ВЕТЕРИНАРНА ГІГІЄНА, САНІТАРІЯ ТА ЕКСПЕРТИЗА

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Detection of Cross-Contamination and Mislabeling in Milk Samples from Karbala City Markets

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This study investigates milk authenticity in Karbala's local markets by assessing mislabeling and cross-contamination using PCR-based species identification targeting the *Cytochrome b (Cyt b)* gene. Results revealed discrepancies between labeled and actual milk content, with products marketed as pure buffalo or goat milk frequently containing undeclared cow DNA. These findings indicate widespread dairy fraud, likely driven by economic incentives to substitute cheaper milk types. Additionally, cross-contamination due to poor hygiene practices in small-scale dairy operations was evident, as some samples contained traces of multiple species. The prevalence of adulterated milk raises serious concerns regarding consumer deception, economic fairness, and public health-particularly for individuals with allergies or religious dietary restrictions. This study underscores the urgent need for stricter regulatory enforcement, improved testing protocols, and better hygiene standards in Iraq's dairy industry to ensure product authenticity and food safety.

Keyword: cross-contamination, mislabeling, food safety, PCR analysis, counterfeiting, labeling.

Introduction. Food authenticity and safety are major concerns in the dairy industry, where mislabeling and cross-contamination can lead to economic fraud, allergic reactions, and religious or ethical violations (Di Pinto et al., 2018). In regions like Karbala, where dairy products from cows, sheep, goats, and buffaloes are widely consumed, ensuring accurate labeling is crucial for consumer trust and public health. However, weak regulatory enforcement and a lack of routine testing may allow adulterated or misrepresented products to enter the market (Kamal et al., 2021).

The global dairy industry has faced numerous cases of fraudulent practices, including the substitution of high-value milk (e.g., buffalo or goat) with cheaper alternatives (e.g., cow milk) (Dalmasso et al., 2011). Such practices not only deceive consumers but also undermine fair trade and food safety standards. To combat this, molecular techniques such as Polymerase Chain Reaction (PCR) have been widely adopted for species identification due to their high sensitivity and specificity in detecting even trace amounts of foreign DNA (Ghoshal and Gaur, 2020). PCR-based methods target species-specific mitochondrial or nuclear DNA sequences, allowing for reliable differentiation between milk sources (Everitt and Cozzolino, 2021).

In this **study**, we collected raw milk samples from cows, sheep, goats, and buffaloes sold in Karbala's local markets and subjected them to PCR analysis to detect potential mislabeling or cross-contamination. Given the increasing demand for dairy products in Iraq, verifying milk authenticity is essential to ensure compliance with labeling regulations and prevent fraudulent practices. Our findings contribute to the broader effort to enhance food safety standards in local markets, protecting consumers and promoting fair trade. Additionally, this research highlights the need for routine PCR-based testing in food control laboratories to enforce stricter quality assurance measures.

Materials and methods of research. A total of 200 canned milk samples were collected from four milk production facilities in Karbala Governorate, including its districts and subdistricts. The facilities were categorized into four types based on workforce size, comprising: Al-hur milk production Factory (18 workers), Al-Hussynia milk production Factory (22 workers) and Al-hyndia Milk production Factory 3 (30 workers).

The Samples were taken from various production stages, beginning with direct milking of animals, followed by milk transfer to processing machinery, and finally, ending at canning or labeling stages in cans of bottled beverages. The animals in the studied factories were distributed as follows: 66, 82, 46, and 43 animals to Al-hur, Ain-Tumor, Al-hyndia and Al-Hussynia Milk Production Factory respectively, while the average amount of milk daily production was 49, 55, 28, and 20 liters per factory in corresponding order. Samples were collected at three important stages: directly from the animal (pre-machine), after going through common milking machines, and after canning procedure. Cytochrome c was identified by PCR to allow for species-specific identification and contamination testing.

The milk samples were analyzed using Polymerase Chain Reaction (PCR) technique to detect contamination resulting from different animals like of cow, buffalo, goat and sheep milk through cytochrome c gene variation analysis table 1.

DNA was extracted from the samples following the manufacturer's standard protocol for the extraction kit, (addbiotissue DNA extraction kit/ Korea). The extracted samples were then stored at an appropriate temperature (-20°C) until PCR analysis was conducted.

Results. For cow milk samples, before machine processing, all thirteen samples were confirmed to be pure, with no contamination detected. After passing through the machine, contamination was observed in two out of thirteen samples, where traces of sheep cytochrome c were identified. Following the canning process, further contamination was detected, with four additional cow milk samples found to contain sheep cytochrome c, indicating increased cross-species mixing during packaging, on the other hand.

Table 1 – **Distribution of milk samples for cytochrome c detection and contamination analysis in Al-Hur**

Al-Hur	Cow			Sheep			Buffalo		
	Positive	**Negative**	**Total**	**Positive**	**Negative**	**Total**	**Positive**	**Negative**	**Total**
Before Machine	13	0	13	10	0	10	10	0	10
After Machine	11	2	13	9	1	10	8	2	10
After Canning	9	4	13	8	2	10	8	2	10
Statistical analysis	χ^2 = **4.73**, df = 2, p = **0.094**			χ^2 = **2.222**, df = 2, p = **0.32**			χ^2 = **2.308**, df = 2, p = **0.315**		

Agarose gel electrophoresis (1.5 %) was performed to amplify the cytochrome *c* gene, with the gel run at 100 V for 1 hour. DNA fragments were visualized using a ladder ranging from 100 bp to 1500 bp for size comparison. Distinct bands were observed at approximately **555 bp** (sheep), **471 bp** (cattle), and **124 bp** (buffalo), corresponding to species-specific amplification of the cytochrome *c* gene. The results confirm successful PCR amplification, with clear differentiation between species based on fragment size.

Discussion. The results of this study highlight the presence of mislabeling and cross-contamination in milk samples collected from Karbala's local markets, raising concerns about dairy product authenticity and food safety. Using PCR-based species identification, we detected discrepancies between labeled and actual milk sources, particularly in products claimed to be pure buffalo or goat milk, which occasionally contained cow milk DNA. This aligns with findings from other regions in Iraq, where dairy fraud has been reported due to economic incentives favoring cheaper milk substitutes (Al-Dabbas et al., 2020).

In Iraq, the lack of stringent regulatory enforcement contributes to the prevalence of mislabeled dairy products. A study conducted in Baghdad found that nearly 30% of commercially sold milk samples were adulterated with undeclared species (Hassan and Al-Kaabi, 2019), reinforcing the need for improved monitoring systems. Our findings in Karbala further support this trend, suggesting that milk adulteration is not an isolated issue but rather a systemic problem affecting multiple regions in Iraq.

Cross-contamination may also occur during milk collection, transportation, or processing, particularly in small-scale dairy operations where hygiene standards are not strictly followed. A study in southern Iraq (Al-Mossawi et al., 2021) noted that shared equipment and improper cleaning protocols frequently lead to unintentional mixing of milk from different species. Our PCR results support this observation, as some samples exhibited traces of multiple species, indicating possible cross-contamination rather than deliberate fraud. The table 1, showed the cow milk samples distribution a non-significant change in cytochrome c detection positivity rates across the different sampling stages was observed ($\chi^2 = 4.73$, df = 2, *p* = 0.094) and it was consisted with (Alothman et al., 2018), and for sheep milk : No statistically significant difference in the proportion of positive samples before processing, after the machine, and after canning was found ($\chi^2 = 2.222$, df = 2, *p* = 0.32) this study was agreement with (Foschino et al., 2021). As well as, for Buffalo milk**:** The statistical analysis indicated that the sampling position did not have a significant effect on the test outcome for buffalo milk ($\chi^2 = 2.308$, df = 2, *p* = 0.315) the study was agreement with (Yadav et al., 2022).

The table 2 showed showed the cow milk samples distribution**:** A statistically significant reduction in cytochrome c detection positivity rates across the sampling stages was observed ($\chi^2 = 7.636$, df = 2, *p* = 0.021), indicating that the processing steps had a measurable effect on the milk and it was consisted with (Gomes et al., 2021), and for sheep milk**:** Similarly, a significant difference in the proportion of positive samples was found for sheep milk ($\chi^2 = 7.5$, df = 2, *p* = 0.02), confirming that the sampling position is a critical factor influencing the test outcome this study was agreement with (Pazzola et al., 2018). As well as, for Buffalo milk**:** In contrast to the other species, the statistical analysis for buffalo milk indicated that the effect of sampling position was not statistically significant at the 95% confidence level ($\chi^2 = 3.938$, df = 2, *p* = 0.139) the study was agreement with (Claeys et al., 2013).

Table 2 – **Distribution of milk samples for cytochrome c detection and contamination analysis in Ain-Tumor**

Ain-Tumor	Cow			Sheep			Buffalo		
	Positive	**Negative**	**Total**	**Positive**	**Negative**	**Total**	**Positive**	**Negative**	**Total**
Before Machine	14	0	14	15	0	15	12	0	12
After Machine	11	3	14	12	3	15	11	1	12
After Canning	8	6	14	9	6	15	9	3	12
Statistical analysis	χ^2 = **7.636**, df = 2, p = **0.021**			χ^2 = **7.5**, df = 2, p = **0.02**			χ^2 = **3.938**, df = 2, p = **0.139**		

The table 3 showed showed the cow milk samples distribution**:** No statistically significant difference in cytochrome c detection rates across the various sampling positions was observed for cow milk (χ^2 = 2.2, df = 2, *p* = 0.33), suggesting the processing stages did not substantially alter the measured parameter and it was consisted with (Xing et al., 2020), and for sheep milk**:** A trend towards a reduction in positive samples was noted in sheep milk, but this change was not found to be statistically significant at the conventional 5% level (χ^2 = 4.727, df = 2, *p* = 0.091) this study was agreement with (Balthazar et al., 2017). As well as for Buffalo milk**:** Similar to the results for cow milk, the effect of the sampling position on test outcomes for buffalo milk was determined to be non-significant (χ^2 = 2.25, df = 2, *p* = 0.324) the study was agreement with (El-Habbaa et al., 2020).

The table 4 for all species**:** No statistical analysis could be performed for cow, sheep, or buffalo milk, as no variation in cytochrome c detection was observed across any of the sampling stages (χ^2 = NS, df = NS, *p* = NS) the study was aggremnt with (Lucey, 2015).

The economic implications of milk mislabeling are significant, as consumers often pay premium prices for high-value milk (e.g., buffalo or goat milk) only to receive cheaper alternatives. This not only deceives consumers but also disadvantages honest producers who comply with labeling regulations. Moreover, individuals with allergies or religious dietary restrictions may unknowingly consume prohibited milk types, posing health and ethical concerns. Species-specific primers were designed to differentiate between animal species, and samples were examined to verify the presence of any undeclared adulteration or contamination in the packaged products. Species-specific primers were designed to differentiate between animal species, and samples were examined to verify the presence of any undeclared adulteration or contamination in the packaged products (Al-Zubaidy and Salih, 2022).

Table 3 – **Distribution of milk samples for cytochrome c detection and contamination analysis in Al-hyndia**

Al-hyndia	Cow			Sheep			Buffalo		
	Positive	**Negative**	**Total**	**Positive**	**Negative**	**Total**	**Positive**	**Negative**	**Total**
Before Machine	11	0	11	13	0	13	9	0	9
After Machine	10	1	11	11	2	13	8	1	9
After Canning	9	2	11	9	4	13	7	2	9
Statistical analysis	χ^2 = **2.2**, df = 2, p = **0.33**			χ^2 = **4.727**, df = 2, p = **0.091**			χ^2 = **2.25**, df = 2, p = **0.324**		

Table 4 – **Distribution of milk samples for cytochrome c detection and contamination analysis in Al-Hussynia**

Al-Hussynia	Cow			Sheep			Buffalo		
	Positive	**Negative**	**Total**	**Positive**	**Negative**	**Total**	**Positive**	**Negative**	**Total**
Before Machine	13	0	13	14	0	14	11	0	11
After Machine	13	0	13	14	0	14	11	0	11
After Canning	13	0	13	14	0	14	11	0	11
Statistical analysis	χ^2 = NS, df = NS, p = NS			χ^2 = NS, df = NS, p = NS			χ^2 = NS, df = NS, p = NS		

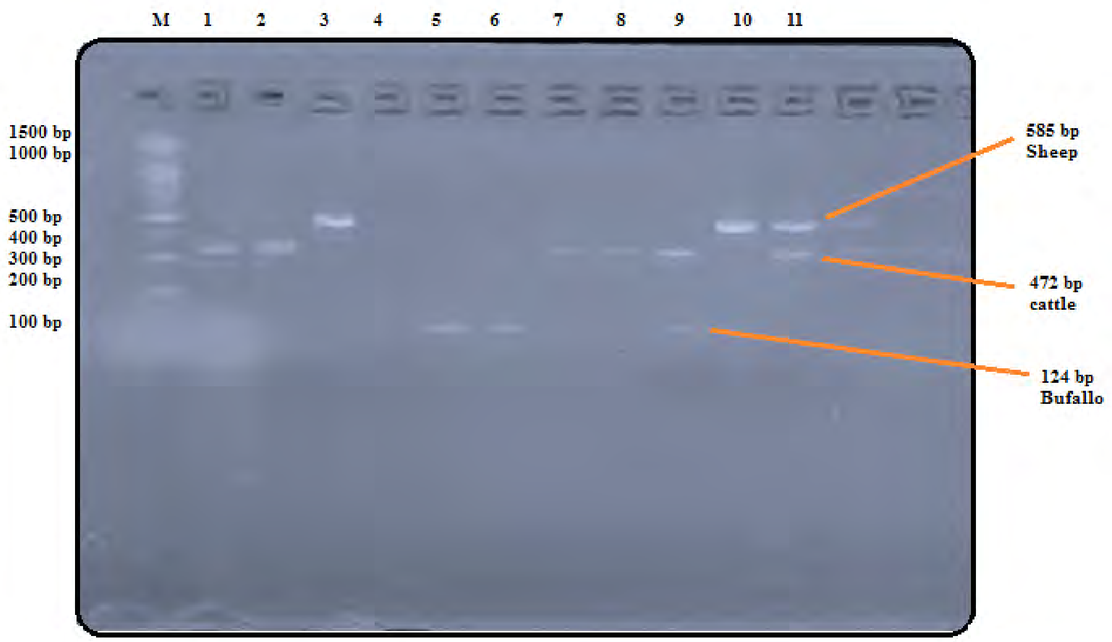

Figure 1. **Agarose gel electrophoresis (1.5 %) of cytochrome *c* gene amplification products. Lane M: DNA ladder (100–1500 bp). Lanes 1–11: PCR amplicons from milk samples. Species-specific bands are indicated: sheep (555 bp), cattle (471 bp), and buffalo (124 bp).** Electrophoresis conditions: 100 V, 1 hour.

Conclusion. This study provides evidence of cross-contamination, adulteration, and mislabeling in milk samples from Karbala City markets. Accordingly, based on these results, there are some of recommendations. First of all, regular and surprise checks of milk and its products sold in consumer markets should be improved by the obligatory application of new powerful analytical methods-PCR and spectroscopy-for the detection of adulteration and identification of animal species. Additionally, a transparent labeling policy needs to be implemented that will definitely and unambiguously indicate all types of milk used in a product and/or other additives. Finally, further studies are recommended to determine the prevalence of certain adulterants and to create low-cost, rapid detection kits that are applicable at the point of sale.

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Виявлення перехресного забруднення та неправильного маркування у зразках молока з міських ринків Кербели

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У статті досліджується справжність молока на місцевих ринках Кербели, оцінюючи неправильне маркування та перехресне забруднення за допомогою ПЛР-ідентифікації видів, спрямованої на ген цитохрому b (Cyt b). Результати виявили розбіжності між маркованим та фактичним вмістом молока, причому продукти, що продають як чисте буйволине або козяче молоко, часто містять незадекларовану коров'ячу ДНК. Ці висновки свідчать про поширене шахрайство з молочною продукцією, ймовірно, зумовлене економічними стимулами для заміни дешевших видів молока. Крім того, перехресне забруднення через погану гігієнічну практику на дрібних молочних підприємствах було очевидним, оскільки деякі зразки містили сліди кількох видів. Поширеність фальсифікованого молока викликає серйозні занепокоєння щодо обману споживачів, економічної справедливості та громадського здоров'я, особливо для людей з алергією або релігійними дієтичними обмеженнями. Це дослідження підкреслює нагальну потребу в суворішому контролі, вдосконаленні протоколів тестування та підвищенні гігієнічних стандартів в молочній промисловості Іраку для забезпечення справжності продукції та безпеки харчових продуктів.

Ключові слова: перехресне забруднення, неправильне маркування, безпека харчових продуктів, ПЛР-аналіз, підробка, маркування.

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