Global Veterinaria 6 (6): 527-529, 2011
ISSN 1992-6197

Determination of Tylosin Residues by ELISA in Pasteurized Milk Marketed in Tabriz

Amir Reza Karamibonari and Mohammad Hosein Movassagh

Department of Veterinary Medicine, Shabestar Branch, Islamic Azad University, Shabestar, Iran

Abstract: Antibiotic residues may lead to allergic reactions in sensitive consumers and induce the resistant of bacteria in consumers. Tylosin is widely used to treat infectious disease of cattle in field. The study was carried out to analyze tylosin residues in pasteurized milk from Tabriz city. A total of 90 samples of commercial pasteurized milk were collected from various markets in Tabriz and were analyzed by enzyme-linked immunosorbent assay (ELISA) technique. Results showed that in12 samples (17.6%) tylosin concentrations were between 3 and 10 ng/ml and in 35 samples (51.5%) tylosin was found between 10 and 30 ng/ml and in 21 samples (30.8%) tylosin was not detected. The antibiotic levels detected were below the limit permitted by the European Union (EU).

Key words: Tylosin · Residues · Pasteurized Milk · Tabriz

INTRODUCTION

Antibiotics are used in veterinary practice for the treatment of bacterial disease. They are also used for prophylactic and prevention purposes. Mastitis is a common disease in dairy cows which requires antimicrobial treatment [1]. Antibiotics are also used as feed additives in order to enhance feed efficiency [2]. In the last decade, improper use of these drugs has expanded risk of bacterial resistance to antibiotics. This risk situation may spread from animals to humans through the food chain (meat, milk, etc.), leading to the failure of treatment programs in human infections. Antimicrobial residues induce allergic reactions and alter the intestinal flora in some consumers. Also, the presence of antibiotic residue in milk can cause problems for producers because these residues may inhibit bacterial fermentation process [3]. Tylosin is a macrolide antibiotic structurally related to Erythromycin that is active against certain Geram-positive and Geram-negative bacteria, especially different members of Mycoplasma spp. It is produced from *Streptomyces fradiae* and it is used clinically in cattle for infections caused by susceptible organisms. This drug is slightly soluble in water and soluble in alcohol. Tylosin is considered highly lipid soluble and it is widely distributed in body fluids and tissues. The ability of Tylosin to pass through the biological membranes is facilitated by low/moderate plasma protein binding. To prevent the negative effects of the antibiotic residues on human health and on the entire ecosystem, procedures for the establishment of maximum residue limits (MRLs) of veterinary drugs in foodstuffs of animal origin are adjusted by European Council (EC) Regulation no. 2337/90. Some analytical methods have been developed in order to control the presence of antibiotics in food and feed samples such as: 1- microbiological approaches based on bacterial growth inhibition; 2- immunochemical techniques; 3-chromatographic methods. Microbiological tests are frequently used for screening. Chromatographic techniques include high performance liquid chromatography (HPLC) - UV spectroscopy and /or HPLC- mass spectometery (MS), usually used to confirm the results of samples suspected of being contaminated. These methods have high specificity and excellent detectability and are also used for the determination of drug residues concentrations [4, 5]. Immunochemical methods can offer important advantages as screening methods owing to their simplicity, high- throughput capabilities and low cost. Enzyme - linked immunosorbent assay (ELISA) test is an example of the methods most commonly used to detect antibiotic contaminants in milk samples [6].

Corresponding Author: Amir Reza karamibonari, Department of Pharmacology and Toxicology,
Faculty of Veterinary Medicine, Shabestar Branch, Islamic Azad University, Shabestar, Iran.
Tel: +989144172698, E-mails: pharmakarami@yahoo.com & amirkarami@iaushab.ac.ir.

To date, published information on the status of Tylosin residue in pasteurized milk in Tabriz is unavailable. The present study was therefore conducted to investigate the presence of Tylosin residue in pasteurized milk samples marketed in Tabriz city by I'screen Tylosin test.

MATERIAL AND METHODS

A total of 90 samples of commercial pasteurized milk were purchased from supermarkets of Tabriz city. Approximately 15 ml. of pasteurized milk was aseptically collected into sterile plastic containers and transported to the laboratory ice-cooled, within 1h. All samples were kept in the fridge and were analyzed before their expiration date. Milk samples were centrifuged for 10 minutes at 3000×g and fat on the top discarded.

ELISA Kit: Determination of Tylosin was based on an ELISA, using Enzyme immunoassay test kit (Tecna, Trieste, Italy). The code number was AB620. Each test kit contained a 96 - well microtitre plate (12 strips of eight wells, breakable in single wells) coated with anti-Tylosin antibodies, standard Tylosin: 1 plastic vial containing 0.2 ml of 9 mg/ml of Tylosin, enzyme conjugate (0.2 ml), enzyme conjugate diluent (12ml), washing - buffer10X (50 ml), developing solution (15ml), dilution buffer 10X (50ml), stop solution(9ml).

Preparation of the Milk Samples: Preparation of samples was conducted according to the instruction of the Enzyme immunoassay test (Tecna) kits for Tylosin. The milk samples were diluted (1/10) in the dilution buffer 1x.

Elisa Procedure: Before use, all reagents were located at room temperature. Standard solutions containing 0, 0.5, 1, 3, 10 and 30 ng/ml were prepared from the stock solution of Tylosin (9mg/ml).

RESULTS AND CONCLUSION

The standard curve for Tylosin is given in Figure 1. The absorption is inversely proportional to the drug concentration in the sample. The calibration curve was found to be virtually linear in the 0/5 - 30 ng/ml range.

The detection limit was 5ng/ml. The occurrence of Tylosin concentration obtained presented in Table 1.

Table 1: The occurrence of Tylosin concentration

Range of Tylosin concentration (ng/ml)	Pasteurized milk
Not detected	21 (30.8)
0.5-3	0
3-10	12 (17.6)
10-30	35 (51.5)
>30	0
Exceeding EU Commission maximum residue levels	0
Range (0-23)	

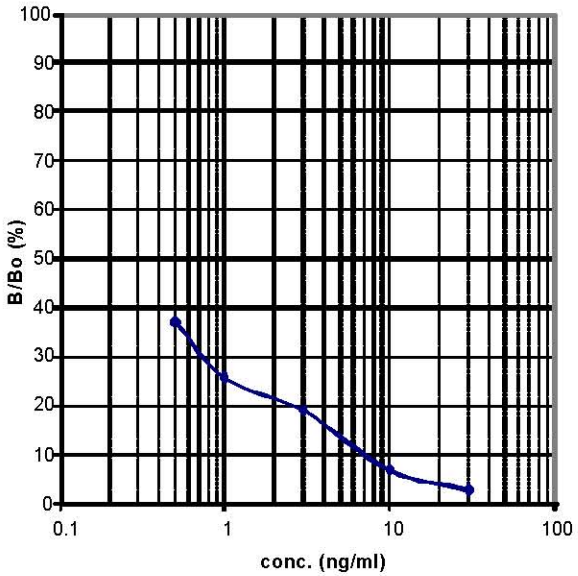

Fig. 1: Calibration curve of Tylosin, Absorbance (%) versus concentration (ng/ml)

Data Presented as N (%): Analysis of 68 samples showed that 12 (17.6%) samples contained 3-10 ng/ml, 35(51.5%) samples contained 10-30 ng/ml and in 21(30.8%) samples Tylosin was not detected. There was a high incidence rate of Tylosin with 43 milk samples (63.2%) being contaminated. The mean value of Tylosin concentration in positive samples was 12.41 ± 4.2 ng/ml. In the (European Union) EU, the maximum residue limit of Tylosin for milk is 50 ppb [7, 8]. The antibiotic levels detected were below the limit permitted by the EU. Since this is the first report of Tylosin residue in pasteurized milk in Tabriz (northwest of Iran), it can be followed by other researches in all parts of the country. Different sensitivities of detection systems have influenced the reported incidence of antibiotic residues in milk [9]. Variable incidence of antibiotic residues in milk has been reported for different countries; In 2010, Ergin Kaya *et al.* [10] showed that of all samples, 1.25 % was positive for antibiotic residues in Turkey. Kim *et al.* [11] reported that in 478 raw milk samples, 5% was positive for antibiotic residues in Korea. In 1988 two studies showed the

incidence of antibiotic residues in milk was 63%- 85% [9]. In a study by Ceyhan and Bozkurt, from a total of 200 milk samples collected around Ankara region, 5.5% of all samples were reported as penicillin positive [12]. Sanli *et al.* [10]analyzed 89 milk samples in Turkey and found 6.9% to be contaminated with chloramphenicol.

In 2009, Unusan showed that the incidence rate of chloramphenicol and tetracycline in milk samples were 46.8% and 66.8% respectively in Turkey [13].

In 2005, Tolentino *et al.* showed the percentage of positive samples sulfonamide residues in four milk brands of Mexico were 47.2% (Milk A), 58.3% (Milk B), 44.7% (Milk C) and 50% (Milk D) [14]. There are little published data on tylosin residue in animals. In cows that received intramuscular injections of tylosin at a dose of 10 mg/kg b.w. once daily for three days, at the end of the treatment, residues was found in one sample at the morning milking (60 mg/kg) and was below the limit of quantification (50 mg/kg) in all samples taken at the afternoon milking [5].

In the present study, tylosin residue in milk was more than that reported in other countries and the incidence of tylosin residue was higher than most of the other antibiotics but all of the samples were below the maximum acceptable limits (50ppb) of the EU.

Antimicrobial residues can induce allergic reactions and may induce resistant populations of bacteria in consumers of contaminated milk. To avoid many unwanted results, farmers must be trained for the correct and proper use of medication and, veterinarians must continuously monitor the livestock.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support of Shabestar Branch, Islamic Azad University. This work is a part of the research project (Research project code. 51955890225002).

REFERENCES

1. Althaus, R.L., A. Torres, A. Montero, S. Balasch and M.P. Molina, 2003. Detection limits of antimicrobials in ewe milk by delvotest photometric measurements. J. Dairy Sci., 86: 457-463.
2. Kantiani, L., M. Farre and OD. Barcel, 2009. Analytical methodologies for the detection of b-lactam antibiotics in milk and feed samples. Trac-Trend Anal Chem., 28: 729-744.
3. Sierra, D., A. Sánchez, A. Contreras, C. Luengo, J.C. Corrales, C.T. Orales, *et al.*, 2009. Detection limits of four antimicrobial residue screening tests for β-lactams in goat's milk. J. Dairy Sci., 92: 3585-3591.
4. Plumb, D.C., 2008. Plumb's veterinary drug handbook. Blackwell Publishing, pp: 913.
5. Lewick, J., 2006. Tylosin a review of pharmacokinetics, residues in food animals and analytical methods. [viewed 2011 May 23]. Available at: ftp:// ftp.fao.org/ ag/ agn/ food/ tylosin_2006.pdf.
6. Adrian, G., D.G. Pinacho, B. Granier, J.M. Diserens and F. Sánchez-Baeza, 2008. A multianalyte ELISA for immunochemical screening of sulfonamide, fluoroquinolone and ß-lactam antibiotics in milk samples using class-selective bioreceptors. Anal. Bioanal. Chem., 391: 1703-1712.
7. Nisha, A.R., 2008. Antibiotic Residues - A Global Health Hazard. Veterinary World, 1: 375-377.
8. European Medicines Evaluation Agency (EMEA), 2002.Tylosin (Extension to all food producing species). EMEA/ MRL/829/02- FINAL 2002. [viewed 2011 May 23]. Available: http: www.ema.europa.eu/ pdfs/vet/mrls/073200en.pdf.
9. Adesiyun, AA., LA. Webb and V. Balbirsingh, 1997. Prevalence of antimicrobial residues in preprocessed and processed cows' milk in Trinidad. J. Food Safety, 16: 301-310.
10. Kaya, S.E. and A. Filazi, 2010. Determination of antibiotic residues in milk samples. Kafkas Univ Vet Fak Derg, 16: S31-S35.
11. Chang- Soo, K., L. Sung-Kwon, K. Tae- Oh and K. Sin -Il, 1996. Determination of sulfonamide residues in raw milk from southern Kyeonggi area. Korean J. Vet. Serv., 19: 39-45.
12. Ceyhan, I. and M. Bozkurt, 1987. Ankara piyasasdnda satdlan sütlerde penisilin araştdrmasd. Türk Hij. Den. Biyol. Derg., 44: 1-5.
13. Unusan, N., 2009. Occurrence of chloramphenicol, streptomycin and tetracycline residues in ultra-heat- treatment milk marketed in Turkey. Informa, 60: 359-364.
14. Tolentino, R.G., M.N. Perez, G.D. Gonzalez, S.V.Y. Leon, M. Gonzalz and G.P. Flore, 2005. Determination of the presence of 10 antimicrobial residues in Mexican pasteurized milk. Interciencia, 30: 291-294.