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## Tetracycline Residues in Milk and Vitreous Humor: Risks of Indirect Antibiotic Resistance

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### Abstract:

**Background:** Since antibiotics became widely used in both human and veterinary medicine, they have been instrumental in fighting bacterial diseases that were once difficult or impossible to treat. However, misuse inevitably increases the risk of selecting resistant bacteria, which poses a major public health concern. The "One Health" concept highlights the interconnectedness of human, animal, and environmental health, recognizing that the environment can serve as a reservoir for resistant microorganisms.

**Objectives:** This study aimed to evaluate indirect antibiotic contamination from animal-derived food, focusing on tetracyclines, which are widely used in animal feed and veterinary treatments. Residues of tetracyclines can remain in animal-based food products such as meat and milk. Notably, their transmission poses a threat to human health.

**Methods:** A comprehensive analysis was conducted using High-Performance Liquid Chromatography (HPLC) to detect tetracycline residues in milk and meat samples collected from commercial sources. The methodology included sample preparation and analysis to quantify the levels of contamination.

**Results:** HPLC analysis revealed tetracycline residues exceeding 100 µg/L in milk and over 20 µg/L in sheep's vitreous humor, indicating a concerning prevalence of contamination in animal-derived food products.

**Conclusion:** These findings highlight the public health risks of indirect antibiotic contamination in food products of animal origin. The study calls for stricter monitoring of antibiotic residues and a reevaluation of current veterinary practices, emphasizing the importance of the "One Health" approach in addressing antibiotic resistance.

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## INTRODUCTION

For centuries, pure fresh milk has been regarded as a healthy source of essential nutrients. Today, milk production represents an important economic activity worldwide. In 2020, over 853 million tons of milk were produced globally, with the European Union, India, and the United States being the main producers [1]. Ensuring milk safety is a critical public health concern, as antibiotic residues and microbial contamination can lead to bacterial resistance, cause allergies or hypersensitivity in humans, and disrupt dairy industry processes. For instance, residues may inhibit or slow the growth of lactic cultures during yogurt and cheese production, resulting in substantial financial losses.

Since the discovery of antibiotics, it has been well established that their misuse accelerates the development of bacterial resistance—a risk further exacerbated by unregulated prescriptions and over-the-counter availability in many regions. Van Boeckel *et al.* (2015) highlighted that globally, antibiotic consumption in food-producing animals far exceeds that in humans [2]. This intensive use spans cattle farming, poultry, aquaculture, and apiculture, not only for therapeutic purposes but also for prophylaxis and growth promotion, and is expected to increase further as livestock production shifts toward large-scale intensive systems.

Among veterinary antibiotics, tetracyclines, particularly chlorotetracycline and oxytetracycline, are among the most widely used. They are administered to livestock for growth promotion, stress management, and treatment of respiratory, intestinal, renal, and skeletal disorders [3]. Oxytetracycline is also employed in crop

protection as a pesticide and in aquaculture to stimulate fish growth and control infectious diseases [4].

Pharmacologically, tetracyclines distribute broadly in tissues and body fluids, cross the placenta and serous membranes, and are eliminated via the kidneys, bile, and milk, sometimes undergoing enterohepatic recirculation [5,6]. Their transfer from blood to milk occurs through diffusion across the mammary gland epithelium, which separates blood (pH 7.4) from milk (pH 6.6).

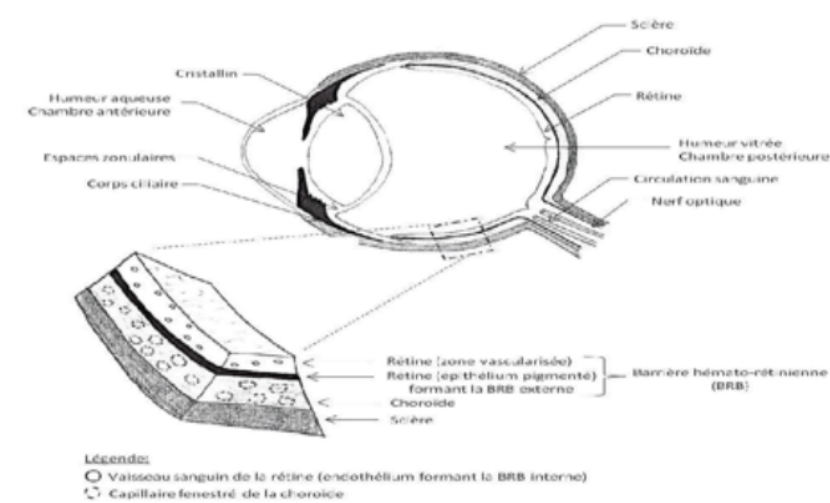
The medical risks associated with veterinary drug residues are well documented. Allergic reactions, particularly due to penicillins and tetracyclines, have been frequently reported [7]. Antibiotic residues in milk may disrupt intestinal microflora [8], while fetal or neonatal exposure to tetracyclines can lead to tooth discoloration, dental bud abnormalities, or delayed skeletal development.

For these reasons, regulatory assessment of antibiotic residues is based on the determination of the No-Observed-Effect Level (NOEL), the Acceptable Daily Intake (ADI), and the Maximum Residue Limit (MRL).

## MATERIALS AND METHOD

Risk assessment was conducted by quantifying "tetracycline" in raw milk samples collected either directly from farms or indirectly from vendors at points of sale. Packaged commercialized milk samples were also analyzed.

Additionally, analyses were performed on the vitreous humor of cows subjected to slaughter.



**Figure 1:** Anatomy of the animal eye and location of vitreous humor (VH) sampling in animals.

The vitreous humor is an ideal medium for measuring substances that diffuse, degrade, or are rapidly eliminated from the general circulation [9].

All samples were stored at -20°C prior to analysis.

Felby concluded that analyzing vitreous humor (VH) is technically easier than analyzing blood, especially in cases of decomposition [10].

The kinetic properties support the feasibility of measuring substances in VH, making it an ideal medium for the quantification of compounds that diffuse, degrade, or are rapidly eliminated from general circulation, due to the slow metabolic exchange between the general circulation and the VH [11].

Sampling is performed using a syringe. To avoid collecting epithelial cells from the retina or iris, aspiration must be done slowly at the center of the eye, and the volume extracted should be limited to 2 mL per eye, even though the total volume of VH is higher [12].

Quantitative analysis was performed using a SHIMADZU HPLC system (SPD-820A) equipped with an Ultra C18 column (5 µm, 150 × 4.6 mm). This system was selected due to its high sensitivity, reproducibility, and reliability for detecting antibiotics such as tetracycline in food and biological samples. This model has been previously reported in similar analytical studies [13].

The operating conditions were defined according to the protocol [13]:

- Wavelength: 270 nm
- Pressure: 14.5 MPa
- Temperature: 35 °C
- Flow rate: 1.5 mL/min
- Injection volume: 5 µL

The method was validated in accordance with the SFSTP (Société Française des Sciences et Techniques Pharmaceutiques) guidelines, 2006, ensuring reliability, accuracy, and reproducibility of the results.

## RESULTS

A total of 42 milk samples (from street vendors, farms, and commercialized sources) and 18 vitreous humor

(VH) samples were analyzed. Although the sample sizes are relatively limited, the obtained data provide useful insight into the presence of tetracycline residues in the studied matrices.

### 1. Concentrations in Milk Sold by Street Vendors

**Table 1: Tetracycline Residue Concentrations in Milk Sold by Street Vendors**

Statistic	Value (µg/L)
Mean	102.64
Standard Deviation	47.01
Minimum	40
Maximum	223

### 2. Concentrations in Farm Milk

**Table 2: Tetracycline Residue Concentrations in Farm Milk**

Statistic	Value (µg/L)
Mean	86.30
Standard Deviation	45.73
Minimum	44
Maximum	187

### 3. Concentrations in Commercialized Milk

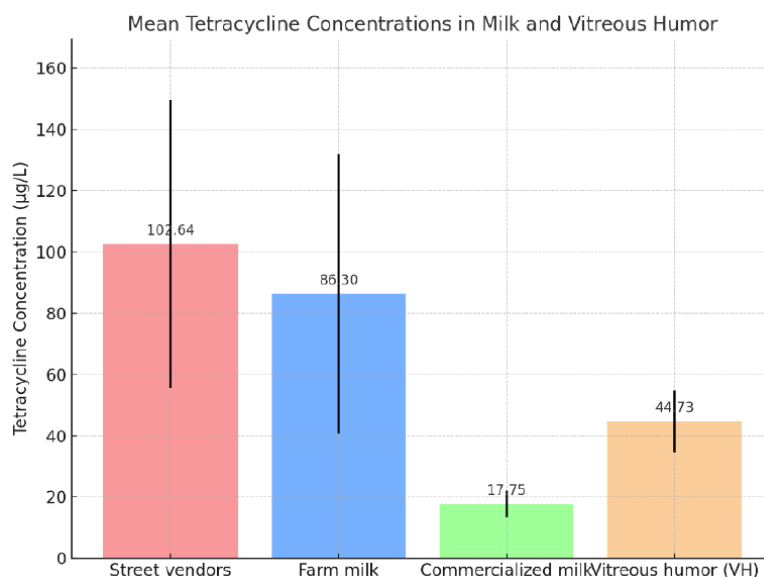
**Table 3: Tetracycline Residue Concentrations in Commercialized Milk**

Statistic	Value (µg/L)
Mean (excluding below LOD)	17.75
Standard Deviation	4.27
Minimum	12
Maximum	22

### 4. Concentrations in Vitreous Humor (VH)

**Table 4: Tetracycline Residue Concentrations in Vitreous Humor (VH)**

Statistic	Value (µg/L)
Mean (excluding below LOD)	44.73
Standard Deviation	10.09
Minimum	29
Maximum	62



**Figure 2:** Distribution of mean tetracycline concentrations ( $\pm$  SD) in street vendor milk, farm milk, commercialized milk, and vitreous humor.

Figure 2 shows that tetracycline concentrations are markedly higher in milk from street vendors and farms, whereas commercialized milk exhibits the lowest levels. Vitreous humor displays intermediate concentrations, suggesting possible systemic exposure to residues.

## DISCUSSION

The maximum residue limits (MRLs) are based on the type and amount of residue considered toxicologically safe for human health. They are expressed as acceptable daily intakes (ADIs) or, in some cases, as temporary ADIs using an additional safety factor. The FDA has set a concern level for tetracycline residues in milk at 80  $\mu\text{g/L}$  [14]. In 2019, a survey revealed that MRLs are exceeded in less than 1% of cases in Europe but in more than 65% of cases in Africa, including Algeria. A 2013 study conducted by the Algerian Program for Monitoring Food Residues and Contaminants (PASCRA) did not result in any publication [15]. In the United States, a 2007 study reported that the MRLs for tetracycline should be 2 ppm in muscles, 6 ppm in the liver, and 12 ppm in the kidneys, while the limit is set at 100  $\mu\text{g/L}$  in milk and meat for European Union countries [16]. More recently, Costa *et al.* (2024) confirmed that tetracyclines remain the most prevalent antibiotic residues in raw milk globally, with higher non-compliance rates in Africa compared to Europe [17].

The results of this study show that raw milk sold through informal markets is the most contaminated,

with 40.9% of samples exceeding the MRLs, compared to 30% for milk collected from farms. These findings are close to those of Mesgari (2011) in Iran, who reported a contamination rate of 28.6% [18], but higher than those reported by Al Zuhair (2012) in Palestine, at 18.7% [19]. A five-year study (2015–2020) revealed that 1.43% of collected milk samples were non-compliant, with contamination by tetracyclines in 30% of cases [20]. In Algeria, Hamiroune reported that 30.6% of milk samples from informal markets in the Jijel and Blida regions contained antibiotic residues [21]. In another study, tetracycline residues in milk from informal markets were reported to be lower, at 24.7%, compared to the results obtained in our study [22]. The findings of this study are also consistent with those from another study conducted in central Algeria, where informal sales circuits showed a contamination rate of 33.8% [23]. The same author revealed that the contamination by tetracyclines in raw milk was 15.79% [24].

This can be explained by the misuse and self-prescription of antibiotics without veterinary supervision. Additionally, milk production from unlicensed farmers is not subject to the compliance controls required by state agricultural services [25]. A survey revealed that 25% of the antibiotics used in cattle farming are tetracyclines. These results are similar to those obtained in France by Cazeau [26], and in Senegal by Bada Alamedji [27]. Several recent studies have corroborated these findings by detecting tetracyclines in marketed milk: Alnassrallah *et al.*

(2022) developed and validated a sensitive HPLC method for their detection in Saudi Arabia [28]; Meklati *et al.* (2022) confirmed their occurrence in Algerian raw milk using LC-MS/MS [29]; and Butovskaya *et al.* (2023) reported similar contamination patterns in Italy using high-resolution spectrometry [30]. In parallel, surveys conducted in France have highlighted inappropriate use of antibiotics in bovine farming, emphasizing the gap between recommended practices and actual implementation [31,32]. Strengthening regulatory frameworks and promoting rational use of veterinary antimicrobials are therefore essential to safeguard public health and mitigate the emergence of antimicrobial resistance.

The assessment of tetracycline levels in marketed milk samples showed concentrations at the detection limit. Regarding tetracycline levels in the vitreous humor, more than 70% of the samples had elevated concentrations. This may be due either to excessive treatment or non-compliance with the slaughter withdrawal period. Generally, in such cases, tetracyclines are used at higher dosages or applied in ways other than those authorized, a practice known as "off-label use." Non-compliance with the withdrawal period by farmers is the primary cause of these residues in milk [33]. For oxytetracycline, the withdrawal period is set at 14 days before slaughter for meat and offal, and 3 days for milk [34]. The survey revealed that the main error committed by farmers during antibiotic treatment is non-compliance with the withdrawal period (28.7%). Similar conclusions were recently drawn in other regions, where off-label use and poor farmer awareness were identified as key contributors to antibiotic residues [35].

The sample size (42 samples for milk and 18 for vitreous humor), as well as the sampling period (January to April), does not allow us to conclude the absence of antibiotic residues throughout the year.

### Broader Implications

The presence of tetracycline residues in both milk and vitreous humor highlights a significant public health risk, particularly in regions where veterinary practices are poorly regulated. These findings underscore the need for reinforced monitoring programs, stricter enforcement of withdrawal periods, and improved farmer education on the responsible use of antibiotics. Strengthening regulatory frameworks within a "One Health" perspective is essential to mitigate indirect

antibiotic resistance and to ensure consumer protection [36,17].

### CONCLUSION

The findings emphasize the urgent need for stricter regulation and monitoring of antibiotic use in veterinary and agri-food practices. The detection of tetracycline residues in milk and vitreous humor highlights the critical importance of compliance with withdrawal periods and appropriate dosage regimens to minimize consumer exposure. Reinforcing regulatory frameworks and promoting the prudent use of veterinary antimicrobials are essential measures to safeguard public health and curb the emergence of antimicrobial resistance.

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