Veterinary antibiotics in dairy products from Kumasi, Ghana

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ANALYTICAL CHEMISTRY | RESEARCH ARTICLE

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Godfred Darko1*, Lawrence Sheringham Borquaye1, Akwasi Acheampong1 and Kwasi Oppong1

Abstract: Consumption of traces of veterinary drug residues through dairy products could have adverse effects on human health. There is, therefore, the need for routine monitoring of drug residues in dairy products on the market. In this study, fresh milk, cheese, and yogurt being sold at different points in the Kumasi metropolis were analyzed for residues of chloramphenicol, sulfathiazole, sulfamethoxazole, and oxytetracycline — four commonly used veterinary drugs in the country. The concentrations found were generally very low and close to the method detection limit of 0.1 μg/kg. Concentration of chloramphenicol in yogurt (0.8 μg/L) was the highest among all the residues studied. However, with the residue levels being hundred folds lower than the maximum residue limit, they are highly unlikely to pose any health risks to the consumers. Therefore, as far as chloramphenicol, sulfathiazole, sulfamethoxazole, and oxytetracycline in fresh milk, cheese, and yogurt are concerned, this study did not indicate any risk of consumption.

Subjects: Agriculture & Environmental Sciences; Environmental Studies; Food Additives & Ingredients; Food Chemistry; Food Laws & Regulations; Chemistry

Keywords: veterinary drug residue; chloramphenicol; sulfathiazole; sulfamethoxazole; oxytetracycline

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Lawrence Sheringham Borquaye is a bioorganic chemist with interest on science at the interface of chemistry and biology. His research interest includes exploring biologically active natural products from marine organisms and from plant sources. Other research interests include the development of methods for the analysis of pharmaceutical and personal care products in the environment and the characterization of essential oils from plants.

Akwasi Acheampong, a senior lecturer, is excited about developing cheap and robust HPLC analytical methods for the simultaneous determination of active principles.

Kwasi Oppong is a Master of Philosophy candidate.

PUBLIC INTEREST STATEMENT
Some of the medications administered on or fed to farm animals stay in their system and build up in their body parts such as milk and muscles. Humans beings who consume such meat and milk products that contain the drugs may gradually build them up in their own bodies and could be affected if the drug’s build-up is high enough. In this work, we investigated how much of some of the popularly used drugs administered on animals are found in milk, cheese, and yogurt sold in three major markets in Kumasi, Ghana. In general, the concentrations of the drugs found in those commodities were very low and would not affect the health of the consumers.
1. Background
Veterinary drugs are used in livestock to treat diseases, maintain herd and flock health, promote growth, improve meat quality, and reduce production costs (1). In modern agricultural practices, veterinary drugs are used on a large scale for both therapeutic and prophylactic purposes. The majority of the drugs are administered as feed additives or via drinking water in order to prevent the outbreak of diseases or to improve the growth of the animals (2). Also, therapeutic drugs are given in case of disease, for drying-off purposes, or for the prevention of losses during transportation. Due to the extensive use of drugs in animal husbandry, their residues are likely to remain in the products of treated animals (3). Misapplication of the drugs, overdose, non-compliance of withdrawal times after application, and other irresponsible use of veterinary drugs has resulted in the occurrence of unwanted residues of drugs in food of animal origin. The factors which determine the occurrence of drug residues in animal products are the route of administration of the drug, contamination of feeds or water, the physicochemical properties and metabolism of the drug, and the physical condition of the animal (4). Antimicrobial drug residues have been proven to accumulate in tissues of food animals and their respective by-products in different parts of the world (5).

Residues of veterinary drugs or their metabolites in foods of animal origin may cause adverse toxic effects to consumers’ health (6) and endanger human health. Short-term health effects include allergic and toxic reactions while a long-term exposure could result in chronic toxic effects such as bacterial resistance development in humans (7). These resistant bacteria might then cause difficult-to-treat human infections and disruption of normal human flora in the intestines (8). Antibiotics might also reduce the total number of useful bacteria or selectively kill some important species. Other major health threats include the development of cancer and mutation in humans, bone marrow aplasia and leukemia (9). Veterinary drug residues in meat have been reported to cause toxic or allergic reactions in humans (10). There has also been report of genetically determined renal tubular functional defect due to consumption of tetracycline (11). The most notable recent occurrence of veterinary drug toxicity involved consumption of Mexican beef containing residues of the illegal growth promoter, clenbuterol (12). As a result of reported occurrences in different parts of the world, coupled with low awareness reported in the developing world, the World Health Organization encourages effective reporting of drug residues in foods of animal origin destined for human consumption (13).

Governmental regulatory authorities control the use of veterinary drugs by approving, registering safe use, and monitoring food for unsafe or prohibited residues. In Ghana, a great deal of concern has been demonstrated over the presence of chemical residues in food items (14), mainly veterinary drugs (4) and pesticides in meat (15) and milk supplies (16). Concerns over veterinary residues in food are both economic and public health related. For example, milk contaminated with penicillin can affect starter cultures used to make fermented milk products such as cheese, buttermilk, and sour cream, which can result in economic losses to those processors. Government, producers, and consumers all have active roles in minimizing antibiotic residues in animal products (17, 18). The use of veterinary drugs in food-producing animals can affect the public health and the international trade of food products because of the presence of residues of the drug. Drug residues present in foods are known to have global health threat. In Ghana, veterinary drugs may be used indiscriminately for the treatment of bacterial diseases or as feed additives for domestic animals. The threat of veterinary drug contamination is a worldwide public health challenge (18). Such residues are spreading rapidly, irrespective of geographical, economical, or legal differences between countries. The aim of this study is to determine residues of commonly used antibiotics (chloramphenicol, sulfathiazole, sulfamethoxazole, and oxytetracycline) in milk, locally made cheese and yogurt and to compare the levels of the drug residues to that of the maximum residual limits given by the Joint FAO/WHO Expert Committee on Food Additives (JECFA).

2. Materials and methods

2.1. Sample collection
Samples were collected from points of sales at Kumasi Central Market, Asafo Market and KNUST in two batches. The areas were selected to represent a variation in consumer concentration, market
access, and dairy production intensity. Kumasi which has a high population density and a relatively more intensive dairy production was chosen to represent high market access. The sampling was based on the assumption that mass treatment is the method of choice when treating animals and residues are equally distributed in all the dairy products taken at a specific time. A total of 90 samples (30 each of raw milk, cheese, and yogurt) were collected between January and March 2014.

2.2. Chemicals and reagents
Anhydrous potassium dihydrogen phosphate (KH₂PO₄), (HPLC grade, Fizmerk Chemicals, Germany), methanol and hexane and acetonitrile (HPLC grade, Fisher Scientific, USA) were the main chemicals used. All other chemical reagents and solvents were of analytical grade. Pure standards (>99.9%) of chloramphenicol, sulfathiazole, sulfamethoxazole, and oxytetracycline were purchased from Sigma-Aldrich Quimica, (Madrid, Spain).

2.3. Preparation of standards
A 40-mg portion of each standard was dissolved in acetonitrile/methanol (3/7 v/v) and topped up to 25 mL in a volumetric flask to make a stock solution. Portions of the stock were serially diluted with acetonitrile/0.05 M sodium dihydrogen phosphate (3/7, v/v) to make 2, 1, 0.5, and 0.1 μg/mL working solutions.

2.4. Sample extraction
The extraction method was based on that of (19) with a slight modification. Specifically, a 30-mL portion of milk or yogurt or 30 g cheese samples was homogenized in 50 mL of acetonitrile for 3 min. The mixture was allowed to stand for 30 min and then filtered using filter paper. After filtration, the residue was mixed with another 50 mL of acetonitrile. The mixing and filtration procedures were repeated. The combined filtrate was transferred into a separating funnel containing 30 mL of acetonitrile-saturated n-hexane and shaken for 5 min. The acetonitrile layer was collected into a concentration bottle and evaporated to dryness at 40°C using a rotary evaporator.

2.5. Sample clean-up
The dry residue was reconstituted in 20 mL of 0.05 M sodium dihydrogen phosphate and applied onto a 1 g ENVI-18 SPE cartridge, which was pre-conditioned with 10 mL of methanol and 10 mL of 0.05 M sodium dihydrogen phosphate. The concentration bottle was washed twice with 5 mL of sodium dihydrogen phosphate, which was then applied onto the same cartridge. The eluant was discarded. The same concentration bottle was then washed twice with 5 mL of methanol and the resulting solution was passed through the same cartridge.

2.6. Instrument conditions
HPLC analysis was carried out on an Agilent 1260 automated HPLC series with a diode array detector operating at 270 nm wavelength with a 50 nm bandwidth. A 20 μL aliquot of samples was injected into Zorbax Eclipse Plus C-18 column (100 × 4.6 × 3, 5 μm) maintained at 40°C. A 90:10 binary system composed of 0.05 M NaH₂PO₄ (A) at pH of 5.10 adjusted with Na₂HPO₄ and acetonitrile (B) flowing at gradient is shown in Table 1.

| Table 1. Elution gradient of mobile phase (A): 0.05 M NaH₂PO₄ at pH of 5.10 adjusted with Na₂HPO₄ and phase (B): acetonitrile (90:10) |
|---|---|---|
| Time (mins) | Phase A | Phase B |
| 0 | 90 | 10 |
| 5 | 85 | 15 |
| 20 | 85 | 15 |
| 35 | 70 | 30 |
| 45 | 90 | 10 |
| 60 | 90 | 10 |
2.7. Standard calibration

The analytes were identified by comparing their retentions with those of the standards. Five concentrations (0.2, 0.5, 1.0, 1.5, and 2.0 μg/mL) of mixed standard were prepared and 20 μL of each was injected. Standard curves were plotted according to the peak areas versus concentrations. Sample and standard solutions were individually injected into the HPLC. Peak identification was made by comparing the retention times and spectra of samples with those of standards. Quantification was done using calibration curves prepared.

2.8. Recoveries and quality assurance

Recovery test was performed in triplicates by spiking standards at 3 different concentrations (0.1, 0.2, and 0.4 μg/L) of the drugs to 10 g samples. A 2-mL portion of a homogenized sample was spiked with 1 mL of the mixed standard. The spiked and blank samples were then analyzed by HPLC. Prior to sample analysis, standards for all drugs were analyzed to verify adequate system performance. Agreement of HPLC data with analyzed standards prior to sample analyses and in between five sample runs were satisfactory. Blank analyses were also performed in order to check interference from the sample. All analyses were done in three replicates. Each batch of sample analysis was prepared to include reagent blank in triplicate to control for background contamination and three spiked samples in triplicate to confirm satisfactory recovery greater than 70%.

The average recoveries of veterinary drugs in milk and dairy products were in the range of 78–97% and the correlation coefficient was ≥0.9991. Of the veterinary drugs, sulfamethoxazole showed the least recovery (78%) while chloramphenicol showed the highest average recovery (97%). The detection limits of the method were found by measuring, in five batches, the lowest concentrations of the analytes in each of the matrices that could be reproducibly determined at the operating conditions of the HPLC using a signal-to-noise ratio ≥10 to avoid false positive observations. The method detection limits were 0.1 μg/kg. Peaks were well resolved within 30 min run (Figure 1).

3. Results and discussion

The concentrations of the antibiotics determined were compared with the maximum residue limits (MRL) set by JECFA to ascertain possible health risks to consumers. All the concentrations were hundredfold lower than their MRL (Table 2).

Chloramphenicol is the only drug that was detected in both cheese and milk from the two major markets (Figure 2). It was also detected in yogurt samples from the KNUST sales point at the average concentration of 0.8 μg/L. Compared with its MRL of 100 μg/L (20), the low concentrations of
chloramphenicol found in yogurt is highly unlikely to cause any direct human health effects. Chloramphenicol is used both as human and veterinary antibiotic, although its veterinary use has been banned in many developed nations, including the USA. Chloramphenicol is allowed for restricted use in many developing countries, including Ghana. However, many poultry farmers interviewed in Nigeria were not aware of the legislation governing the use of chloramphenicol in food for animals (9). The presence of trace quantities of chloramphicol in human food could lead to bacteria resistance which could compromise its antibiotic use in humans.

Sulfathiazole was not detected in any of the samples. Apart from milk from Asafo and Kejetia that recorded 0.2 μg/L each, sulfamethoxazole was not detected in any other samples. The concentrations of sulfamethoxazole detected in the raw milk samples were too low to present health risks to human consumers. Sulfanamides including sulfamethoxazole, sulfathiazole, sulfamerazine, sulfadimidine, sulfadimidine, sulfadimethoxine, sulfanomethoxine, sulfaquinoxaline are commonly used on food-producing animals as growth promoters and as therapeutic and prophylactic drugs because they have antibiotic effects against a wide variety of bacterial and protozoan infections (21). Sulfanamides are synthetic antibiotics with a wide spectrum action against most gram-positive and many gram-negative organisms. Sulfanamides inhibit bacteria growth by competitively blocking the para-amino benzoic acid in the folic acid metabolism cycle (22, 23). The extensive use of sulfanamides as a result of their low cost has resulted in the increase of many sulfonamide-resistant strains of bacteria. As a result of their extensive usage, considerable attention has been paid to the potential human health risk due to their carcinogenic potency. People with sulfur allergy may also develop a type of hepatitis and kidney failure as a result of sulfur medications. Sulfonamides can affect the lung, with pneumonia-like reactions, worsening asthma, and vasculitis occurrence (22).

Table 2. Mean concentrations of residue detected in different products

<table>
<thead>
<tr>
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<th>Milk (μg/L)</th>
<th>Cheese (μg/kg)</th>
<th>Yoghurt (μg/kg)</th>
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<tbody>
<tr>
<td></td>
<td>Kejetia</td>
<td>Asafo</td>
<td>KNUST</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>0.12 ± 0.01</td>
<td>0.23 ± 0.01</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>Sulfathiazole</td>
<td>0.14 ± 0.01</td>
<td>0.11 ± 0.00</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>0.25 ± 0.01</td>
<td>0.22 ± 0.01</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>0.21 ± 0.00</td>
<td>0.26 ± 0.02</td>
<td>0.15 ± 0.01</td>
</tr>
</tbody>
</table>
Oxytetracycline was detected in milk samples from Asafo and Kejetia as well as in cheese from all the sampling sites. The mean milk concentration was 0.2 μg/kg. It was found at average concentrations of 0.1, 0.05, and 0.03 μg/kg, respectively, in cheese from Kejetia, Asafo and KNUST. These concentrations are lower than the MRL of 100 μg/kg (24) and do not present any risks to consumers. Of the 8 commercially available tetracycline, chlortetracycline, oxy-tetracycline, tetracycline and deoxytetracycline are most commonly applied to food-producing animals (19, 25–27). Oxytetracyclines are widely used in veterinary practice as feed additives for growth promotion in cattle and poultry because of their broad range of activity against bacteria and low cost (28). Overuse in cows and laying hens may result in oxytetracycline residues being present in meat, milk, and eggs can lead to human health problems. Side effects are mainly gastrointestinal and photosensitive allergic reactions common to the tetracycline antibiotics group. It can also damage calcium-rich organs, such as teeth and bones (29), although this is said to be very rare (30). It sometimes causes nasal cavities to erode (31). A cross-sectional study conducted in Ethiopia (32) detected oxytetracycline at a mean concentration of 125.25 μg/L in 12% of the 400 milk samples analyzed.

In general, samples from Kejetia and Asafo had more residues than those from KNUST. Except for chloramphenicol that was detected in yogurt and oxytetracycline in cheese, no other residues were found in samples from KNUST. This work, however, did not trace the original sources of the products. It was expected that cheese and yogurt being concentrated forms of milk will have higher concentrations of the residues but that trend was not very clear from the results obtained. The reason for this departure from the expected could be that the products are from different sources.

The use of antibiotics for growth promotion in livestock is unknown or seldom practiced in Ghana and many other African countries (33) as was observed in this study. This possibly explains why the antibiotic residues detected in this study were below their respective MRLs. This situation is contrary to what is found in poultry where antibiotics are incorporated into manufactured feeds which farmers might use without intentionally having growth promotion in mind (33). High antibiotic use is often common in countries where there is increased pressure on milk producers to increase milk production since antibiotics are widely used to treat common infections like mastitis. Dairying is, however, a young and developing industry in Ghana, and it is thus likely that the high use of antibiotics is not widespread; hence, the low levels of the antibiotic residues recorded in the various dairy products sampled in this study.

The low levels of the antibiotic residues recorded in this study could also be attributed to factors such as observance of withdrawal of antibiotic treatment during the milking stages of animals. High levels of antibiotic residues in milk products in other studies have been attributed to the non-observance of withdrawal periods from antibiotic therapy during milking. Another possible reason for the low levels of antibiotic residues recorded in this study could be the low or no usage of antibiotic treatments in dairying in Ghana. Low usage of antibiotics in dairying in Ghana, however, appears to be the main factor accounting for the low levels of residues found in the raw milk and milk products sampled in this study. A study in Ghana (34) indicated that only about 17% of livestock farmers in the Ashanti Region knew about withdrawal periods and antibiotic residues. That study also revealed that even though most farmers commonly used antibiotics without regard to the infecting organism or the part of the body that was affected (e.g. upper respiratory or gastrointestinal), the use of higher doses of antibiotics for treatment or growth promotion was not a common practice. The study concluded that antibiotics use for growth promotion and prophylaxis were largely unknown among livestock farms in the Ashanti Region. In measuring out the antibiotics, the farmers lacked adequate measuring instruments to mete out correct dosages (35). It is thus possible that most livestock farmers give less-than-required dosages for economic and cost-saving reasons and hence, the low levels of accumulated residues recorded in this study.

4. Conclusions
This study shows veterinary drugs residues such as chloramphenicol, sulfathiazole, sulfamethoxazole, and oxytetracycline are present in some milk, yogurt, and cheese being sold on the market. However,
their concentrations are far below the levels at which they could pose risks to the people who consume the diary product. Therefore, cheese, milk, and yogurt sold in Kumasi are safe for consumption as far as chloramphenicol, sulfathiazole, sulfamethoxazole, and oxytetracycline are concerned.

List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organisation</td>
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<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
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<tr>
<td>JECFA</td>
<td>Joint FAO/WHO Expert Committee on Food Additives</td>
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<tr>
<td>KNUST</td>
<td>Kwaame Nkrumah University of Science and Technology</td>
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<tr>
<td>M</td>
<td>Molar</td>
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<tr>
<td>mL</td>
<td>Millilitre</td>
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<tr>
<td>MRL</td>
<td>Maximum Residue Limits</td>
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<tr>
<td>n-hexane</td>
<td>Normal Hexane</td>
</tr>
<tr>
<td>SPE</td>
<td>Solid Phase Extraction</td>
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<tr>
<td>USA</td>
<td>United States of America</td>
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<tr>
<td>v/v</td>
<td>Volume per Volume</td>
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<tr>
<td>μg</td>
<td>Microgram</td>
</tr>
<tr>
<td>μL</td>
<td>Microlitre</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
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</table>

Authors’ contributions

Godfred Darko and Lawrence Sheringham Borquaye conceived the study and drafted the manuscript. All experiments were designed by Godfred Darko, Lawrence Sheringham Borquaye, Akwasi Acheampong, and Kwesi Oppong. Samples were collected by Godfred Darko and Kwesi Oppong. All the experiments were carried out by Kwesi Oppong under the guidance of Godfred Darko, Lawrence Sheringham Borquaye, and Akwasi Acheampong. All authors proof-read and approved the final manuscript.

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References


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