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Quality Study of Raw Milk Produced in Private Farm by Estimate, Bacterial Contamination and the Prevalence of Antibiotic Residues

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Annotation: Antibiotic residues can lead to the development of antibiotic-resistant bacteria and the loss of their ability to be controlled. The study's goal was to determine the level of bacterial contamination and antibiotic residues in raw milk from a few private farms in Iraq's Al-Diwaniyah province. The raw milk of 120 cattle was aseptically obtained from a private farm in the Iraqi province of Al-Diwaniyah. 60 of them were taken at the morning milking, while the rest were taken at the nighttime milking. The samples were collected 10, 20, and 30 days after delivery. At a temperature of 4 °C, all of the milk samples were kept. Samples were brought to the lab in a cooler set at a temperature of no more than 4 °C. Until analysis, they were stored at 4 °C in a refrigerator. The microbiological tests started as soon as possible. After that, all samples were kept at -20 °C in a deep freezer and tested within 48 hours. Between 30 and 45 minutes passed between the time the sample was collected and it was placed in the -20 °C environment. High levels of bacterial contamination and amoxicillin and oxytetracycline residues were found in the results. The investigation came to the conclusion that antibiotics are being misused, which could be harmful to people's health.

Keywords: bacterial contamination, prevalence, amoxicillin, oxytetracycline, raw

milk.

Introduction

Milk and dairy products are among the foods with great nutritional, social, and economic value that are produced globally utilizing a variety of production techniques and technology. [1].

Consuming milk polluted with antibiotic residues in excess of the maximum allowed residue level can induce human poisoning and the development of resistant bacteria, resulting in antibiotic treatment failure and threatening human life. Long-term exposure may also alter the bacteria that live in the gut. , increasing the risk of several illnesses.(2) antibiotics have been used in livestock care to treat and prevent common diseases (such mastitis, respiratory and foot ailments, etc.). these compounds having antibacterial activity that can be applied topically, orally, orally and naturally and they can be produced, semi-synthetic, or naturally occurring. Since the 1960s, Mastitis remains the most prevalent infectious disease in dairy cows despite the deployment of mastitis control measures over the past 30 years, and it still causes the dairy industry to suffer large financial losses. [3].

Antibiotic residues are defined as "pharmacologically active substances (whether active principles, recipients, or degradation products) and their metabolites which remain in foodstuffs obtained from animals to which the drugs in question have been administered" [4]. Sachi et al. [5] reviewed the scholarly literature on the topic of antibiotic residues in milk from 1960 to 2017. They found 224 studies that looked at the quantitative and qualitative makeup of antibiotic residues in cow milk samples. However, the majority of study (82.14%) focused on detection methods that only looked at a few samples, and in most cases, milk was contaminated with known antibiotic concentrations to advance the method (6) . For the purpose of analyzing drug residues in milk, several analytical techniques have been developed, which may be further classified into confirmatory and screening assays. The microbial inhibition test, High-Performance Liquid Chromatography (HPLC), and thin layer chromatography (TLC) are all examples of qualitative screening techniques that are often used to identify residues. (7) The study's goals were to estimate the bacterial contamination and to determine the amoxicillin and oxytetracycline residues in the raw milk produced by private farms.

Materials and methods

1- Collection of milk sample

In Al-Diwaniyah province, Iraq, 120 samples of 10 ml each of raw bovine milk were aseptically collected. 60 of them were taken at the morning milking, while the rest were taken at the nighttime milking. Samples were collected 10, 20, and 30 days following delivery. At a temperature of 4 °C, all of the milk samples were kept. Samples were brought to the lab in a cooler set at a temperature of no more than 4 °C. Until analysis, they were stored at 4 °C in a refrigerator. The microbiological tests started as soon as possible. The Merck Company provided all of the materials needed for microbiological cultivation. After that, all samples were kept at -20 °C in a deep freezer and tested within 48 hours. Between 30 and 45 minutes passed between the time the sample was collected and it was placed in the -20 °C environment.

2- Selection and preparation of antibiotics

To screen milk samples using TLC, two veterinary antimicrobials: commonly used were selected amoxicillin, oxytetracycline, These antimicrobial standards have been bought from Sigma (Fluka and Vetranal), a United States-based corporation, by followed standard operating procedures for storing and monitoring standards. by dissolving 0.1 g of standard in 2 mL of methanol. Stock solutions were prepared and further diluted using the same solvent in order to produce a standard working solution with varied concentrations., and maintained in a deep freezer at -20.

3- Physical examination:

Taste panel scores: With the assistance of a panel of experts, an organoleptic test of raw milk was carried out to identify color, flavor, and texture visually, nasally, and lingually. Using a lactometer, specific gravity was also calculated. A panel of experts used a standard scorecard to evaluate the look, texture, smell, and taste attributes of each batch of raw milk in order to determine its organoleptic quality (ISO, 1995).

4- Bacterial examination

Total bacterial count: Raw milk samples were serially diluted up to 1:10,000 in tubes containing 9 ml of sterile% 0.1 peptone water [8]. The samples were then grown on Nutrient agar using 0.1 mL of each sample's dilution. After 48 hours of aerobic incubation at 37 °C, a total mesophilic bacterial count was performed on the plates [9].

5- Chromatography analysis

5.1 Thin layer chromatography (TLC): One milliliter of milk and one milliliter of acetonitrile-methanol deionized water were mixed 40:20:20 in a centrifuge tube. After thoroughly mixing the liquid, it was centrifuged for 10 minutes at 3000 rpm. The supernatant was subsequently used in the TLC procedure described by [10]. Positive samples were kept at 20 °C until they were ready to be analyzed by UHPLC.

5.2 Ultra high-performance liquid chromatography (UHPLC):

Liquid Chromatography-Diode Array Detector can identify sub-MRL oxytetracycline and amoxicillin residues. We approved the method's linearity, recovery, specificity, and accuracy. The extracted samples were centrifuged at 3000 rpm for 15 minutes in an Eppendorf tube before filtering with 0.2nm MFS filters. The samples were extracted again in UHPLC using oxytetracycline [11] and amoxicillin [12]. Chromatography usually using a stainless column C 18 (P/N 891-5002, 2 mm ID_10 0 mm, L No. 22G2C-001). 0.2 and 1.5 ml/min amoxicillin and oxytetracycline were added to the mobile phase. Amoxicillin was found at 254 nm and oxytetracycline at 360 nm. Both oxytetracycline and amoxicillin needed 20 l UHPLC injections. [13, 14]

Results

Table 1 listed the times when milk samples were collected after delivery; at 10 days, there were 20 morning milkings and 20 evening milkings; at 20 days, there were 20 morning milkings and 20 evening milkings; and at 30 days, there were 20 morning milkings and 20 evening milkings. 120 different samples were used in all periods.

Table-1: List of milk samples that were examined at different study periods.

Milk collection periods	Morning milking	Evening milking	Total samples
10 days	20	20	40
20 days	20	20	40
30 days	20	20	40

Table 2 lists the physical characteristics of raw milk samples taken at various points after delivery. At 10 days, the colors were deep yellowish white; at 20 days, they were medium yellowish white; and at 30 days, they were light yellowish white. At 10 days, the flavors were sweet scent and 50% normal, returning to normal at 20 and 30 days. At 10, 20, and 30 days, the textures were normal thick, normal, and normal thin, respectively. At 10, 20, and 30 days, the specific gravities were 1.0290, 1.0273, and 1.0247, respectively.

Table- 2: Physical parameters of raw milk samples collected at different periods post parturition.

Physical parameters	Periods collection post parturition		
	10 days	20 days	30 days
Color	Deep yellowish white	Moderate yellowish white	Light yellowish white
Flavor	Normal50%,sweet aroma50%	Normal	Normal
Texture	Normal thick	Normal	Normal thin
Specific gravity**	1.0290	1.0273	1.0247

Mean±SD (Standard deviation) **

The total number of bacterial colonies (CFU/ml) found in the raw milk samples taken at various points after delivery. At 10 days, morning milking represented 21 (52.5%) and evening milking represented 17 (42.5%); at 20 days, morning milking represented 16 (40%) and evening milking represented 13 (32.5%); and at 30 days, morning milking represented 8 (20%) and evening milking represented 5 (12.5%).

Table-3: Total bacterial colonies count CFU/ ml. of the raw milk samples at different periods

Milk collection periods	Morning milking		Evening milking	
	Total bacterial count	%	Total bacterial count	%
10 days	21±3.2A	52.5	17±2.21A	42.5
20 days	16±2.68B	40	13±1.72B	32.5
30 days	8±1.76C	20	5±1.53C	12.5
LSD	3.08		2.13	

* Different letters denote to the significant difference (P<0.05).

* Samples No. for each period=40

Tables 4 and 5 compare the prevalence of amoxicillin and oxytetracycline residues at 10 days after delivery. At morning milking, there were 4 positive samples for amoxicillin and 10 positive samples for oxytetracycline. At evening milking, there were 5 positive samples for amoxicillin and 8 positive samples for oxytetracycline. The overall positive ratio was 67.5%. While at 20 days postpartum, morning milking found 2 positive samples for amoxicillin and 7 positive samples for oxytetracycline, and evening milking found 3 positive samples for oxytetracycline and 7 positive samples for amoxicillin, total positive ratio was 47.5%. At 30 days postpartum, morning milking found 1 positive sample for amoxicillin and 6 positive samples for oxytetracycline, and evening milking found 1 positive sample for amoxicillin and for oxytetracycline were 8 samples, total positive ratio was 40%.

Table-4: Comparative prevalence of antibiotics residues in the raw milk

Milk collection periods	Morning milking positive		Evening milking positive	
	Amoxicillin g/Lμ	Oxytetracycline g/Lμ	Amoxicillin μg/L	Oxytetracycline μg/L
10 days	4±0.45A	10±0.64A	5±0.13A	8±0.28A
20 days	2±0.16B	7±1.02B	3±0.22B	7±0.19B
30 days	1±0.11C	6±0.34C	1±0.12C	8±0.41A
LSD	0.67	0.82	0.32	0.44

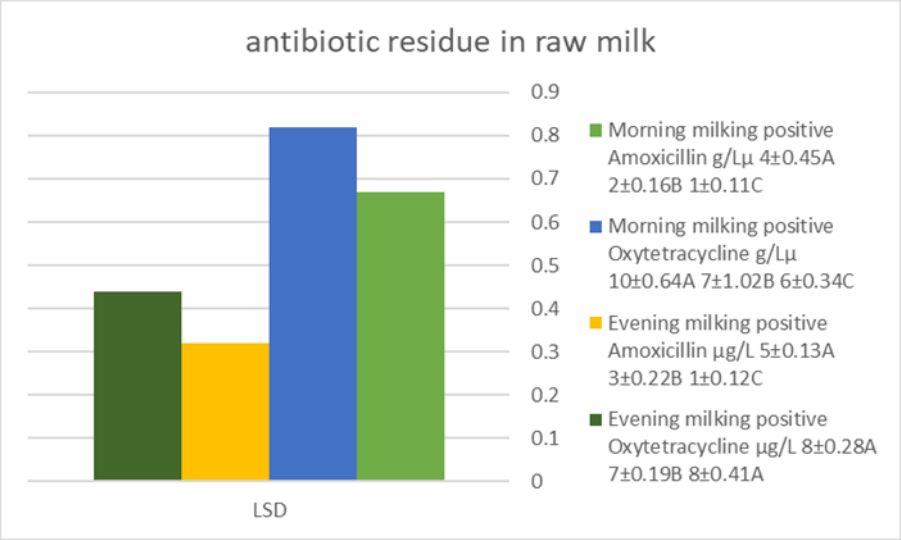

Figure (1) Comparative prevalence of antibiotics residues in the raw milk

Table-5: The % ratio of antibiotic residues prevalence in the raw milk

Milk collection period	Morning milking positive				Evening milking positive			
	Amoxicillin		Oxytetracycline		Amoxicillin		Oxytetracycline	
	Samples No.	%	Samples No.	%	Samples No.	%	Samples No.	%
10days	4	10	10	25	5	12.5	8	20
20days	2	5	7	17.5	3	7.5	7	17.5
30days	1	2.5	6	15	1	2.5	8	20

- No. of samples for each period= 40.

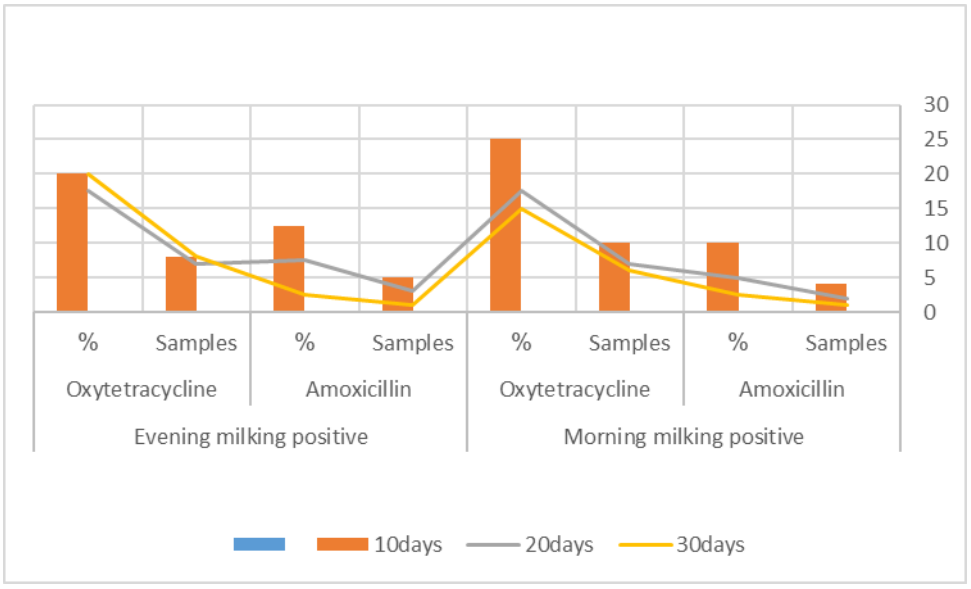

Fig.-2: Amoxicillin and oxytetracycline concentrations (μg/L) in the raw milks at different periods.

Discussion

The findings revealed physical alterations in the raw milk taken at various times at 10, 20, and 30 days following delivery. Because of the lysis of its protein and fat contents and changes in pH, these physical alterations were expected. The texture varied from normal thick due to a high percentage of fat to thin normal, and the color varied from deep yellowish white to light

yellowish white as shown in (table 2) . The flavor was 50% normal and 50% aroma sweet due to its content of sugars as a source of energy to the calf, then to normal. (Table 3) displayed a high bacterial count at various postpartum intervals. These findings point to the existence of germs like Staphylococcus aureus and E. coli that are resistant to antibiotics. Farms give their calves antibiotic injections even one week before parturition to protect them from possible bacterial infections after giving birth. The results of the current investigation demonstrate that many private farmers treated their cattle with antibiotics even when they weren't sick. Furthermore, farms in the Al-Dewaniyah province with a lot of livestock showed greater antibiotic use residual levels. . In turn, the rise of antibiotic-resistant bacteria has been attributed to the high number of cattle per farm, which has increased the presence of antibiotic residues In addition to those who self-prescribe antibiotics, our findings show that farmers self-administer antibiotics frequently. This is important because it can result in under- or overdosing of dairy cattle without their being aware that the antibiotic course has been finished. Dairy farmers' concern of potential financial loss from discarding milk during the withdrawal period may potentially be a factor in their noncompliance (15) . Additionally, the majority of farmers claimed that giving sick cows an antibiotic overload is the only time that it is misused. This attitude reflects their preference to give an antibiotic at a low dose, which they consider to be one of their best practices. Significantly, many scientists claimed that underdosing was the primary cause of the potentially catastrophic rise in antibiotic resistance. Additionally, infections exposed to antibiotics at sub-inhibitory doses will cause more bacterial populations to survive in that animal, resulting in an advanced level of antibiotic resistance [16].

Waste management and the cattle rearing system are crucial for the development and spread of infections that are resistant to antibiotics from animals to humans and the environment [17]. Strict biosecurity procedures on dairy farms, enhanced welfare and hygiene standards, and good antibiotic stewardship could manage antibiotic residues in the products and lessen risks to human health [18]. The results showed how frequently milk contained antibiotic residue. When compared to the overall prevalence discovered in two earlier investigations carried out in Chattogram, who discovered 18% and 18.6%, respectively [19, 20], our findings in raw milk samples were greater. The effectiveness of the screening tests and study time may cause variation. While other investigations employed commercial kits and microbial inhibition assays, we used the TLC approach.

The variation in outcomes is also caused by other factors such as sample size, location, and length. (21) Additionally, the prevalence of diseases in the study location has a significant impact on the usage of antimicrobials in dairy cows and residues identified in milk, which may have led to the inconsistent results.

Conclusion

The usage of antibiotics in dairy cows may be impacted by variables such as sample size, location, and illness prevalence, all of which might alter the study's conclusions.

Antibiotic residues may cause antibiotic-resistant microorganisms and their control loss. The Results showed high bacterial contamination and amoxicillin and oxytetracycline residues, suggesting antibiotic abuse and potential patient harm.

Ethical approval

Approval for the research protocol was granted by the Ethics Committee of the Veterinary College at Al-Qadisiya University (Iraq), . Nonetheless, proper consent was obtained from all subjects prior to their inclusion in the study.

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