RESEARCH NOTE

DETECTION OF RESIDUES IN MILK FROM ANTIBIOTICS COMMONLY USED FOR TREATMENT OF MASTITIS IN SELECTED DAIRY CATTLE FARMS IN CALABARZON, PHILIPPINES USING AN OPTICAL BIOSENSOR

James Allan L. Cerrero, DVM[1], Arrol Jan B. Aquino, DVM[1]*, and Saubel Ezrael A. Salamat, MSc[2]

[1]Department of Basic Veterinary Sciences, College of Veterinary Medicine;
[2]Department of Veterinary Paraclinical Sciences, College of Veterinary Medicine,
University of the Philippines Los Baños, Laguna 4031, Philippines

ABSTRACT

The study was conducted to detect residues of antibiotics commonly used for the treatment of mastitis using an optical biosensor in milk samples collected from selected dairy cattle farms in CALABARZON (Region IV-A), Philippines. Antibiotic residues could cause health problems and could contribute to antimicrobial resistance. Thus, it is important to detect these residues in milk. Out of 129 samples tested, 43 were used in Anti-microbial Array II and all 129 samples were used in Beta-lactam Array Plus. Antibiotic residues from quinolones, ceftiofur, streptomycin, tylosin, tetracyclines, cephalexin, beta-lactams, and cefuroxime were detected. Among the positive results, three samples had residue levels beyond Maximum Residue Limit (MRL) wherein it was hypothesized that the drinking water of animals was the most probable source of the detected antibiotic residues. Tetracycline, beta-lactams, and a combination of beta-lactams and aminoglycoside drugs were the most used antibiotics. Farm factors such as route of antibiotic administration, dry cow therapy, intrauterine treatment, antibiotic cycling, and the presence of veterinarians in farms have no significant association with the occurrence of antibiotic residues beyond MRL. The results of the study suggest sufficient withdrawal period and proper usage of antibiotics during treatment and that milk, from the selected farms, are safe for consumption.

Keywords: *antibiotic residues, cattle, Maximum Residue Limit, milk, optical biosensor*

Philipp. J. Vet. Med., 59(1): 53-59, 2022

INTRODUCTION

Milk is considered important worldwide as it is food with high nutritional value and is consumed by the entire population from newborns to the elderly (Priyanka *et al.,* 2017). Thus, it is important to maintain the cows and their by-products healthy and free from diseases and contaminants as it may impose potential life threats to humans (Rathe and Al-Shaha, 2017).

Antibiotic residues are residual contamination of antibiotics on food of animal origin when animals were treated using antimicrobial drugs (Schlemper and Sachet, 2017). Administration of drugs in an extra-label fashion, not observing the specified withholding period for the drug after treatment, incorrect dosage and lack of records can most likely result in antibiotic residues in milk (Priyanka *et al.,* 2017; Rathe and Al-Shaha, 2017). Even at a low concentration, upon consumption by man, these residues can cause health problems such as allergy, intoxication, and antibiotic resistance.

In consideration of this imposing threat, regulatory authorities have decreed Maximum Residue Limits (MRLs) for some antibiotics in milk wherein detectable residues in milk higher than that of MRLs are deemed illegal (Priyanka *et al*., 2017; Rathe and Al-Shaha, 2017). Thus, it is important to detect in milk residues of antibiotics commonly used for treating mastitis in dairy cows.

To the extent of the author's knowledge, there are no published studies that were conducted in relation to the occurrence of antibiotic residues in milk in the Philippines. In this study, an optical biosensor that utilizes a

***FOR CORRESPONDENCE:**
(e-mail: abaquino@up.edu.ph)

chemiluminescence-based biochip array sensing technique was used in detecting residues in milk from antibiotics commonly used for the treatment of mastitis in selected dairy cattle farms in CALABARZON (Region IV-A), Philippines and to assess whether detectable residue levels exceed the MRL. The results of the study will help in providing data on residue levels of antibiotics in dairy cattle farms in the region and will also help authorities in implementing guidelines and regulations on the proper use of antibiotics.

MATERIALS AND METHODS

Dairy cattle farms

Dairy cattle farms selected for the study were highly dependent on the approval of its owners and availability. A total of eleven (11) different dairy cattle farms in CALABARZON, Philippines were visited and given a survey questionnaire to assess the antibiotics given to animals in treating mastitis, the route of administration in giving antibiotics (parenteral; intramammary), whether the animals were also given antibiotics due to other diseases, is practicing dry cow therapy and intrauterine treatment, and to determine if there is a veterinary consultant or in-house veterinarian in the farm. Dairy cattle farms of interest comprised of both commercial and cooperative farms mostly coming from Batangas province.

Dairy cattle and milk

Convenience and quota sampling methods were used for the milk sample collection. The number of cows for milk collection per farm was based on the availability of the animals during the collection and the owner's approval. A total of 118 raw milk samples from lactating cows were included in the study. In addition, a total of eleven (11) processed (sterilized) milk products from commercial sources were also included.

Milk collection

Milk was collected from lactating cows after specific pre-milking management/procedures were done in accordance with the farm protocol. Sterile tubes labeled with ear tag numbers and initials of the farm were prepared and 3-5 ml random quarter milk samples were collected by hand milking. Collected milk samples were immediately placed in an icebox, transported to the laboratory, and stored in a -80°C freezer until use.

Sample population

A total of 129 milk samples were collected from backyard farms (n = 11), commercial farms (n = 107), and processed milk products (n = 11). Forty-three (43) out of 129 milk samples were tested using Anti-Microbial Array II (AM II) for quinolones, ceftiofur, thiamphenicol, streptomycin, tylosin, and tetracyclines detection. All 129 milk samples were tested in Beta-Lactams Antibiotics Array PLUS (BLACT PLUS) in which amoxicillin, ampicillin, cloxacillin, dicloxacillin, nafcillin, oxacillin, penicillin G, penicillin V, cefacetril, cefazolin, cefoperazone, cefquinome, ceftiofur, cephalexin, cephalonium, cefapirin, cefuroxime, and cefadroxil could be quantitatively analyzed.

Detection of antibiotic residue

An optical biosensor was used to detect antibiotic residues in milk. The platform consists of immunoassay biochip kits (Randox Laboratories Ltd., UK), as well as a biochip analyzer instrument (Evidence Investigator™, Randox Laboratories Ltd., UK). It employs a multiplexed form of competitive chemiluminescent immunoassay and a set of known standards to detect and quantify multiple target antibiotic residues.

Chosen milk samples for testing were first thawed to room temperature before use and centrifuged at 2,880 relative centrifugal force (rcf) for 10 minutes. Biochips for the target analytes (antibiotics) in milk were used following the manufacturer's instructions. Two (2) immunoassay biochip kits were used in the study, namely: AM II and BLACT PLUS. The biochips contain an array of discrete test regions of immobilized antibodies specific to different antimicrobials.

A matching set of nine antimicrobial mixtures of known concentrations were used for each of the AM II and BLACT PLUS kits to generate calibration curves for quantification of the antimicrobial residues. One biochip carrier (containing nine biochips) was used for the standards for each kit. Milk samples were loaded, incubated, and subjected to reading using the biochip analyzer instrument.

Data analysis

Data coming from the optical biosensor in relation with the calibration curve for the specific immunoassay were compared to the published Maximum Residue Limits (MRLs) for antibiotics in milk according to Codex Alimentarius Commission (2015) which is adopted by the Philippines for its MRL for veterinary drug residues in food (Philippine National Standard, 2016).

Farm data coming from the survey

questionnaire were encoded in a spreadsheet for simple descriptive analysis and the Chi-square test ($p < 0.05$) was used to determine the effect of selected farm-level factors that may affect the occurrence of antibiotic residues beyond MRLs in milk.

RESULTS

Antibiotics used for mastitis treatment

A total of eleven (11) farms from Batangas (n = 6), Laguna (n = 4) and Quezon (n = 1) were included in the study. Different families of antibiotics were being used to treat bovine mastitis in the farms such as tetracycline in which three out of 11 farms (27.27%) were utilizing it; whereas five out of 11 farms (45.45%) were mainly using beta-lactam drugs; while, a combination of beta-lactam and aminoglycoside were being used by seven out of 11 farms (63.64%); and in contrast with aminoglycoside, fluoroquinolones, and cephalosporins, only one out of 11 farms (09.09%) were using these drug.

For diseases other than mastitis, either no antibiotics were declared used, else tetracyclines, fluoroquinolones, and a combination of beta-lactam and aminoglycosidewere utilized for treatment. Five (5) out of 11 farms (45.45%) did not administer antibiotics to the animals for other diseases. In four out of the 11 farms, tetracyclines were being used, one out of the 11 farms (09.09%) utilized fluoroquinolones, and three out of 11 farms (27.27%) used a combination of beta-lactam and aminoglycoside to treat other diseases in the farm.

Farm factors that may affect the occurrence of antibiotic residues

Survey results were collated and showed that among farms included in the study, nine out of 11 farms (81.82%) were found to be practicing the intramammary route of administration while only one out of 11 farms (9.09%) were practicing the intrauterine treatment of antibiotics whereas six out of the 11 farms (54.55%) were practicing dry cow therapy. Concerning farms using multiple antibiotics, seven (7) out of 11 farms (63.64%) were identified as using various drugs in treating diseases while only one out of the 11 farms (9.09%) was using only a single drug as a treatment regimen. In addition, nine out of 11 farms (81.81%) have veterinarians as consultants.

Detection of antibiotic residues using AM II

A total of one hundred twenty-nine (129) milk samples were included in the study wherein 43 samples coming from Batangas (n = 34), Laguna (n= 4), and Quezon (n = 5) were subjected to AM II immunoassay biochip (AM II). Among the milk samples that were tested, only one out of 43 (2.33%) milk samples (Table 1) had streptomycin residue level of 314 ppb, which is 1.57x higher than the prescribed MRL (200 ppb) for streptomycin (Table 2). Minimal amounts of antibiotic residues coming from quinolones, ceftiofur, streptomycin, tylosin, and tetracyclines were also detected with residue levels ranging from 0.01 - 0.19 ppb, 0.01 - 0.27 ppb, 0.09 - 20.32 ppb, 0.15 ppb, and 0.54 - 17.00 ppb, respectively.

Detection of antibiotic residues using BLACT PLUS

All one hundred twenty-nine (129) samples from Batangas (n = 85), Laguna (n = 28), Quezon (n = 5), and the processed milk products (n = 11) were utilized in BLACT PLUS immunoassay biochip. Two (2) out of 129 samples (1.55%) tested

Table 1. Number of milk samples with antibiotic residues in AM II beyond the MRL.

Area	Number of samples (%) with residues of antibiotics higher than MRL in AM II						
	No. of samples	QNL *(N/A)	CEFT *(100)	TAF *(N/A)	STR *(200)	TYL *(100)	TCN *(100)
Batangas	34	-	-	-	1/34 (3.00%)	-	-
Laguna	4	-	-	-	-	-	-
Quezon	5	-	-	-	-	-	-
Total	43	-	-	-	1/43 (2.33%)	-	-

(QNL) = Quinolones, (CEFT) = Ceftiofur, (TAF) = Thiamphenicol, (STR) = Streptomycin, (TYL) = Tylosin, (TCN) = Tetracyclines

* - MRL according to Codex Alimentarius Commission (2015) adopted by Philippine National Standard (2016)

Table 2. Range of concentration (ppb) of antibiotic residues in milk samples used in AM II within and above established MRLs.

Residue Level	Concentration (ppb) range of antibiotic residues in AM II					
	QNL *(N/A)	CEFT *(100)	TAF *(N/A)	STR *(200)	TYL *(100)	TCN *(100)
Above MRL	-	-	-	314.00	-	-
Below MRL	0.01 - 0.19	0.01 - 0.27	-	0.09 - 20.32	0.15	0.54 - 17.00

(QNL) = Quinolones, (CEFT) = Ceftiofur, (TAF) = Thiamphenicol,(STR) = Streptomycin, (TYL) = Tylosin, (TCN) = Tetracyclines
* - MRL according to Codex Alimentarius Commission (2015) adopted by Philippine National Standard (2016)

Table 3. Number of milk samples from dairy farms and processed milk products with antibiotic residues in BLACT PLUS above established MRLs.

Milk samples	Number of samples (%) with residues of antibiotics higher than MRL in BLACT PLUS			
	No. of samples	Cephalexin *(N/A)	Beta-Lactam *(4)	Cefuroxime *(N/A)
Batangas	85	-	2/85 (2.35%)	-
Laguna	28	-	-	-
Quezon	5	-	-	-
Processed milk products	11	-	-	-
Total	129		2/129 (1.55%)	

* - MRL according to Codex Alimentarius Commission (2015) adopted by Philippine National Standard (2016)

Table 4. Range of concentration (ppb) of antibiotic residues in milk samples from dairy farms and processed milk products used in BLACT PLUS within and above established MRLs.

Residue Level	Concentration (ppb) range antibiotic residues in BLACT PLUS		
	Cephalexin *(N/A)	Beta-Lactam *(4)	Cefuroxime *(N/A)
Farm milk samples			
Above MRL	-	5.70	-
Below MRL	0.01 - 0.13	0.02 – 2.40	0.01 - 0.92
Processed milk products			
Above MRL	-	-	-
Below MRL	0.01 - 0.02	0.25	0.01 - 0.06

* - MRL according to Codex Alimentarius Commission (2015) adopted by Philippine National Standard (2016)

(Table 3) had beta-lactam residue levels of 5.70 ppb, which is 1.43x higher than the prescribed MRL (4 ppb) for beta-lactams (Table 4). In the farm milk samples, low concentrations of antibiotic residues were also detected from beta-lactams, cephalexin, and cefuroxime with residue levels ranging from 0.02 – 2.40 ppb, 0.01 – 0.13 ppb, and 0.01 – 0.92 ppb, respectively.

Among the processed milk products, no

residues of antibiotics detected were beyond the prescribed MRLs of the drugs tested. The detected residues were only of minimal concentrations from beta-lactam, cephalexin, and cefuroxime with residual levels ranging from 0.25, 0.01 – 0.02, and 0.01 -0.06 ppb, respectively.

DISCUSSION

As the annual overall consumption of milk is steadily increasing together with the increasing population of humans worldwide, it is of utmost importance to make sure that milk for consumption is safe and free of contaminants such as antibiotic residues (Memili and Memili, 2015; Rathe and Al-Shaha, 2017).

Antibiotic residues detected in AM II and BLACT PLUS

In this study, forty-two (42) out of 43 samples (97.67%) utilized in AM II and 127 out 129 samples (98.45%) that were tested in BLACT PLUS have antibiotic residues within MRL. Among the forty-three (43) samples that were tested for residues of antibiotics in AM II, only one of the samples (2.33%) had streptomycin residue level of 314 ppb which is 1.57x higher than the prescribed MRL (200 ppb) for streptomycin, whereas two out of 129 samples (1.55%) utilized in BLACT PLUS both had beta-lactam residue levels of 5.70 ppb which are 1.43x higher than the prescribed MRL (4 ppb) for beta-lactam. The farms, where these milk samples were collected, were initially suspected of not following the proper withholding period and that they also used antibiotics extensively and inappropriately on the treated animals. However, according to the data gathered from the survey, all animals that had levels of antibiotic residues in milk beyond MRL had no history of antibiotic administration over the last three months or more. Nonetheless, the said farms were identified to be administering beta-lactams, tetracyclines, quinolones, and drugs with a combination of beta-lactams and aminoglycosides to animals for the treatment of mastitis and other diseases. It is possible that cross-reactions may have contributed to the detected residues beyond MRLs. False-positive reactions are also considered since the optical biosensor used in the study claims <5% false-positive results (6.45 false -positive results expected in 129 samples tested). The use of a confirmatory diagnostic technique (e.g., liquid chromatography) is essential to confirm the results obtained in the study. In a study conducted by Gaudin *et al.* (2016), it was stated that no cross-reactions were observed among the residues detected in muscle from different animal origin and aquaculture products. Their results also showed 100% sensitivity and 97.72% specificity using spectrophotometric detection as the confirmatory diagnostic method. A probable source of the minimal antibiotic residues detected is the drinking water source in the farm and water being utilized in the milking parlor. Antibiotics used in animals can be excreted in manure and urine thereby predisposing groundwater and surface water to antibiotic residue build-up (Watanabe *et al.*, 2010). In a study conducted by Tong *et al.* (2014) in Jianghan Plain, Central China, 19 antibiotics were detected on surface and groundwater wherein chlortetracycline, doxycycline, and enrofloxacin have the highest concentrations.

Commonly used antibiotics

Among the antibiotic residues detected using AM II, tetracycline incurred the most in which all (100%) of the samples had residues. As for BLACT PLUS, residues of cephalexin were present in the majority of the samples tested occurring in one hundred fourteen (114) out of 129 samples (88.37%). In the study conducted, the top three commonly used antibiotics in treating mastitis among the 11 farms were tetracyclines (27.27%), beta-lactams (45.45%), and a combination of beta-lactam and aminoglycoside drugs (63.64%). Furthermore, antibiotics were also being administered to animals with other diseases besides mastitis, wherein administration of tetracyclines was the most common (36.34%), followed by drugs with the combination of beta-lactam and aminoglycoside (27.23%), and lastly, quinolones (9.09%). As the said drugs were the most common ones to be administered to animals, it is more likely that residue build-up will be observed in the milk samples. In comparison with studies conducted by Korb (2011) and Vieira *et al.* (2012) as cited by Trombete *et al*. (2014), in antibiotic residues in Brazilian milk, they found out that oxytetracycline and penicillin G were the prevailing residues of antibiotics in their milk samples as these drugs were the commonly used antimicrobials in treating bovine mastitis.

Farm factors associated with the occurrence of residues

Intramammary infusion of antibiotics may contribute to the presence of antibiotics in milk (Gradinaru *et al.*, 2011). According to Samarzija *et al*. (2002) and Ruegg (2013) as cited by Fejzic *et al.* (2014), parenteral administration of antibiotics is excreted relatively faster through

milk compared to intramammary administration in which residues stay the longest and in highest concentrations. In comparison, the survey conducted in the present study found nine out of the 11 farms (81.82%) were practicing intramammary infusion when administering antibiotics to the animals which could therefore add up to the impending risk of antibiotic contamination in milk. However, the Chi-square test analysis revealed that there is no significant association between the intramammary route of administration of antibiotics and the occurrence of antibiotic residues in milk samples beyond MRL ($p > 0.05$).

Intrauterine infusion of antibiotics is the practice of administering antimicrobials through the reproductive tract. According to Jones (2009), intrauterine infusions of drugs have resulted in residues of antibiotics in milk after 24-48 hours post-infusion. In the present study, only one (1) out of the 11 farms (9.09%) practiced intrauterine infusion of drugs on their animals. Chi-square test analysis also showed no significant association between intrauterine infusion of antibiotics and the occurrence of antibiotic residues in milk samples beyond MRL ($p > 0.05$).

Dry cow therapy is the treatment of intramammary infections at the end of lactation to prevent new infections during the drying-off period (Berry and Hillerton, 2002). Due to the vast use of antibiotics in this procedure, public health concerns in relation to antimicrobial resistance and antibiotic residues in food of animal origin were being carefully monitored (Berry and Hillerton, 2002). In the study conducted, six (6) out of 11 farms (54.55%) were practicing dry cow therapy which could have led to antibiotic residues being detected in the tested samples. Again, the Chi-square test analysis revealed no significant association between the practice of dry cow therapy and the occurrence of antibiotic residues in milk samples beyond MRL ($p > 0.05$).

Antibiotic cycling is the rotational usage of antibiotics wherein different antibiotics are cyclically used to prevent antimicrobial resistance (Beardmore *et al.,* 2017). A study (Beardmore *et al.*, 2017) suggests that antibiotic cycling has the potential of reducing the risk of residue contamination through decreasing and/or preventing the extended use of antibiotics during treatment. In the study conducted, seven (7) out of 11 farms (63.64%) were using multiple drugs in treating diseases in the farm which could contribute to lower levels of antibiotic residues detected in the study. There was no significant association between the practice of antibiotic cycling and the occurrence of antibiotic residues in milk samples beyond MRL using the Chi-square test ($p > 0.05$).

The use of antibiotics in food animals by non-veterinarians is highly discouraged as this practice may contribute to the presence of antibiotic residues in food of animal origin (Chowdhury *et al.,* 2015). In the study conducted, nine (9) out of 11 farms (81.82%) had veterinarians as consultants capable of giving proper prescription and administration of antibiotics to animals. Again, there was no significant association between the presence of veterinarians on the farm and the occurrence of antibiotic residues in milk samples beyond MRL using the Chi-square test ($p > 0.05$).

The results obtained in the study highly suggest that the milk samples with antibiotic residues within the MRL obtained from the dairy cattle farms in Batangas, Laguna and Quezon are safe for human consumption. The farms where milk samples showed antibiotic residues beyond the prescribed MRLs did not declare recent use of antibiotics however, these farms also previously used beta-lactams, tetracyclines, quinolones, and drugs with a combination of beta-lactams and aminoglycosides to animals with mastitis and other diseases. These findings necessitate the conduct of confirmatory diagnostic methods to these samples such as the use of high-performance liquid chromatography-tandem with mass spectrophotometry (HPLC-MS). The detection of antibiotic residues in milk beyond MRLs in the study is alarming because of its potential threat to direct toxicity in humans once ingested such as immunogenic reactions, alterations of gut microflora, and the possible development of antimicrobial resistance. The findings in the study could help authorities provide further implementing guidelines and regulations on the proper use of antibiotics in the dairy cattle farm industry. These include the discriminate and rational use of antibiotics in these animals, proper observation of withdrawal period following its use, and the recommendation for the usage of antibiotics in food animals only by veterinarians. This entails a consensual effort in the industry including awareness creation, effective surveillance, monitoring, and control, on the use of these veterinary products to prevent the occurrence of drug residues in foods, such as in milk, for human consumption.

ACKNOWLEDGEMENT

The authors would like to extend their sincerest gratitude to the "Profiling of Economically Important Diseases of Swine and

Cattle in the Philippines for Enhanced Disease Management, Surveillance, and Control" project funded by the United States Department of Agriculture through the United States Public Law 480 (PL-480) Title-I Program, and administered through the Department of Agriculture – Philippine Council on Agriculture and Fisheries (DA-PCAF). In addition, the authors would also like to acknowledge Dr. Loinda R. Baldrias and Dr. Jovencio Hubert A. Abalos of the University of the Philippines Los Baños, College of Veterinary Medicine for sharing their knowledge for the improvement of the study, as well as to the National Dairy Authority South Luzon Department for their assistance in the dairy cattle farm contact and sample collection process.

STATEMENT OF COMPETING INTEREST

The authors have no competing interests to declare.

AUTHOR'S CONTRIBUTION

JALC contributed to the development of the methodology, performed the experiment, collected and analyzed the data, wrote the original draft, and reviewed and edited the manuscript. AJBA and SEAS conceptualized the study, designed the methodology, provided some resources, supervised the research activity, assisted in the analysis of data, and reviewed and edited the manuscript.

REFERENCES

Beardmore RE, Peña-Miller R, Gori F and Iredell J. 2017. Antibiotic cycling and antibiotic mixing: which one best mitigates antibiotic resistance?. *Molecular Biology and Evolution* 34 (4): 802-817.

Berry EA and Hillerton JE. 2002. The effect of selective dry cow treatment on new intramammary infections. *Journal of Dairy Science* 85(1): 112-121.

Chowdhury S, Hassan MM, Alam M, Sattar S, Bari MS, Saifuddin AKM and Hoque MA. 2015. Antibiotic residues in milk and eggs of commercial and local farms at Chittagong, Bangladesh. *Veterinary World* 8(4): 467-471.

Fejzic N, Begagic M, Seric-Haracic S and Smajlovic M. 2014. Beta lactam antibiotics residues in cow's milk: comparison of efficacy of three screening tests used in Bosnia and Herzegovina. *Bosnian Journal of Basic Medical Sciences* 14(3): 155-159.

Gaudin V, Hedou C, Soumet C and Verdon E. 2016. Evaluation and validation of a multi-residue method based on biochip technology for the simultaneous screening of six families of antibiotics in muscle and aquaculture products. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment* 33(3): 403-419.

Gradinaru AC, Popescu O and Solcan G. 2011. Antibiotic residues in milk from Moldavia, Romania. *Human and Veterinary Medicine Bioflux* 3(2): 133-141.

Jones GM. 2009. On-farm tests for drug residues in milk. https://www.pubs.ext.vt.edu/404/404-401/404-401.html. Accessed 09 April 2017.

Memili A and Memili E. 2015. Antibiotic residues detected in commercial cow's milk. *Journal of Emerging Investigators* 1-4.

Philippine National Standard. 2016. *Veterinary drug residues in food: Maximum Residue Limits (MRLs).* Quezon City, Philippines: Bureau of Agriculture and Fisheries Standards.

Priyanka, Panigrahi S, Sheoran MS and Ganguly S. 2017. Antibiotic residues in milk- a serious public health hazard. *Journal of Environment and Life Sciences* 2(4): 99-102.

Rathe HAA and Al-Shaha OM. 2017. Detection of antibiotic residues in milk and milk products of cattle in dairy farms in Baghdad region. *Journal of Entomology and Zoology Studies* 5 (3): 1797-1802.

Schlemper V and Sachet AP. 2017. Antibiotic residues in pasteurized and unpasteurized milk marketed in southwest of Parana, Brazil. *Ciencia Rural* 47(12): 1-5.

Tong L, Huang S, Wang Y, Liu H and Li M. 2014. Occurrence of antibiotics in the aquatic environment of Jianghan Plain, central China. *Science of the Total Environment* 497-498(1): 180-187.

Trombete FM, dos Santos RR and Souza ALR. 2014. Antibiotic residues in Brazilian milk: a review of studies published in recent years. *Revista Chilena de Nutrition* 41(2): 191-197.

Watanabe N, Bergamaschi BA, Loftin KA, Meyer MT and Harter T. 2010. Use and environmental occurrence of antibiotics in freestall dairy farms with manured forage fields. *Environmental Science & Technology* 44(17): 6591-6600.