Detection and determination of Oxytetracycline and Penicillin G antibiotic residue levels in bovine bulk milk from Nazareth dairy farms, Ethiopia

Desalegne Abebew [1] Kelay Belihu[2] and Girma Zewde [2]*

[1]Wollo University, School of Veterinary Medicine, Dessie, P.O. Box 1145, Ethiopia.
[2]Addis Ababa University, School of veterinary medicine; Debre Zeit, P.O.Box 34, Ethiopia

**Corresponding author: Wollo University, School of Veterinary Medicine, Dessie, P. O. Box: 1145, Email: dabebew@gmail.com*

Abstract

A cross-sectional study was conducted between October 2007 and May 2008 to detect and determine oxytetracycline and penicillin G residue levels in bulk milk of cows in Nazareth dairy farms. A total of 400 bulk milk samples were randomly collected. All samples were qualitatively screened for antibiotic residues by Delvotest SP assay. Questionnaire survey was carried out by personal interviews with some of the dairy farm owners in Delvotest positive farms (cases) and Delvotest negative farms (controls) to identify various risk factors for the occurrence of antibiotic residue in milk. Farm management practices, disease conditions and treatment factors have been identified as the main risk factors. Concentration of Oxytetracycline and Penicillin G in the positive samples was determined by High Performance Liquid Chromatography. Out of 400 samples analyzed for antibiotic residue, 48 (12 %) milk samples were positive for antibiotic residues. The mean residue level of oxytetracycline was 125.25µg/l and that of penicillin G was 4.52 µg/l. Concentrations of oxytetracycline and penicillin G in all samples were between ranges of 45 -192 µg/l and 0-28 µg/l, respectively. The antibiotic residue positive samples which showed residues of oxytetracycline above the WTO/FAO/CAC established maximum residue limit of 100µg/l were 40 (83.33%). For penicillin G, the number of samples above the maximum residue limit of 4µg/l, were 8 (16.66%). In conclusion, this study revealed that oxytetracycline and penicillin G were imprudently used in dairy farms of the study area.

Keywords: Delvotest SP, High Performance Liquid Chromatography, Milk, Oxytetracycline, Penicillin G, Residue

Introduction

Antibiotics have been used in the dairy industry for more than five decades. They are used in dairy cattle production primarily to treat or prevent disease and to a lesser extent to increase milk production or improve feed efficiency. Antibiotics used as growth promoters are administered at low doses for extended periods. As prophylactics, antibiotics are used at low doses to prevent disease (Gustafson, 1991). The use of antibiotics therapy to treat and prevent udder infections in cows is a key component of mastitis control in many countries (Bramley and Dodd, 1984). However, widespread use of antibiotics has created potential residue problems in milk and milk products that are consumed by the general public. Because of the public health significance, milk and milk products contaminated with antibiotics beyond a given residue levels, are considered unfit for human consumption (Hillerton et al., 1999).

Antibiotic residues are small amounts of drugs or their active metabolites which remain in milk after treating the cows. Problems associated with antibiotic residues in milk include the risk of allergic reactions after consumption by penicillin-sensitized persons, increased resistance of pathogens towards antibiotics, and inhibition of bacterial starter cultures used in dairy production. The concerns arise mainly from the possibility that antibiotic resistant bacteria may be transferred from animals to humans, through contact, through the environment (e.g., water, manure) or through contaminated milk products (Busato et al., 2000; Codex Alimentarius Commission, CAC, 2010). Levels of the drug and their metabolites may persist at unacceptable levels and consumers can be exposed to them. The presence of residues may result from failure to observe the mandatory withdrawal periods, illegal or extra-label use of drugs and incorrect dosage levels. Unauthorized antibiotic use may result in residues of these substances in milk and tissues. Furthermore, many antibiotics used in animal agriculture are poorly absorbed in the animal gut. It is estimated that 25% to 75% of the antibiotics administered to feedlot animals could be excreted unaltered in feces and can persist in soil after application on land (Mackie, et al., 2006).

To detect antibiotic residues, different kinds of methods were developed. These consist of screening methods and chromatographic techniques (Mitchel et al., 1998). The screening procedure is generally performed by microbiological, enzymatic and immunological methods. The screening methods are based on susceptibility of bacteria to different antibiotics. Microbiological assays utilize

bacteria such as *Bacillus stearothermophilus* because of its high sensitivity to the majority of antibiotics (Schenck and Callery, 1998; Farrington, et al., 1991; Gilbertson, 1995). If the milk from a single cow undergoing treatment accidentally enters the herd bulk milk, this may be sufficient to make the content of a tanker unsuitable for human consumption (McEwen et al., 1991).

In order to safeguard human health, the World Health Organization (WHO) and the Food Agriculture Organization (FAO) have set standards for acceptable daily intake and maximum residue limits in foods (FAO and WHO, 1995). Regulatory limits for antibiotic residues have been imposed on the dairy industry in many countries (FDA, 1996). However, Ethiopia has not yet adapted international standards or established specifications for residue limits in the milk. The need for studies on residue levels of various antibiotics in milk should be investigated in the country in order to set standards. Therefore, the objectives of this work were to determine the prevalence, concentration of oxytetracycline and penicillin G residues in milk samples destined for consumption and to assess the knowledge of the dairy farm owners about antibiotic residues in milk.

Materials and methods

Study area

The study was carried out in Nazareth dairy farms between October 2007 and May 2008 and the study population consisted of milking cows found in Nazareth dairy farms.

Study design

A cross-sectional study was undertaken. On each sampling day, usually once a week, about 20 ml of milk samples were randomly selected and sampled from each farm bulk tank. Twenty four dairy farm owners were visited for interview after antibiotic residues were detected in samples of their bulk milk (case farms= 12) along with an equal number of residue free farms (control farms = 12). The interview was administered to determine associations between the occurrence of antibiotic residue in milk and various risk factors like management practices, treatment factors, residue prevention methods and knowledge of the farm owners about the antibiotic residues.

Sampling procedure

Individual dairy farms to be sampled were selected using random sampling technique. A subset of individual farms was chosen from a large set of farms. About 20 ml milk samples were collected in each dairy farm from their bulk milk. Each sample was labeled legibly and accompanied by necessary identification information, which included date of sampling, type of samples, breed of cows from which the samples were obtained and identification code. All milk samples were transported under chilled conditions to the laboratory and stored at –20 0C until analysis.

Sample size determination

The sample size required for the study was determined based on the expected occurrence (prevalence) of drug residue and desired absolute precision according to Thrusfield (2005) and the sample size was about 384, but it was raised to 400.

Methods for oxytetracycline and penicillin G residue analysis

The milk samples were screened qualitatively by using Delvotest SP assay (microbial inhibitor tests with Bacillus stearothermophilus as test microorganism) and quantitatively by High Performance Liquid Chromatography (HPLC).

The qualitative analysis of oxytetracycline and penicillin G residues in milk was done using Delvotest SP assay as described by Suhren (1998). This method is based on the susceptibilities of bacteria to different antibiotics. Delvotest SP ampoules were supplied by DSM (DSM Food Specialties, Delft, and the Netherlands). The method combines the principle of agar diffusion tests with a color change of the indicator resulting from the active metabolism of the testing microorganism in the absence of the indicator. The sample examined is put in to a tube filled with agar medium containing B. stearothermophilus var. calidolactis. The incubation (64 ± 1 0C/2.5-5 h), at which the tested strain is growing, causes that the color of the indicator (bromcresol red) will change from purple to yellow. If the sample examined contains substances inhibiting the growth of the test strain, the color of the indicator remains purple.

The samples positive for Delvotest SP assay were further analyzed by HPLC for quantification as described by Ghidini et al., (2003) for oxytetracycline and penicillin G. A given sample was regarded as positive for oxytetracycline or penicillin G if its retention time and peak corresponded to that of the standard.

Retention time was considered a reasonably unique identifying characteristic of a given substance or chemical constituent that is of interest in an analytical procedure. The area inscribed by the peak is proportional to the amount of substance separated in the chromatographic system. To get the concentration of oxytetracycline or penicillin G, 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. The parameters are indicated in Table 1.

Table 1: Parameters of HPLC

Parameter	Limit
Retention (Capacity) Factor	$k \geq 2$
Injection Precision	RSD < 1% for $n \geq 5$
Resolution	$Rs > 2$
Tailing Factor	$T \leq 2$
Theoretical Plate (Efficiency)	$N > 2000$

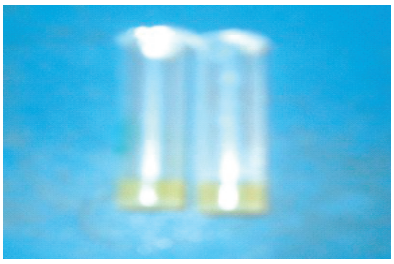
Data management and analysis

The data collected through questionnaire survey, Delvotest SP and HPLC were entered in to databases using Micro-Soft computer program Excel (Version 6. 0, 2000) and analyzed using SPSS (SPSS version 11. 05, 2000). Descriptive statistics were also used to describe the nature and the characteristics of the data.

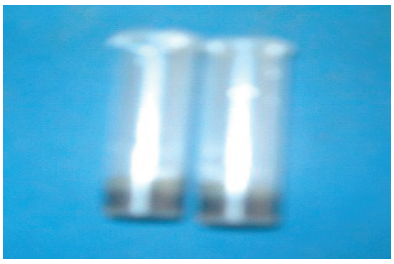
Results

Qualitative analysis with Delvotest SP assay

Out of 400 milk samples, the prevalence of antibiotic residue in the dairy farms was 48 (12%). The colour reaction of the Delvotest kit with standards (positive and negative) and samples (negative and positive) are shown in Fig. 1 and Fig. 2 respectively.



Negative control samples



Positive control samples

Figure 1: Colour reaction of Delvotest kit with controls (- / +)

Yellow colored (-) samples Purple colored (+) samples

Figure 2: Colour reaction of Delvotest kit with milk samples (- / +)

Response to questionnaire survey in the dairy farm

The average number of animals per farm was 2.79 and the mean number of milking cows was 2.29. Proportions of the dairy farm owners which applied different management practices are summarized in Table 2. Twenty five percent farmers reported using of part-time employers for the milking of cows. None of the farmers participated in any training of dairy farm management. Use of medicated feed, post milking teat dips and branding of milking equipment were not recorded in any of the dairy farms.

Table 2: Response to questionnaire survey on farm management practices in Nazareth Diary farms

Farm management practices	(N= 24)		
	Frequency	%	95% CI
Part time help	6	25.0	7.68-42.32
Feed additives	0	0	0
Training	0	0	0
Teat dips	0	0	0
Branding of milking equipment	0	0	0

N = number of samples; CI=Confidence Interval

Commonly observed disease conditions recorded were 33% mastitis, 16 % metritis and 16.7% enteritis. The other disease conditions (dystocia, retained fetal membrane, metabolic problem and foot problem) recorded were 20.1% of the farms. On 33.3%, 25% and 29.2% of the dairy herds surveyed, Oxytetracycline, penistripe and multiject were the antibiotics commonly utilized to treat disease problems. None of the farmers were aware of dry cow therapy for con-

trolling mastitis. Antibiotics were administered by veterinarians, assistant veterinarians and the owners themselves on 23.5 %, 67.7% and 8.8% of farms respectively. Administration of antibiotics was accomplished using the routes of intramuscular, intramammary, intrauterine and per-os (orally) on 41.7 %, 25%, 8.3 % and 16.7 % of the farms (Table 3).

Table 3: Percentage of the treatment factors in Nazareth dairy farms

Treatment factors	(N =24)		
	Frequency	Percent	95% CI
Drugs			
Oxytetracycline	8	33.3	14.47-52.19
Penistripe	6	25	7.68-42.32
Multiject	7	29.2	10.98-47.36
Other drugs	7	29.2	10.98-47.36
Route of administration			
Intramuscular	10	41.7	21.95-61.39
Intramammary	6	25	7.68-42.32
Intrauterine	2	8.3	-2.73-19.39
per-os	4	16.7	1.76-31.58
Person administering drugs			
Veterinarian	4	16.7	1.76-31.58
Assistant Veterinarian	17	70.8	52.64-89.02
Owner	3	12.5	-0.73-25.73
Dry cow therapy	0	0	0

N =total sample number; CI=Confidence interval

In addition, the major antibiotic residues prevention methods used as per questionnaire survey were studied. The study noted that 25%of dairy farms used marking for treated cows. Around 42% of farms used separate equipment for milking treated cows. 58.3% of dairy farms reported withholding milk from all quarters of treated cows to prevent occurrence of antibiotic residue. Around 20 % of dairy farms used keeping records of antibiotic treatment. None of the farms used antibiotic test kit. Nearly 60% respondents thought that antibiotic residues were of public health significance. Table 4 shows descriptive statistics of herd size and number of milking cows in their herd.

Table 4: Summary of descriptive statistics of herd size and number of milking cows in Nazareth dairy farms

Variables	N	Mean	Std. Dev.	95% CI for mean		Minimum	Maximum
				Lower limit	Upper limit		
Herd size	24	2.79	1.668	2.09	3.50	1	7
No of milking cows	24	2.29	1.083	1.83	2.75	1	5

N= number of samples; CI= Confidence interval

Quantitative analysis of residues by High Performance Liquid Chromatography (HPLC)

Chromatograms of reference standards, oxytetracycline HCl and penicillin G procaine salt, and some samples which were positive for oxytetracycline and penicillin G from the dairy farms are shown in Figure 3, 4, 5 and 6.

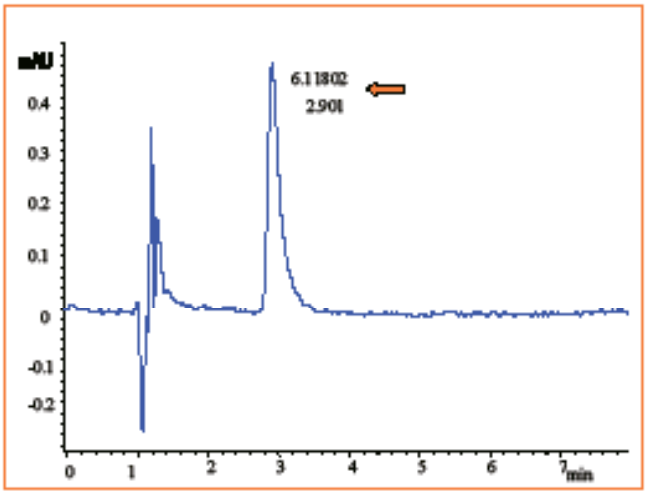


Figure 3: Chromatograms of reference standards of oxytetracycline HCl

The arrow indicates the peak, peak area and its retention time.

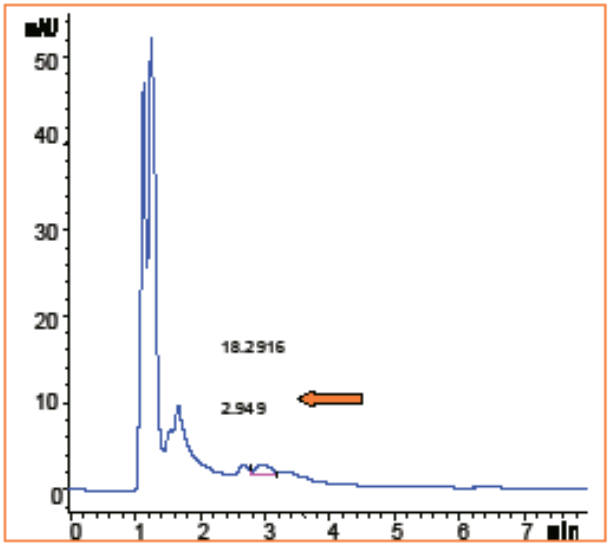


Figure 4: Chromatograms of some samples which were positive for oxytetracycline residue.

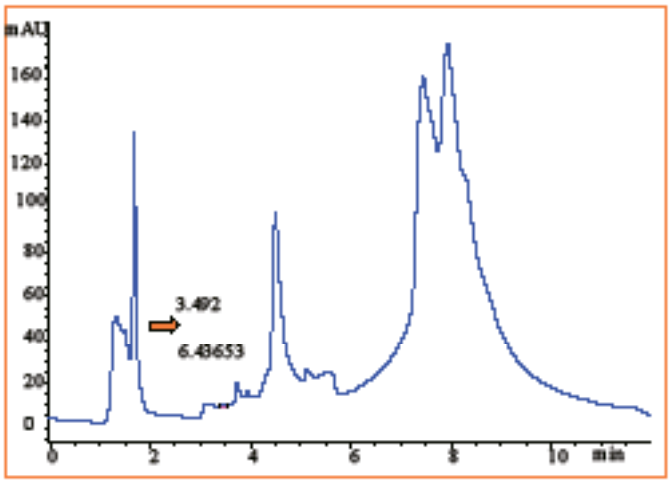


Figure 5: Chromatograms of reference standards of penicillin G procaine salt

The arrow indicates the peak, peak area and its retention time

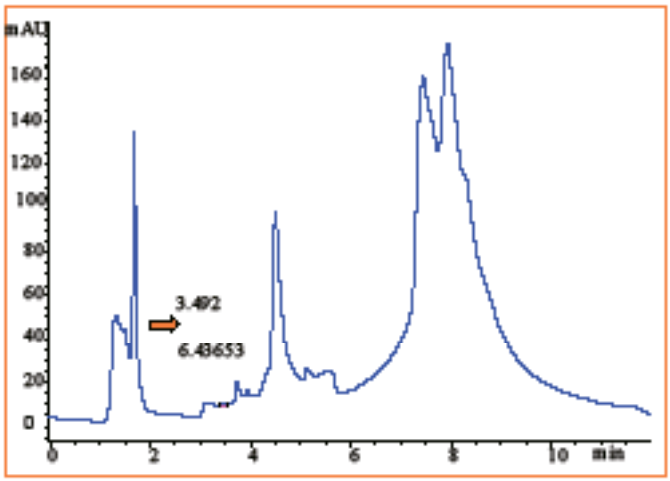


Figure 6: Chromatograms of some samples which were positive for penicillin G residue

The range for oxytetracycline residue level was 45µg/l to 192µg/l. The range for penicillin G residue levels was 0µg/l to 28µg/l. The antibiotic residue positive samples which showed residues of oxytetracycline above Maximum Residue Limit (MRL) were 40 (83.33%). The antibiotic residue positive samples which showed residues of penicillin G above MRLs were 8(16.66%). The descriptive statistics such as mean, range and standard deviation of oxytetracycline and penicillin G residues are shown in Table 5. Oxytetracycline was found being present in all samples. But, no penicillin G was found in some milk samples 20 (41.66%).

Table 5: Summary of descriptive statistics of oxytetracycline and penicillin G residues concentration (µg/l) in Nazareth dairy farms

Antibiotics	N	Mean	Std. Dev.	95% CI for mean		Minimum	Maximum
				Lower limit	Upper limit		
Oxytetracycline	12	125.25	52.091	92.15	158.35	45	192
Penicillin G	12	4.52	8.097	-0.63	9.66	0	28

N =total number of sample; CI= Confidence Interval

Discussion

Out of the total 400 milk samples analyzed by Delvotest SP assay, 48 (12 %) had detectable levels of antibiotic residues. The proportion of positive samples was higher when compared to other reports elsewhere. In the study undertaken by Sternesjö and Johnsson, (1998) in Sweden, it was also indicated that 0.08%-0.26% milk samples were positive for antibiotic residues which were much lower than the present study. Another study conducted by Sudershan and Bhat, (1995) in India also indicated 9% milk samples had residues of antibiotic and a study by McEwen et al., (1992) revealed 9.4% prevalence of antibiotics, which are both comparable with the results of this study. But, prevalence of antibiotics in our finding 12 % was smaller than the finding of Seymour et al., (1988) which was 21%. This could be due to the low access of antibiotics as feed additives in our studying areas.

Small dairy producers in Kenya were observed producing milk with beta-lactam residues exceeding the established maximum residue levels (Shitandi, 2004). But, the use of antibiotics in Sweden and Norway for mastitis treatment had been influenced by national policies and recommendations. In these countries, the preference for using beta-lactams (i.e., procaine and benzyl penicillin) was based on the withdrawal period. Dairy producers in Sweden use long-acting drug treatment for subclinical mastitis and dry cow therapy, whereas the same formulations are not accepted in Norway (Grave et al., 1999). These examples indicate that antibiotic usage varies among and within countries and also between farms, depending on policies.

The questionnaire survey conducted during the study period included questions that were helpful to gain insights into farm management practices associated with antibiotic usage. In general, twenty six percent 26.09% of the respondents used contract laborers or part-time employee for milking activities. However, in most cases, contract laborers were not aware of giving much attention to the importance of hygienic conditions during milking. All the respondents indicated that they never practiced post milking teat dipping. Nearly all respondents reported that they never used feed additives in their dairy farms and branding of milking equipment.

Mastitis is a complex disease and the difference in results could be due to difference in management systems among farms. The high prevalence of mastitis may be attributed to improper milking hygiene, lack of use of post milking

teat dipping and practices of milking by contract laborers with different skills. Antibiotics, particularly tetracyclines and penicillin G, are extensively used for prevention and treatment of diseases in dairy farms. Oxytetracycline was the first antibiotic used in most farms 46.74 % followed by penicillin (36.96 %) according to the respondents. The use of antibiotics continues to be a predominant in the treatment and control of mastitis (Owens et al., 1991). But, dry cow therapy was not reported in any farms which could have a great effect in protecting against new intramammary infections (Sanchez and Watts, 1999).

Health services were given mostly by the practitioners coming to the farms or sometimes by taking the animals to veterinary clinics. Regular health programs by professionals were not practiced; this might be due to the income they get from sale of milk that allows them to pay for the veterinary services. Only 12% of the dairy producers said that they always completed the course of antibiotic treatment by themselves. The tendency to rely on personal experience for antibiotic use, dosage, and withdrawal period was also observed in dairy producers surveyed by Zwald et al., (2004) which could lead to improper antibiotic usage. One important finding of this study was the observation that about 60% of respondents thought that antibiotic residues were of public health significance. Only 20% of the farms surveyed kept records of antibiotic treatment that could be verified. Similar survey of dairy producers in Pennsylvani indicated that 50% of dairy farms maintained antibiotic treatment records (Sawant et al., 2004).Therefore, insufficient record keeping and poor knowledge about drug withdrawal periods among producers were important factors leading to drug residues in milk.

Oxytetracycline was found in all milk samples collected in dairy farms with concentration range of 45-192 µg/l. This finding was higher as compared to Owens et al., (1991) who found oxytetracycline concentration within range of 13-106µg/l in USA. For penicillin G, the concentration range was 0-28 µg/l. A study by Sudershan and Bhat (1995) found out that penicillin G was the most common type of antibiotic residue in milk, with levels often exceeding the maximum residue limit established in the European Union (4 µg/l). But, in this study penicillin G was not detected in 20 (41.66%) of the milk samples. It might be due to lack of such type of antibiotic in the market for the dairy farmers.

The study also showed that oxytetracycline and penicillin are imprudently used in those areas which are the basic means for treatment of many diseases. As a

result, these drugs will be out of use in the near future due to treatment failures by creating resistance to many species of bacteria. In general, the excessive use of antimicrobials has led to the development of multi-drug resistance in animal and human pathogens. Furthermore, milk contaminated with even low concentrations of antimicrobial drug residues Sarmah et al., (2006) may also create problems in the production of fermented milk by products, because such compounds inhibit the growth of the starter cultures (Keys et al., 1997).

Conclusion

This study showed higher prevalence and amount of oxytetracycline and penicillin G residues in Nazareth dairy farms. Excessive use of antimicrobials for prevention and treatment of diseases in dairy farms, treatment of diseased cows by owners without professional advice, insufficient record keeping and poor knowledge about drug withdrawal periods, lack of proper management of dairy cows and poor awareness of the people concerning health impact of antibiotic residue in milk were the major contributing factors. The antibiotic screening tests should be provided to be used by dairy producers, milk processors and veterinarians to ensure the production of antibiotic residue-free milk. Because milk and milk products are essential foodstuffs for small children, attention has to be paid to the presence of drug residues in milk. In addition, the use of effective enforcement of their standards is essential to fulfill the objective of consumer providing them with safe and wholesome milk and milk products.

Acknowledgments

We are grateful to Faculty of Veterinary Medicine, Chemistry department in Science Faculty of Addis Ababa University and Drug Administration and Control Authority (previous name) of Ethiopia for their support and facility. This study was also financially supported by International Livestock Research Institute (ILRI).

References

Bramley, A. J. and Dodd, F. H., 1984. Reviews of the progress of Dairy Science: Mastitis control-progress and prospects. *J. Dairy Res*., 51(3), 481-512.

Busato, A., Trachsel, P., Schällibaum, M. and Blum, J. W., 2000. Udder health and risk factors for subclinical mastitis in organic dairy farms in Switzerland. *Prev. Vet. Med*., 4:4(3-4), 205-20.

Codex Alimentarius Commission (CAC), 2010. Reports of the nineteenth session of the codex committees on residues of veterinary drugs in Foods. Burlington, USA.

Farrington, W. H., Tarbin, J., Bygrave, J. and Shearer, G., 1991. Analysis of trace residues of tetracyclines in animal tissues and fluids using metal chelate affinity chromatography/HPLC. *Food Addit Contam*., 8(1): 55-64.

Food and Agriculture Organization (FAO) and World Health Organization (WHO), 1995. Application of risk analysis to food standards issues. Report of the Joint FAO/ WHO expert consultation. Geneva, Switzerland, WHO/FNU/FOS/95.3, Pp13-17.

Food and Drug Administration (FDA) USA, 1996. Evaluation and use of milk. Antimicrobial drug screening tests report. Centre for food safety and applied nutrition. FDA, 20855.

Ghidini, S., Zanardi, E., Varisco, G., and Chizzolini, R., 2003. Residues of ß-lactam antibiotics in bovine milk: Confirmatory analysis by liquid chromatography tandem mass spectrometry after microbial assay screening. *Food Addit Contam*., 20(6), 528-534.

Gilbertson, T. J., Mejeur, R. L., Yein, F. S., and Jaglan, P. S., 1995. Modified Microbiological Method for the Screening of Antibiotics in Milk. *J. Dairy Sci.*, 78(5), 1032-8.

Grave, K., Greko, C., Nilsson, L., Odensvik, K., Mork, T. and Ronning, M.,1999. The usage of veterinary antibacterial drugs for mastitis in cattle in Norway and Sweden during 1990-1997. *Prev Vet Med*., 42, 45–55.

Gustafson, R. H., 1991. Use of antibiotics in livestock and human health concerns. *J. Dairy Sci.,* 74(4), 1428-32.

Hillerton, J. E., Halley, B. I., Neaves, P., and Rose, M. D., 1999. Detection of antimicrobial substances in individual cow and quarter milk samples using Delvotest microbial inhibitor tests. *J. Dairy Sci*., 82 (4), 704-711.

Keys, J. E., Pearson, R. E. and Weinland, B. T., 1997. Starter Culture, Temperature and Antibiotic Residue in Fermentation of Mastitic Milk to Feed Dairy Calves. *J. Dairy Sci.*, 62(9): 1408-1414.

Mackie, R. I., Koike, S., Krapac, I., Chee-Sanford, J., Maxwell, S. and Aminov, R. I., 2006. Tetracycline Residues and Tetracycline Resistance Genes in Groundwater Impacted by Swine Production Facilities. *Animal Biotechnolog*., 17 (2), 157-176.

McEwen, S. A., Black, W. D., and Meek, A. H., 1991. Antimicrobial residue prevention methods, farm management, and occurrence of antibiotic residues in milk. *J. Dairy Sci.*, 74(7), 2128-2137.

McEwen, S. A., Black, W. D. and Meek, A. H., 1992. Antibiotic residues (bacterial inhibitory substances) in the milk of cows treated under label and extra label conditions, *Can Vet J*., 33(8), 527 – 34.

Mitchell, J. M., Griffiths, M. W., McEwen, S. A., McNab, W. B. and Yee, A. J., 1998. Antimicrobial Drug Residues in Milk and Meat: Causes, Concerns, Prevalence, Regulations, Tests, and Test Performance. *J. Food Prot*., 61(6), 742-56.

Owens, W. E., Nickerson, S. C ., Washburn, P. J. and Ray, C. H., 1991. Efficacy of a cephapirin dry cow product for treatment of experimentally induced Staphylococcus aureus mastitis in heifers. *J. Dairy Sci.,* 74, 3376–3382.

Sanchez, M. S. and Watts, J. L., 1999. Enhancement of the activity of novobiocin against Escherichia coli by lactoferrin. *J. Dairy Sci.,* 82, 494–499.

Sarmah, A. K., Meyer, M. T. and Boxall, A. B., 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics in the environment. *Chemosphere.,* 65, 725–759.

Sawant, A. A., Sordillo L. M. and Jayarao, B. M., 2005. A Survey on Antibiotic Usage in Dairy Herds in Pennsylvania. *J. Dairy Sci*., 88 (8), 2991-2999.

Schenck, F. J. and Callery, P. S., 1998. Chromatographic methods of analysis of antibiotics in milk. *J. Chromatogr A*., 812 (1 – 2), 99 – 109.

Schlüsener, M. P., and Beste, K., 2006. Persistence of antibiotics such as macrolides, tiamulin and salinomycin in soil. *Environ Pollut*., 143 (3), 565-571.

Seymour, E. H., Jones, G. M. and McGilliard, M. L., 1998. Persistence of residues in milk following antibiotic treatment of dairy cattle. *J. Dairy Sci.,* 71(8), 2292-6.

Shitandi, A., 2004. Factors contributing to the occurrence of antimicrobial drug residues in Kenyan milk. *J. Food Prot*., 67 (2), 399–402.

Sternesjö, A., and Johnsson, G., 1998. A novel rapid enzyme immunoassay (Fluorophos BetaScreen) for detection of beta-lactam residues in ex-farm raw milk. *J. Food Prot*., 61 (7), 808-811.

Sudershan, R. V. and *Bhat*, R. V., 1995. A *survey on veterinary drug use and residues in milk* in.*Hyderabad*. *Food Addit Contam*., *12(5), 645-650*.

Suhren, G., and Beukers, R., 1998. Delvotest SP for detection of cloxacillin and sulfamethoxazole in milk. IDF interlaboratory study. *J. AOAC Int.,* 81, 978-990.

Thrusfield, M., 2005. Veterinary epidemiology. 3rd ed. Blackwell Science Ltd., Oxford, Great Britain. Pp 182-198.

Zwald, A. G., Ruegg, P., Fossler, C. and Halbert, L. W., 2004. Management practices and reported antimicrobial usage on conventional and organic dairy farms. *J. Dairy Sci.,* 87,191–201.