Detection of oxytetracycline residues in milk of cows in rural area in Tetovo, Macedonia

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Summary

Our study was focused on detection of oxytetracycline residues in milk cows produced by cows reported with retained fetal membranes. Oxytetracycline is antibiotic routinely used in an extra-label manner for intrauterine therapy. Our research evaluated level and presence of oxytetracycline residues in milk from cows collected from rural areas of Tetovo, Macedonia. There are many cases with retained fetal membranes diagnosed in cows by veterinary doctors and treated with oxytetracycline once a day with 5 g of oxytetracycline by intrauterine infusion for at least 2 days, or until the membranes were expelled. 161 milk samples were collected during 2010 at rural areas of Tetovo in Macedonia in ten cow farms. All samples were frozen and submitted every 4 weeks for high performance liquid chromatography (HPLC) for oxytetracycline analysis in Institute of Food Safety and Veterinary in Tirana. Oxytetracycline was detected in milk of cows at the incidence 3,1% (5/161) and 2 milk samples 1,24% (2/161) had a maximum concentration ranging from 100 to 1,600 ug/kg (ppb). There are studies confirming treatment of milk cows with retained fetal membranes by intrauterine infusion of oxytetracycline which may contain the drug for variable lengths of time. Milk should be discarded to avoid illegal residues of this antibiotic and appropriate withdrawal measures should be taken by food and veterinary inspectorate.

Key words: residues, oxytetracycline, milk, cows, Tetovo.

Introduction

Residues cause 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). For these reasons it is important effectively control of antibiotic residues in milk and therefore, regulatory authorities have enacted maximum residue limits (MRLs) for a number antibiotic agents in milk (EMEA; 1995). National monitoring programs to control the veterinary drug residues in various animals and their products, including milk, are compulsory in all EC countries and Macedonia (Council directive 96/23 EC).

Detectable concentrations of antibiotic residues in milk supplies higher than the MRLs are illegal. On this context, oxytetraxicline is a broad spectrum antibiotic that is effective against a wide variety of bacteria. However, some strains of bacteria have developed resistance to this antibiotic, which has reduced its effectiveness for treating some types of infections (Heeschen et al., 1996; Lee, et al., 2000). Oxytetraxicline still used to treat infections caused by chlamydia and those caused by mycoplasma (eg pneumonia), Gram negative and Gram positive bacteria and. Oxytetraxicline is used to treat the skin infection that cause acne (Propionebacterium acnes) (Lewis, 1997; Lee, et al., 2000). It is also used to treat chronic bronchitis, due to its activity against bacteria that are usually responsible. Oxytetraxicline can also be used to treat other rare infections, such as those caused by a group of organisms called
riketie (eg Q fever). To ensure that the bacteria that cause infection are susceptible to oxytetracycline diagnostic test are carried out (Petkovska et al., 2006).

Commonly observed disease conditions recorded in the dairy farms are mastitis in average incidence 38.2%. Other infection such metritis are evaluated to have the incidence ranged from 12- 17 % (Lewis et al, 1997). From some authors are reported that 10.3 % of infections in farms have as cause enteritis. 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 16.2%.

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 dairy cows is commonly used and risk of residues of this group of antibiotics is higher.

Materials and method

Study was focused in 10 dairy farms located in Tetovo district in Macedonia. 161 raw milk samples were collected every month from different dairy farms in Tetovo. Attention was paid to collection of milk samples analyzed were of different farms from different locations. Milk samples were kept in the refrigerator (4°C) until analysis and were analyzed within two days at most.

HPLC analysis:

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 (Buchi, 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 quantitation 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 oxytetraxicline residues in 2010 in Tetovo, Macedonia.

<table>
<thead>
<tr>
<th>No. farms</th>
<th>No. milk samples</th>
<th>Detection of oxitetraxicline residues with HPLC (%)</th>
<th>Above MRL(100 μg/L) in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>161</td>
<td>3.1% (5/161)</td>
<td>1.24% (2/161)</td>
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</table>
Milk samples before analytical process was tested with ELISA and all positive cases were performed with HPLC to quantify the level of oxytetracicline residues. The antibiotic residue positive samples which showed residues oxytetracicline above MRLs were 2 (1.4%). Oxytetracycline was found being present in all positive samples in a concentration range of 27-1600μg/l. Levels of oxiteteracycline in tow positive milk samples above MRL were respectively 290 μg/L and 1600 μg/L.

The positive milk samples were analyzed by HPLC for oxitetraxicline 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. Some authors are reported the incidence 1, 4-2, 7% in Europe (Grave et al., 1999; Allara et al., 2001).

There are many studies confirming the low incidence from 0, 01%- 1. 5%. In some specific cases the incidence of level of oxytetracycline was higher than 1. 8%. Comparing of chromatograms of reference standards, oxitetracycline HCl and some samples those were positive for oxitetracycline 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). The antibiotic residue positive samples which showed residues of oxytetracycline were 5 (3.1%).

**Conclusions**

The oxytetracycline residue of milk samples collected in dairy farms in Tetovo, Macedonia showed residues of oxytetracycline in 3.1% of total samples. Oxytetracicline residues above MRLs were confirmed in 2 samples or 1.4%. Even though the values of incidence regarding to level of oxytetracycline above the MRL the risk of oxytetracicline residues by consumption of cow milk remains higher.

**References**


