Control for Oxytetracycline Residues in Cow's Milk in Tetovo, Macedonia

Veterinary Medicine

Keywords: residues, oxyteteracycline, milk, cows, Tetovo.

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Abstract

The analytical control for qualitative and quantitative oxyteteracycline residues evaluation in cow's milk was performed in 2012 in cattle farms in Tetovo, Macedonia. The fresh cow's milk samples were collected from 43 milking cows' farms in Tetovo. This research was focused on detection of oxatetracycline residue in 123 fresh milk samples using ELISA test for qualitative evaluation. After this check positive samples were frozen and analyzed with high performance liquid chromatography (HPLC) method for qualitative evaluation of oxytetracycline residues. The control carried out by ELISA test confirmed that 3.2% (4/123) of raw milk samples were positive for oxytetracycline residues. The qualitative evaluation was carried out in all positive milk samples using HPLC. Positive samples had following values of quantity in 1 liter milk: 60ug/l, 120ug/l, 200ug/l, 1100ug/l (ppb). Regarding to positive results confirmed treatment of milking cows with high doses of oxytetracycline in Tetovo remaining a serious risk for consumers.

Introduction

As antibiotic with broad-spectrum of tetracycline, oxytetracycline works by interfering with the ability of bacteria to produce proteins that are essential to them. 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). 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). Oxytetracycline as broad-spectrum veterinary antibiotic is used to treat many animal infections caused by pathogen bacteria. It is used in lactating and non-lactating dairy cattle for the treatment of bacterial infections such as enteritis, pneumonia, diphtheria, infections infection caused by chlamydia, genital infections, urethritis and other infections in animals. Regulatory limits protect consumers from over-exposure to oxytetracycline above maximum residue limit (MRL) of 100ug/l milk (Lewis, 1997; Alica et al., 2003). Oxyteteracycline treatment of different infections remains a very effective way to control many infections in milking cows. Concerning to public health treatment with oxyteteracycline residues are causing adverse effects on people exposed to this kind of antibiotics. Uses of this antibiotic build up antibiotic-resistant organism in humans and cause inhibition of starter cultures in processed milk products such as yogurt and cheeses (Alica et al., 2003). On this context effectively control of oxitetracycline residues in milk is necessary and therefore, regulatory authorities have enacted maximum residue limits (MRLs) for oxytetracycline (EMEA; 1995). Many countries set up national residues monitoring programs the control for residues of oxytetracycline in cow s' milk used for human consumption. The Republic of Macedonia is realizing each year national residues control 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. 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). The most commonly observed diseases recorded in the dairy farms are mastitis in average incidence until 30% (Alica et al., 2003). According to the results of other studies disease conditions such as dystocea, retained fetal membrane, metabolic problem and foot problem recorded in dairy farms are having the incidence above 15%. Other authors are reporting that more than 50% of the farmers interviewed utilized oxytetracycline, and only 5% of the farmers were aware of dry cow therapy for controlling mastitis (Forst et al., 1991). Administration of antibiotics using the routes of intramuscular, intramammary, intrauterine and peros in 52.9%, 30.9%, 8.8% and 14.7% of the farms respectively (Grave et al., 1999). For above reason oxitetraxycline in milking cows is commonly used and risk of residues of this group of antibiotics is higher.

Materials and method

Study was focused in 43 cattle farms located in Tetovo district in Macedonia. 123 raw milk samples were collected in 6 months 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 is 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 vacum 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 in 2012 in Tetovo, Macedonia.

No. farms	No. milk samples	Detection of oxitetraxycline residues with HPLC (%)	Above MRL (100 μg/L) in %
43	123	3.2% (4/123)	2.4% (3/123)

Table 2. Qualitative evaluation of raw cow s' milk samples confirmed positive by HPLC

Milk samples	Qualitative evaluation by HPLC
1.West part of Tetovo	80 ug/l
2.Central part of Tetovo	120 ug/l
3.South part of Tetovo	200 ug/l
4.West part of Tetovo	1100 ug/l

The control of raw cow s' milk samples with ELISA found 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. In our study we use this kit to dectect the presence of oxitetracycline in milk. Then all positive cases were performed with HPLC to quantify the level of oxytetracycline residues. The antibiotic residues of positive samples which showed residues oxytetracicline above MRLs were 3/1123 or 2.4%. Oxytetracycline was found in all positive samples in a concentration from 80-1100μg/l. Levels of oxiteteracycline in three positive milk samples above MRL were respectively 120ug/l, 200ug/l and 1100

ug/l. The positive milk samples were analyzed by HPLC for oxitetraxycline quantification. 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, 01%- 1.5%. 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 1600µg/l (Grave et al., 1999).

Conclusions

The oxytetracycline residues of milk samples collected in dairy farms in Tetovo in Macedonia showed positive cases of oxytetracycline in 3. 2 % of total samples confirmed by MaxSignal® Oxytetracycline ELISA Test. Performing analytical control with HPLC oxytetracycline residues above MRLs were confirmed in 3 samples or 2. 4%. The most of milk samples originated from west part of Tetova confirming values of incidence of oxytetracycline above the MRL and the risk of oxytetracycline residues in cow s' milk produced in this area.

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