HPLC to quantify the level of oxytetracycline residues. The antibiotic residues of positive samples showed residues oxytetracicline above MRLs in 5/138 or 3.6%. The positive milk samples were checked by HPLC for quantification of oxitetraxycline. A given sample was regarded as positive for oxytetracycline if its retention time and peak corresponded to that of the standard. Retention time was considered a reasonably unique identifying characteristic of a given samples (Ding and Mou; 2000; Cinquina et al., 2003). The area inscribed by the peak isproportional to the amount of substance separated in the chromatographic system. To get the concentration of oxytetracycline, a reference standard of a known concentration had been injected in to the HPLC and concentration of the sample was extrapolated from the curve peak area. Studies in Europe carried out for detection of oxytetracycline residues in raw milk produced by milking cows reported values of incidence from 0, 2 % to 2, 7 % (Grave et al., 1999; Allara et al., 2001). There are many studies confirming the low incidence from 0, 001%- 3.5%. In some cases in Germany and USA the incidence of level of oxytetracycline was higher than 5% and the cause was attributed the genital infections in milking cows (Heeschen et al., 1996). Comparing of chromatograms of reference standards, oxytetracycline HCl and some samples those were positive for oxytetracycline from the dairy farms were performed in these study to detect level of this antibiotic in milk. The range for oxytetracycline residue level was 0μg/l to 2400μg/l (Grave et al., 1999).

Conclusions

Analytical control in milk samples performed with MaxSignal® Oxytetracycline ELISA confirmed oxytetracycline residues in dairy farms in Tetovo in Macedonia. Study results Oxytetracycline residues were present in 5.8% of total samples. Instrumental check with HPLC confirmed oxytetracycline residues above MRLs in 5 samples or 3. 6%. Confirming values of oxytetracycline residues above the MRL are signalizing the risk for consumers in this area.

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