Food Sci. Preserv., 31(6), 1057-1062 (2024)
pISSN: 3022-5477, eISSN: 3022-5485

Food Science and Preservation
https://doi.org/10.11002/fsp.2024.31.6.1057

Research Note

Tetracycline residues in retailed pork meat and liver in public markets of Davao city, Philippines

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Abstract The misuse of antibiotics, including tetracycline, is a significant issue in veterinary medicine because of their widespread use, leading to antibiotic residues in animal tissues. Residues pose health risks to consumers and promote antimicrobial resistance. This study aimed to detect tetracycline residues in retail pork meat and liver samples from selected public markets in Davao city. A total of 146 pork samples were tested, comprising 74 meat and 72 liver samples. Detection was performed using enzyme-linked immunosorbent assay (ELISA). The results showed that all samples were positive for tetracycline residue with a mean concentration level of 67.12±11.29 ng/g for meat samples and 74.01±16.43 ng/g in liver samples. However, none reached or exceeded the maximum residue levels of 200 ng/g and 600 ng/g for meat and liver, respectively. Liver samples had significantly higher mean tetracycline concentrations than meat samples from three public markets in Davao city. The detected levels were within the regulatory limits, and the significant difference between liver and meat samples highlights the need for continued monitoring of antibiotic residues in different animal tissues.

Keywords tetracycline residues, maximum residue level, food safety

Citation: Develos KMS, Nerio KJO, Fuentes KDC, Lanaban AB, Mapundo KJB, Guillen CAKO, Dumagan HMB, Buelis GCL, Cadotdot NM, Insular NL, Northrup CMA, Rivera JM, Hinay AA Jr. Tetracycline residues in retailed pork meat and liver in public markets of Davao city, Philippines. Food Sci. Preserv., 31(6), 1057-1062 (2024)

Received: October 21, 2024
Revised: November 10, 2024
Accepted: November 22, 2024

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1. Introduction

Antibiotic misuse in veterinary medicine leads to residues in animal products, posing significant human health risks owing to exposure to these residues or the spread of antibiotic resistance among foodborne pathogens (Araby et al., 2020). Tetracycline (TC), a commonly used broad-spectrum antibiotic developed in 1940, is effective against various infectious diseases owing to its antibacterial properties, affordability, minimal side effects, and therapeutic efficacy (Xue et al., 2023). It works well against gram-positive and gram-negative bacteria, such as *Ornithobacterium rhinotracheale*, *Pasteurella*, *Clostridium*, *Mycoplasma,* and protozoa (Granados-Chinchilla and Rodríguez, 2017). However, tetracycline accumulates in soil, water, and food items, posing risks to human health and the ecosystem.

Despite their widespread use, bacterial resistance has reduced the efficacy of tetracyclines in veterinary and human medicine. The use of these antibiotics as animal growth promoters is becoming increasingly controversial owing to concerns about resistance to human diseases (Kavya et al., 2023;

Prestinaci et al., 2015). The presence of tetracycline residues in pork and other animal products is a significant concern with potential health implications for consumers. International regulatory bodies have established maximum residue limits (MRLs) for tetracyclines in animal products, with the FDA setting an acceptable daily intake (ADI) of 25 ng/g body weight per day. At the same time, the European Commission's MRLs range from 100 ng/g to 600 ng/g depending on the tissue (Sánchez et al., 2004). The Philippine national standards (PNS) have set MRLs at 1,200, 600, and 200 ng/g for kidney, liver, and pork muscle tissues, respectively (Toralba et al., 2020). To detect these residues, high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS) is considered the gold standard due to its high sensitivity and specificity. Other detection methods include HPLC with UV or fluorescence detection, capillary electrophoresis, microbiological assays, and spectroscopic methods (De Wasch et al., 1998). Enzyme-linked immunosorbent assays (ELISA) have gained popularity owing to their rapid results, cost-effectiveness, high-throughput capability, minimal sample preparation, and high sensitivity, making it an excellent tool for screening large numbers of samples (De Wasch et al., 1998; Kim et al., 2013; Ramatla et al., 2017). The consumption of contaminated pork poses several health risks, including antimicrobial resistance, disruption of the gut microbiota, allergic reactions, and potential long-term health effects. Addressing this issue requires attention from regulatory bodies, farmers, and consumers alike to implement stricter controls, improve farming practices, and raise awareness of the risks associated with antibiotic residues in food products.

In the Philippines, a significant producer and consumer of pork, the use of antibiotics for food safety is a major issue. Therefore, the detection of antibiotic residues in food is essential for human consumption. With the pressing issues stated above, this study aimed to determine tetracycline residues in pork meat and liver in Davao city public markets, inform the public of potential health risks, and encourage regulatory authorities to enforce strict guidelines on drug use and ensure proper withdrawal times before slaughter.

2. Materials and methods

2.1. Research locale

Davao city is strategically positioned in the southeastern part of Mindanao Island, the Philippines. As the regional capital of the Davao Region (Region XI), the city spans 2,443.61 square kilometers with a population of 1,776,949 as of the 2020 census. The city is administratively divided into 182 barangays and encompasses a diverse urban landscape comprising three distinct geographical districts.

The public markets A, B, and C selected for this study represent each of these geographical districts, offering a comprehensive view of the city's retail pork distribution. Market A, located in the urban center, represents District I and reflects the most densely populated commercial zone. Market B, situated in a transitional area, represents District II and may exhibit intermediate market characteristics. Market C, located in a more peripheral district, represents District III and demonstrates different market dynamics and pork-handling practices. These markets are managed by city economic enterprises (CEE), an organization responsible for overseeing market operations, vendor regulations, and economic activities. The CEE's systematic approach to market management ensures standardized practices across districts. By selecting these representative markets, this study aims to capture nuanced variations in pork quality, handling practices, and market dynamics across Davao city's diverse urban landscape, thereby providing critical insights into local food safety and economic systems.

2.2. Sampling procedure

Retailed pork, particularly muscle and liver, served as study samples. The selection of pork tissue was based on a previous study that showed a concentration of tetracycline residue in muscle and higher amounts in liver samples (Toralba et al., 2020). Stratified random sampling with proportional allocation was then performed. Each of the three public markets in the three districts of Davao city, Philippines was considered a stratum. Pork stalls served as the sampling unit, and pork muscle and liver served as the elementary units. Interval systematic sampling was used to select stalls to purchase pork muscle and liver samples. Proportional allocation of samples from each market was employed. The researchers did not consider other variables such as the meat distributor, specific parts of the pork meat, or other practices related to how the retail pork meat and liver were prepared. The sample size was determined using OpenEpi ver. 2.3.1, with an assumed prevalence of 29.4% (Ramatla et al., 2017).

https://doi.org/10.11002/fsp.2024.31.6.1057

At a confidence level of 95%, 36 pork meat and 34 liver samples were obtained from Market A 14 meat and 14 liver samples from Market B, and 24 pork and 24 liver samples from Market C. A total of 74 pork meat and 72 liver samples were collected.

2.3. Sample preparation and assay

Each sample of pork meat and liver was homogenized and weighed 2 g each, as indicated by the Tetracycline Elisa Kit Package insert (ELABSCIENCE, Houston, TX, USA). Two grams of the homogenized sample was placed in a centrifuge tube, and 4 mL of dimethylformamide (DMF) was added. The sample was vortexed for 5 min to fully mix with the liquid before it was centrifuged at 2,000 ×*g* for 10 min at room temperature. Subsequently, 250 μL of the supernatant was added to 750 μL of reconstitution buffer and mixed thoroughly.

Each well contained 50 μL of lower aqueous phase. An ELISA plate reader (ELx50, Bio Tek, CA, USA) was used to measure absorbance at 450 nm (reference wavelength 630 nm). Six calibration standard solutions were used in this study, and a negative control was used. The dilution factor used in this assay was 8. TC levels were expressed in μg/kg of the examined tissues. Meat samples with recovery rates of around >99% and a mean lower detection limit of about 0.05 μg/kg for the Elabscience TC test.

2.4. Data analysis

Each sample was analyzed in triplicate. Statistical analysis was performed using SPSS for Windows (version 16.0, SPSS Inc., Chicago, IL, USA). The Mann-Whitney U test was used to compare the results of the total TCs between the meat and liver samples. The significance level was set at $p<0.05$. A 95% confidence interval (CI) was calculated based on the mean concentration and standard deviation (SD) of the swine meat and liver samples.

3. Results and discussion

3.1. Concentration of tetracycline residues in retailed pork meat and liver

Table 1 presents the mean concentrations of tetracycline residues in retail pork meat and liver samples collected from public markets in Davao city. The market with the highest mean concentration of tetracycline residues in pork meat is market B, with a mean of 71.50±8.81 ng/g. The market with the lowest mean concentration of tetracycline residues in pork meat is market A, with a mean of 63.35±14.02 ng/g. Consequently, the pork liver samples collected from market A had the highest average concentration of tetracycline residues, with a mean of 78.34±18.02 ng/g. In contrast, market C had the lowest mean concentration of tetracycline residues in pork liver, at 67.17±17.27 ng/g.

The elevated tetracycline residue levels observed in the meat and liver of Davao city pork samples likely reflect the widespread use of tetracyclines in local swine production, both for therapeutic purposes and as growth promoters. Several factors may contribute to this prevalence, including the accessibility and cost-effectiveness of tetracyclines, their broad-spectrum efficacy against various pathogens, and their potential growth-promoting effects (Amangelsin et al., 2023; Nguyen et al., 2014).

These findings align with broader trends observed in other Southeast Asian countries. For instance, a study in Hanoi,

Table 1. Mean concentration level of tetracycline residues in retailed pork meat and liver in Davao city public markets

Swine parts	MRL[1)] in ng/g	Market A		Market B		Market C		Average		p-value
		n	Tetracycline residues	*n*	Tetracycline residues	*n*	Tetracycline residues	N	Tetracycline residues	
Meat	200	36	63.35±14.02[2)] [59.92, 66.77][3)]	14	71.50±8.81 [69.34, 73.65]	24	66.52±11.05 [63.82, 69.21]	74	67.12±11.29 [64.36, 69.87]	0.008[4)]
Liver	600	34	78.34±18.02 [73.74, 82.93]	14	76.52±13.99 [72.94, 80.09]	24	67.17±17.27 [62.76, 71.57]	72	74.01±16.43 [69.81, 78.20]	0.008

[1)]MRL, maximum residue limit.
[2)]Mean±standard deviation.
[3)]Confidence interval [CI] at 95%.
[4)]Significant difference in total tetracycline resides between liver and meat at $p<0.05$.

Vietnam, found that 5.5% of pork samples contained tetracycline residues, with higher proportions in suburban areas than in urban districts (Van Nhiem et al., 2006). Similarly, research on porcine stomach tissues in the United States has demonstrated that tetracycline residues can persist even after an established withdrawal period, highlighting the complexity of managing antibiotic use in livestock (Ghimpețeanu et al., 2022).

This study employed an ELISA to detect tetracycline. Although ELISA is widely used because of its rapid and cost-effective screening capabilities, it is important to acknowledge its limitations in precision compared to HPLC. Nevertheless, ELISA remains a valuable tool for initial screening and has been successfully used in similar studies (De Wasch et al., 1998; Kim et al., 2012; Pham Kim et al., 2013). Despite potential limitations, the results of this ELISA-based study provide substantial evidence for the use of tetracycline in swine production in Davao city. While the levels detected in Davao city were below the established MRL of 200 ng/g for pork and 600 ng/g for the liver, their presence underscores the need for monitoring and management of antibiotic use in livestock, contributing to broader efforts to address antimicrobial resistance and ensure food safety.

3.2. Significant difference in tetracycline concentration between meat and liver sold in Davao city public markets

Table 1 presents that the mean tetracycline concentration in pork liver (74.263 ng/g) was significantly higher than that in pork meat (66.809 ng/g). This difference was statistically significant at the 5% level ($p<0.05$). The higher concentration in the liver than in muscle meat is consistent with the role of the liver in drug metabolism (Cammilleri et al., 2019). This pattern has also been observed in other studies, such as those that found higher streptomycin residues in liver samples (87.5%) than in meat samples (38.46%) (Abdullah et al., 2012; Araby et al., 2020). The function of the liver as the primary organ for detoxification explains why it often accumulates higher levels of antibiotics. Moreover, Tetracycline residues are present in pork meat primarily due to the administration of these antibiotics in livestock farming. Farmers often use tetracyclines for therapeutic and growth-promoting purposes in pigs, which can lead to residual levels remaining in the meat at the time of slaughter.

The findings regarding antibiotic residues in liver and meat samples, with mean concentrations of 74.263 ng/g in the liver and 66.809 ng/g in meat, although below the MRLs, raise significant concerns about food safety. The higher concentration of residues in the liver suggests that it may serve as a sensitive indicator of antibiotic use in livestock, emphasizing the need for targeted monitoring. Consumers who frequently consume liver products could be exposed to higher levels of these residues, particularly because some studies have indicated the presence of multiple antibiotic residues in single samples, leading to cumulative exposure risks. This situation highlights the ongoing use of antibiotics in livestock and raises questions about current practices and the potential development of antibiotic resistance.

However, it is crucial to note that there is a pressing need to increase the sample size in future studies. A larger sample size would provide more robust and statistically significant results, thereby offering stronger support for these findings. Expanding the scope of sampling to include a wider range of livestock sources, geographical locations, and time periods would enhance the reliability and generalizability of the results. This increased sample size would allow for more accurate assessment of the prevalence and distribution of antibiotic residues across different animal products and production systems.

4. Conclusions

In conclusion, although the detected levels were within regulatory limits, the significant difference between liver and meat samples highlights the need for continued monitoring of antibiotic residues in different animal tissues. It also underscores the importance of proper antibiotic use in livestock and adherence to withdrawal periods before slaughter.

Funding

This research was funded by the University of Immaculate Conception Research and Innovation Center, Davao city, Philippines.

Acknowledgements

None.

Conflict of interests

The authors declare no potential conflicts of interest.

https://doi.org/10.11002/fsp.2024.31.6.1057

Author contributions

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Ethics approval

This research was approved by IRB of The University of The Immaculate Conception Protocol Code UG-0060-02-24.

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https://doi.org/10.11002/fsp.2024.31.6.1057