DETECTION AND DETERMINATION OF OXYTETRACYCLINE AND PENICILLIN G ANTIBIOTIC RESIDUE LEVELS IN BOVINE BULK MILK FROM DEBREZEIT AND NAZARETH DAIRY FARMS

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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 Debre Zeit dairy farms. A total of 400 bulk milk samples were randomly collected in the respective study dairy farms. A questionnaire survey was carried out by personal interviews with dairy farm owners in Delvotest positive farms (cases) and Delvotest negative farms (controls) to identify various risk factors and to determine associations among the occurrence of antibiotic residue in milk. All samples were qualitatively screened for antibiotic residues by Delvotest SP assay. Concentration of the positive samples was determined by high performance liquid chromatography. Concentrations were established using linear calibration reference curves. Out of 400 samples analyzed for antibiotic residue, 34 (8.5%) milk samples were positive for antibiotic residues. The mean residue level of oxytetracycline was 142.00µg/l that of penicillin G was 4.77µg/l. Oxytetracycline concentrations in all samples is ranged at a concentration between 27µg/l - 251µg/l. The antibiotic residue positive samples which showed residues of oxytetracycline above the WTO/FAO/CAC established maximum residue limit of 100µg/l were 24 (70.58%). For penicillin G with maximum residue limit of 4µg/l, they were 7 (20.58%). Penicillin G was found in some milk samples of (58.8%) dairy farms. The result obtained confirmed that oxytetracycline and penicillin G were imprudently used in dairy farms.

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

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. Although the duration of antibiotic use differs for growth promotion and prophylaxis, the dosage for both is typically less than 200 g/ton, and is considered subtherapeutic (IOM, 1989).

The therapeutic regimen is dictated by label instructions by the manufacturer or in accordance with extra-label instructions by a veterinarian. Antibiotics are administered to animals through injections (e.g., intramuscular, intravenous, or subcutaneous), orally, topically, or via intramammary or intrauterine infusion. Several types of antibiotics are commonly used in food animals (Mitchell et al., 1998).

The use of antibiotics therapy to treat and prevent udder infections in cows is a key component of mastitis control in many countries. Due to the widespread use of antibiotic for treatment of mastitis in dairy cows, much effort and concerns have been directed towards the proper management and monitoring of antibiotics usage in treatments in order to prevent contamination of raw milk. 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 (CAC, 1998). 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 (CAC, 1998). Residues are illegal and milk supplies containing detectable concentrations are not acceptable. It has been estimated that antibiotic contaminated milk costs the US dairy industry $50 million annually (Rice et al., 1984).

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 (Ivona and Mate, 2002). 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 (Elmund et al., 1971, Feinman and Matheson, 1978) and can persist in soil after application on land (Donoho, 1978; Gavalchin and Katz, 1994). There is little information available concerning the fate of antibiotics in the environment and their link to the emergence of resistant genotypes found there. The annual production of livestock and poultry waste in the United States is nearly 180 million tons (dry weight basis) (Hagedorn et al., 1999) and coupled with antibiotic usage, this waste is a potentially large source of both antibiotics and antibiotic-resistant bacteria released into the environment.

Antibacterial drugs such as oxytetracycline and penicillin G are routinely used in veterinary medicine for prevention and control of disease. Oxytetracycline is applied for the purpose of prevention or treatment of diseases such as bronchopneumonia, mastitis and metritis in cows. As a result, there is concern that residues of these compounds may be presented in the milk and milk products. The penicillins are widely used to treat or prevent local and systemic infections of farm animals. The use of penicillins as intramammary infusions or formulations to treat or prevent bovine mastitis is widespread (Haapapuro, 1997).

To detect antibiotic residues, different kinds of methods were developed. These consist of screening methods and chromatographic techniques to detect as many antibiotics as possible. The screening method is generally performed by microbiological, enzymatic and immunological methods. The screening methods are based on the various susceptibility of bacteria to different antibiotics. The antibiotic residue detection assays that are currently available use different methods and test microorganisms (Mitchell et al., 1998). Microbiological assays for the detection of antibiotic residues utilize bacteria such as *Bacillus stearothermophilus* because of its high sensitivity to the majority of antibiotics. Both microbiological and chromatographic methods have been described for monitoring tetracyclines and penicillins in milk and animal tissues. Although the microbiological assay techniques have been recommended as official and conventional methods because of their simplicity, the bioassay methods lack specificity and provide only semi-quantitative measurements of residues detected and sometimes produce false positives (Kurittu, Lonnberg, Virta and Karp 2000). Therefore, chromatographic techniques, such as TLC, and HPLC, and capillary electrophoresis (CE), have been developed to replace microbiological assays (Chen and Gu, 1995; Cinquina et al., 2003; Ding and Mou, 2000; Furasawa, 2003; Huang et al., 1997; Petkovska et al., 2006; Posyniak et al., 2005; Zhao et al., 2004).
Residues of antibiotic agents may be of toxicological significance for the consumer and may influence the technological properties of milk used for manufacturing fermented products. To guarantee consumers safe and high quality dairy products, raw milk is regularly analyzed for the presence of antibiotic residues. 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; Folly and Machado, 2001). However, Ethiopia has not yet adapted international standards or established specifications for residue limits in the milk. The Ethiopian dairy industry has not adopted any control programs to ensure the safety of the milk. The drug residue limits, which apply to both the parent drug and its metabolites, need to be enforced at all levels in Ethiopia dairy industry in order to protect the health of consumers.

In Ethiopia, no studies have been conducted on oxytetracycline and penicillin G residue levels in milk and as such there are no data on the residual levels of these drugs in milk. Therefore the objectives of this work were to determine the prevalence of oxytetracycline and penicillin G residues in milk samples destined for consumption, quantitatively determine concentration of oxytetracycline and penicillin G residues in milk samples with qualitatively positive results and to assess the knowledge of the dairy farm owners about antibiotic residues in milk.

MATERIALS AND METHODS

Study Area

The study was conducted in Debre Zeit dairy farms between October 2007 and May 2008.

Debre Zeit

The town is located at 9°0N and 40°0E. It is 47 km South East of Addis Ababa, the capital of Ethiopia. The altitude is about 1850m above sea level. It experiences bimodal patterns of rainfall with the main rainy season extending from June to September with an average rainfall of about 800 mm. The mean annual minimum and maximum temperatures are 12.3 °C and 27.7 °C, respectively with an overall average of 18.7 °C (CSA, 2001). The mean relative humidity is 61.3%.

Study population

The study population consisted of milking cows found in Debre Zeit dairy farms.

Study design

A cross-sectional study was undertaken in. On each sampling day, usually once a week, about 20 ml of milk samples were randomly selected and sampled from each farm bulk tank. Sixty four dairy farms were visited after antibiotic residues were detected in samples of their bulk milk (case farms= 34) along with an equal number of residue free farms (control farms = 34). A questionnaire survey was conducted by personal interview of the dairy farm owners in case farms and equal number of control farms. It 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. In management practices of the farm owners, the information collected were the use of feed additives, use of post-milking teat dips and training on dairy management.

Information on treatment factors included sources of antibiotics, type person who administers antibiotics to cows, route of antibiotic administration, record keeping, use of dry cow
therapy and number of milking cows. Regarding residue prevention methods, the information gathered were marking of treated cows, milking of treated cows using separated equipment, use of antibiotic test kit and knowledge of withdrawal periods of antibiotics. Each milk sample was aseptically collected in separate containers and transported in ice-box packed to the AAU, Faculty of Veterinary Medicine (FVM) and Drug Administration and Control Authority (DACA) laboratory.

**Sampling Procedure**

Individual dairy farm owners to be sampled were selected using random sampling technique. 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°C, until analysis.

**Sample Size Determination**

The sample size required for the study were determined 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 type of antibiotics in milk samples was screened qualitatively by using Delvotest SP assay (microbial inhibitor tests with *Bacillus stearothermophilus* as test microorganism) and quantitatively by high performance liquid chromatography (HPLC).

**Detection of Oxytetracycline and Penicillin G Residue in Milk using Delvotest SP Assay**

The qualitative analysis of oxytetracycline and penicillin G residues in milk was done using Delvotest SP assay as described by Suhren and Beukers (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 was carried out according to the instructions by the manufacturer.

**Determination of the Residue Levels of Oxytetracycline and Penicillin G Antibiotics with High Performance Liquid Chromatography (HPLC).**

The qualitatively positive samples (section 3.5.1) were further quantitatively analyzed using HPLC as described by Ghidini *et al.* (2003) for oxytetracycline and penicillin G.

**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). Differences between proportions of groups with certain determinant factors were assessed by Chi-square (χ²) test. Descriptive statistics were also used to describe the nature and the characteristics of the data.

**RESULT**

**Qualitative Analysis of with Delvotest SP assay**
In Debre Zeit dairy farm, out of 400 milk samples, 34 (8.5%) of them were positive for antibiotic residue. The colour reaction of the Delvotest kit with standards (positive and negative) and samples (negative, positive and doubtful) have been shown.

**Fig1:** Colour Reaction of Delvotest Kit with Controls (-/+)

![Positive control samples](image)
Colour reaction of Delvotest kit with milk samples(-/+)

**Response to Questionnaire Survey in the Dairy Farm**

Proportions of the dairy farms which applied different management practices are summarized in Table 1. 18(26.47%) farmers reported using of part-time employers for the milking of cows. The farmers were asked if they participated in any training of dairy farm management. Around forty-six percent of the dairy producers said as they had participated. Use of medicated feed, post milking teat dips and branding of milking equipment were not recorded in this farm.

**Table 1:** Response to Questionnaire Survey on Farm Management Practices in Debre Zeit Diary Farms

<table>
<thead>
<tr>
<th>Farm management practices</th>
<th>(n=68) frequency</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Part time help</td>
<td>18</td>
<td>26.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.98-36.96</td>
</tr>
<tr>
<td>Feed additives</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Training</td>
<td>31</td>
<td>45.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>33.75-57.43</td>
</tr>
<tr>
<td>Teat dips</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Branding of milking equipment</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Commonly observed disease conditions recorded in the dairy farms were 38.2% mastitis, 17.6% metritis, 10.3% enteritis and other types of diseases were recorded in 16.2% of farms. The other disease conditions (dystocia, retained fetal membrane, metabolic problem and foot problem) recorded in dairy farms was 16.2% of the farms. 51.1%, 41.2% and 14.7% 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. On 70.8%, 16.7% and 12.5% 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 (Table 2).

Table 2: Percentage of the Treatment Factors in Debre Zeit Dairy Farms

<table>
<thead>
<tr>
<th>Treatment factors</th>
<th>(n=68)</th>
<th>Frequency</th>
<th>Percent</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Drugs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>35</td>
<td>51.4</td>
<td>39.59-63.35</td>
<td></td>
</tr>
<tr>
<td>Penistripe</td>
<td>28</td>
<td>41.2</td>
<td>29.48-52.88</td>
<td></td>
</tr>
<tr>
<td>Multiject</td>
<td>10</td>
<td>14.7</td>
<td>6.29-23.13</td>
<td></td>
</tr>
<tr>
<td>Other drugs</td>
<td>12</td>
<td>17.7</td>
<td>8.59-26.71</td>
<td></td>
</tr>
<tr>
<td><strong>Route of administration</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intramuscular</td>
<td>36</td>
<td>52.9</td>
<td>41.08-64.8</td>
<td></td>
</tr>
<tr>
<td>Intramammary</td>
<td>21</td>
<td>30.9</td>
<td>19.9-41.86</td>
<td></td>
</tr>
<tr>
<td>Intrauterine</td>
<td>6</td>
<td>8.8</td>
<td>2.08-15.56</td>
<td></td>
</tr>
<tr>
<td>Perous</td>
<td>10</td>
<td>14.7</td>
<td>6.29-23.13</td>
<td></td>
</tr>
<tr>
<td><strong>Who administer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veterinarian</td>
<td>16</td>
<td>23.5</td>
<td>13.45-33.61</td>
<td></td>
</tr>
<tr>
<td>Assistant</td>
<td>46</td>
<td>67.7</td>
<td>56.53-78.77</td>
<td></td>
</tr>
<tr>
<td>Owner</td>
<td>6</td>
<td>8.8</td>
<td>2.08-15.56</td>
<td></td>
</tr>
<tr>
<td><strong>Dry cow therapy</strong></td>
<td>4</td>
<td>5.9</td>
<td>0.29-11.47</td>
<td></td>
</tr>
</tbody>
</table>

n=total sample number; CI=Confidence interval

The major antibiotic residues prevention methods used as per questionnaire survey are presented in Figure 3. The study noted that 52% of dairy farms marked as treated cows. 64.7% of dairy farms in 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 3: The Summary of Descriptive Statistics of Herd Size and Number of Milking Cows in Debre Zeit Dairy Farms

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Mea</th>
<th>Std.</th>
<th>95% CI for mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower limit</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Upper limit</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mini</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Maxi</td>
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</tbody>
</table>

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Table 3 shows descriptive statistics of herd size and number of milking cows. The average animals per farm were 3.94 animals per farm. The mean number of milking cows was 3.34.

**Result of Quantitative Analysis by High Performance Liquid Chromatography**

The samples positive for Delvotest SP assay were further analyzed by HPLC for quantification. 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 analyte. 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. Chromatograms of reference standards, oxytetracycline HCl and penicillin G procaine salt and some samples those were positive for oxytetracycline and penicillin G from the dairy farms

**Fig. 2:** Chromatogram of Reference Standards of Oxytetracycline HCl
The arrows indicate the peak, peak area and its retention time.

**Fig 3:** Chromatograms of some Samples that were Positive for Oxytetracycline Residue

![Chromatograms of some Samples that were Positive for Oxytetracycline Residue](image1)

The range for oxytetracycline residue level was 0µg/l to 251µg/l. The range for penicillin G residue levels was 0µg/l to 47µg/l. The antibiotic residue positive samples which showed residues

**Fig 4:** Chromatograms of Reference Standards of Penicillin G Procaine Salt

![Chromatograms of Reference Standards of Penicillin G Procaine Salt](image2)
of oxytetracycline above MRLs were 24 (70.58%). The antibiotic residue positive samples which showed residues of penicillin G above MRLs were 7 (20.58%). The Descriptive statistics such mean, range and standard deviation of oxytetracycline and penicillin G residues are shown in Table 18. Oxytetracycline was found being present in all samples in a concentration range of 27-251µg/l.

But, no penicillin G was found in some milk samples in some dairy farms (58.8%). The concentration range of penicilline G was 0-47 µg/l.

**Table 4: Summary of Descriptive Statistics of Oxytetracycline and Penicillin G Residues Concentration (µg/l) in Debre Zeit dairy farms**

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>n</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Lower limit</th>
<th>Upper limit</th>
<th>Minim um</th>
<th>Maxim um</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxytetracycline</td>
<td>34</td>
<td>142.00</td>
<td>67.206</td>
<td>118.55</td>
<td>165.45</td>
<td>27</td>
<td>251</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>34</td>
<td>4.77</td>
<td>10.787</td>
<td>1.01</td>
<td>8.53</td>
<td>0</td>
<td>47</td>
</tr>
</tbody>
</table>

n=total number of sample; CI= Confidence Interval

**DISCUSSION**

Out of the total 400 milk samples analyzed by Delvotest SP assay during the study period, 34(8.5%) had detectable levels of antibiotic residues. The proportion of positive samples was higher when compared to other reports elsewhere. A similar study carried out by Borges et al. (2000) in Brazil country reported that 4.3 % milk samples had detectable level of residue for antibiotic. The result of Rybinska et al. (1995) in Poland revealed that 13-22 % milk sample showed violative concentration of chemical residues. In the study undertaken by Sternesjo (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 (1998) in India also indicated that 9% milk samples had residues of antibiotic which is comparable with the results of this study.

The occurrence of antibiotic residues may be attributed to absence of use of post milk teat dipping, milking by contract laborers and poor record keeping systems. The presence of minute amount of antibiotics in milk, as a result of therapeutic use in dairy cows, can cause a wide variety of dairy manufacturing problems, including inadequate milk curdling or sub optimal ripening during cheese production, inadequate acidity and flavor attributes during the manufacture of butter milk, suboptimal starter culture growth, false results during quality control testing due to the presence of interfering drug metabolites (EEC, 1992).

Small dairy producers in Kenya were observed producing milk with beta-lactam residues exceeding the established maximum residue levels (Shitandi, 2004). 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. Nearly 26.47% of respondents reported use of part-time employee for milking activities. This finding was lower than the finding of Tesfaye (2007) at Nazareth who reported a proportion of (39.0%). However, in most cases, contract laborers were either not aware of giving much attention to the importance of hygienic conditions during milking, proper milking practices and the necessary precautionary majors while milking treated cows.

All the respondents indicated that they never practiced post milking teat dipping. Post milking teat dipping was carried out by 3.9% of the respondents in Addis Ababa dairy farms as reported by Mungube (2001). Those farms that practiced teat dipping used ammonium compounds (3.9%) and lugols iodine (5.9%). Nearly all respondents reported that they never used feed additives in their dairy farms. Branding of milking equipments was not also used.

The prevalence of mastitis at herd level was 38.2% which is comparable with that of Workineh et al. (2000) who reported 25.1% in Addis Ababa. However, the present study was in higher proportion than that reported by Bishi (1998) and Munguba et al. (2001) who reported 5.35% and 6.6%, respectively in Addis Ababa dairy farms. In addition, the prevalence was much higher than Gizat (2004) who reported 3.9% in Bahardar. 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. Cases of metritis and enteritis in lactating cattle were 17.6% and 10.5% respectively. Cows with other types of diseases like dystocia, retained fetal membranes and various metabolic disorders are more likely to lead to metritis (Lewis, 1997).

Based on the findings of this survey, it can be inferred that 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%) second to 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., 1997). Dry cow therapy was reported in 4.35% farms which was similar to that report by Mungube (2001) in Addis Ababa dairy farms. For dry cow therapy on farms, preferred drugs were cephapirin, penicillin G procaine and cloxacillin. These antibiotics are effective in protecting against new intramammary infections (Sol and Melenhorst, 1990; 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, but in general, it can be hypothesized that all farms had access to health services provided by professionals when needed. This might be due to the income they get from sale of milk that allows them to pay for the veterinary services. Twenty two percent of the farmers always sought veterinarian advice before administering antibiotics. Other than the veterinarian, antibiotics were administered primarily veterinary assistants and owner or herdsmen on 69% and 10% of farms, respectively. Only 9% 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). This lapse 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. Similarly, 78.4% of the respondents had knowledge on residue (Mungube, 2001) in Addis Ababa dairy farms. Only 20% of the farms surveyed kept records of antibiotic treatment that could be verified. Kaneene and Ahl’s (1987) survey of dairy producers in Michigan USA indicated that insufficient record keeping and poor knowledge about drug withdrawal periods among producers were important factors leading to drug
residues in milk. All respondents in all dairy farms indicated that they never used antibiotic test kit for detection of residues.

42% of respondents said that they used separate equipment for milking treated cows. Half (52%) of the respondents reported marking of separated cows. The average animals per farm was 3.94 which is in agreement with the existing reports by Devendra (2001) and Mekonnen et al. (2006) who reported average 4.56 animal per farm in Debre Zeit.

Milk samples that were Delvotest assay positive had detectable levels of oxytetracycline and penicill G residues by HPLC analysis. In most countries, limits have been established for antimicrobial drug residues in food products such as milk; for instance, in the European Union (EU), the maximum residue limits (MRLs) have been set by the European Commission. FAO/WHO Expert Committee on Food Additives established MRLs for oxytetracycline and penicillin G in milk at 100 µg/l and 4 µg/l, respectively. The accurate detection of low levels of antimicrobial drug residues in milk is not only of great importance for governmental control, laboratories and the dairy industry but also for farmers to enable them to ensure that contaminated milk from individual cow is not consigned to the bulk tank (EEC, 1990).

This study revealed that out of 46 milk samples found positive for antibiotic residues, 34 (73.91%) had oxytetracycline residues and 8 (17.39%) penicillin G residues above the recommended MRLs. The mean concentration of oxytetracycline residue was 142 µg/l. For penicillin G residue, the mean concentration was equal to 4.77µg/l. Oxytetracycline was found in all milk samples collected with area-specific concentration range of 27-251µg/l. This finding was higher as compared to Zhao et al. (2004) who found oxytetracycline concentration within range of 13-106µg/l in USA. A study by Shitandi (2004) found out that penicillin G was the most commonly type of antibiotic residue in milk, with levels often exceeding the maximum residue limit established in the European Union (4 µg/l). Penicillin G was not detected in 58.8 % of the milk dairy farms. The concentration range of penicillin G was 0-47 µg/l.

The presence of antibiotic residues in milk is strongly associated with several variables such as milk production at time of treatment, type and amount of antibiotic used, type of vehicle used in antibiotic formulations and the disease state of the animal (Mercer, et al., 1970). Antimicrobials, anti-inflammatory and hormones are the pharmacologically active substances most used for these purposes, but an illegal or unsuitable use increases the risk of introducing harmful residues into the human food chain. Adverse effects to consumers are connected with the intrinsic toxicity of a drug and its metabolites. The use of antimicrobial agents in food animals has caused concern regarding the impact of them on human health.

The use of tetracyclines in the United States exceeds 5.6 million pounds annually (Mellon and Benbrook, 2001). The main applications of tetracyclines in animal husbandry are for prophylaxis of bacterial infections and increase in growth rates. Although the public health risks are difficult to define, it is accepted that antimicrobial drug residues may induce allergic reactions in sensitized individuals and may have negative effects on the composition of the human intestinal flora. In general, the excessive use of antimicrobials has led to the development of multi-drug resistance in animal and human pathogens (Sarmah, Meyer and Boxall, 2006). Furthermore, milk contaminated with even low concentrations of antimicrobial drug residues may also create problems in the production of fermented milk by products, because such compounds inhibit the growth of the starter cultures.

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 even to those species which are isolated for the fist time in Ethiopia; for example, S. Braenderup, S. Hall (Becker et al., 1996).
Suspicious carcinogenicity of growth-promoting agents has prompted the European Union (EU) to ban the use of these compounds and to forbid the importation of meat and milk products from countries that authorize their use for fattening purposes. Therefore, drug residues remain very significant from the prospective of international trade and consumer confidence, because it results in international trade barrier (Kanneene and Miller, 1997). To increase considerable foreign currency, milk and milk products need to be exported, the requirement by World Trade Organization (WTO) and Codex Alimentarius Commission (CAC) should be adhered. One of the requirements is that antimicrobial residues in food should be below MRLs. But, the indiscriminate use of veterinary drugs can hinder the country’s interest to fulfill the need to export to those WTO member countries. Therefore, attempts should be made to reduce the magnitude of the problem at various levels through the prudent use of antimicrobials such as oxytetracycline and penicillin G. Awareness need to be created at different levels including controlling authorities, concerned organizations and the consumers.

CONCLUSION

This study showed higher prevalence and amount of oxytetracycline and penicillin G residues in Debre Zeit dairy farm and lack of proper management and awareness of the people 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. And 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.

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