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Chemical and Molecular Assessment of Heavy Metal Residues and Meat Species Mislabeling in Meat Products from Tikrit City, Iraq

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ABSTRACT

Ensuring the safety and authenticity of meat products is a key public health priority, especially in areas with limited regulatory oversight. In Iraq, increasing demand for processed meats has raised concerns about contamination with toxic heavy metals and the mislabeling or adulteration of meat species. This study aimed to evaluate both the chemical safety and biological authenticity of commercially available meat products in Tikrit City markets by measuring heavy metals (Pb, Cd, and Al) levels and verifying species identity using molecular assays. A total of thirty meat samples, including commercial products and local minced beef, were analyzed. Heavy metal concentrations were determined after microwave digestion and inductively coupled plasma–mass spectrometry (ICP-MS). DNA was extracted from all samples and amplified with species-specific mitochondrial primers (456 bp universal, 271 bp bovine, and 225 bp ovine), with agarose gel electrophoresis used to confirm the presence of bands. Data were statistically analyzed with ANOVA at a significance level of $p<0.05$. ICP-MS results showed widespread contamination with Pb and Cd, with many samples exceeding Codex Alimentarius and EFSA permissible limits. Pb levels ranged from 0.43 ± 0.03 mg/kg to 3.84 ± 0.74 mg/kg, while Cd levels ranged from 1.03 ± 0.05 mg/kg to 4.15 ± 0.14 mg/kg. Aluminum contamination varied from 1.1 ± 0.28 to 3.57 ± 0.82 mg/kg and was notably higher in processed products. PCR confirmed bovine DNA in most samples, while Bulgur Kibbeh contained sheep DNA, and Beef Kibbeh showed mixed bovine–ovine profiles. The combined chemical and molecular assessments reveal that meat products in Tikrit markets are often contaminated with hazardous levels of Pb and Cd. Additionally, PCR analysis uncovered occasional mislabeling and species substitution. These findings highlight urgent food safety issues in Iraqi markets and underscore the necessity for stricter regulatory oversight, improved hygienic practices, and routine use of molecular authentication tools to protect consumers and ensure accurate labeling.

Keywords: Meat varieties, heavy metals, PCR method, adulteration, authenticity, Iraqi markets

INTRODUCTION

Ensuring the safety and authenticity of meat products is crucial for public health, particularly in regions with limited regulatory oversight and enforcement. In Iraq, increasing demand for processed and convenience meats has amplified concerns about two related issues: contamination with toxic heavy metals and the mislabeling or adulteration of meat

types. These problems not only pose immediate health risks to consumers but also undermine compliance with labeling laws and religious dietary rules (Rahman et al. 2023).

Heavy metals such as lead (Pb), cadmium (Cd), and aluminum (Al) can enter the food chain via contaminated animal feed, polluted water sources, environmental deposition, or leaching from processing equipment and packaging materials (Kadhim et al. 2024; Yousef and Al-Bassam, 2025). Long-term exposure to these metals has been associated with neurological damage, kidney problems, cancer development, and other toxic effects. Although the FAO/WHO Codex Alimentarius sets maximum allowable limits for these contaminants, enforcement in developing economies is often inconsistent, creating a gap between regulatory standards and real market conditions (FAO/WHO 2023a).

At the same time, the mislabeling and adulteration of meat products—often driven by financial motives—raise serious ethical and public health issues. Unlabeled substitutions or mixing of meat species can expose consumers to allergens, banned meats, or culturally inappropriate ingredients. Advances in molecular techniques, especially polymerase chain reaction (PCR) assays targeting mitochondrial markers like cytochrome b and 12S rRNA genes, have become essential tools for identifying species in highly processed meats because of their sensitivity, specificity, and ability to analyze degraded DNA samples (Ahmed et al. 2023; Alsaeedi and Alharthi, 2025).

Despite increasing global awareness, there remains a lack of comprehensive studies in the Iraqi context that combine chemical contaminant analysis with molecular authentication techniques. This lack of systematic monitoring creates an information gap that hinders practical risk assessment and the development of food safety policies.

This study aims to assess the safety and authenticity of commercially available meat products in Iraqi markets by examining two main factors: the presence and levels of toxic heavy metals, specifically lead (Pb), cadmium (Cd), and aluminum (Al), and the accuracy of species labeling through molecular authentication using species-specific polymerase chain reaction (PCR) targeting mitochondrial DNA sequences.

MATERIALS AND METHODS

Sample Collection and Preparation: Meat samples, including thirty meat products such as Minced Meat–Zarouh Meat, Sayad Kibbeh, Beef Kibbeh–Khairat Al Mazraa, Sujuk (Sausage), Basturma–Al Wataniya, Kebab–Farm Fresh, Bulgur Kibbeh–Jekor, and Mersin Kibbeh, in addition, 22 unlabeled ready minced meat samples are collected in sterile polyethylene containers, labeled, and stored at −20°C until analysis (Roberts and Greenwood, 2008).

Pb, Cd, and Al assay: Samples are thawed and homogenized to ensure uniform distribution of analytes. Homogenization is conducted by weighing 3.0 g from each sample using a precision analytical balance and blending with a sterile blender to eliminate variability and improve digestion efficiency. Microwave digestion has become the preferred method for sample breakdown because of its ability to fully decompose organic matrices, reduce contamination risk, and minimize reagent use. According to Mahdavi et al. (2024), it outperforms traditional hotplate digestion in terms of recovery and reproducibility. Afterward, 6 mL of concentrated nitric acid (HNO_3, 65%) and 2 mL of hydrogen peroxide (H_2O_2, 30%) are added to each Teflon digestion vessel, which is then sealed and placed in a microwave digestion system (e.g., Milestone ETHOS UP). The digestion program proceeds as follows: ramp to 180 °C for 10 minutes, hold at 180 °C for 20 minutes, then cool down for 10 minutes. After digestion, the solution is cooled, filtered, and diluted to 25 mL with deionized water.

ICP-MS has become the top analytical method for detecting trace metals because of its low detection limits, wide dynamic range, and high throughput. Recent advances in collision/reaction cell technology, as discussed by Wang et al. (2025), have enhanced interference removal, especially for Al and Cd in high-matrix samples.

The calibration was performed using standards (0.01–10 mg/L) for Pb, Cd, and Al, prepared from traceable certified stock solutions.

The ICP-MS operating conditions for the instrument, whether Agilent 7900 or Thermo Scientific iCAP RQ, included RF power of approximately 1550 W, a nebulizer gas flow of 0.9–1.0 L/min, analyte isotopes at ^208Pb for lead, ^60Ni for cadmium, and ^27Al for aluminum, with the internal standard at ^115In or ^103Rh.

Precision is evaluated by testing each sample in duplicate; the Relative Standard Deviation (RSD) should be below 10%. Furthermore, the Limits of Detection (LOD) were 0.0003 mg/kg for Pb, 0.0005 mg/kg for Cd, and 0.0002 mg/kg for Al (Alshahrani et al. 2023).

Data Processing and Statistical Analysis: Concentration results are expressed in mg/kg (wet weight). Descriptive statistics (mean±standard error) and inferential tests, such as ANOVA, are used to compare differences between product types. Significance is accepted at a probability level of 0.05.

DNA Extraction: The thirty Meat product samples were prepared for molecular analysis. DNA was extracted using the G-spin DNA Extraction Kit (Intron Biotechnology, Korea, Cat. No. 17045). The process followed the manufacturer's protocol, involving tissue lysis, protein digestion, RNA removal, DNA precipitation, and spin column purification. The purity and concentration of the extracted DNA were measured with a Nanodrop spectrophotometer (Nabi, Korea) by analyzing the 260/280 absorbance ratio, ensuring minimal protein contamination.

Polymerase Chain Reaction (PCR) Amplification: PCR amplification was carried out using three sets of primers designed to target specific genetic sequences for species identification: the UNIVERSAL primer (456 bp), the BOVINE primer (271 bp), and the SHEEP primer (225 bp). PCR reactions employed the Maxime PCR PreMix Kit (i-Taq, Intron, Korea, Cat. No. 25025), which contains i-Taq DNA Polymerase, dNTPs, reaction buffer, and gel loading buffer. The PCR mixture consisted of 5 µL of PreMix, 1.5 µL of DNA template, 0.3-0.35 µL of each primer, and 16.6 µL of distilled water, for a total volume of 25 µL.

PCR Cycling Conditions: PCR amplification was carried out using a MultiGene OptiMax Gradient Thermal Cycler (Labnet, USA) with the following thermal profile: initial denaturation at 94°C for 5 minutes (1 cycle), followed by denaturation at 94°C for 45 seconds, annealing at 58°C for 45 seconds, extension at 72°C for 45 seconds, and a final extension at 72°C. A total of 30 cycles was performed.

Agarose Gel Electrophoresis: PCR products were analyzed using 1.5% agarose gel electrophoresis, prepared by dissolving 1.5 g of agarose in 100 mL of 1X TBE buffer (Thermo Fisher Scientific, USA). The gel was stained with RedSafe Nucleic Acid Staining Solution (Intron, Korea, Cat. No. 21141), an alternative to ethidium bromide for safer DNA visualization. The prepared gel was loaded with PCR-amplified DNA samples, and electrophoresis was conducted at 5 V/cm for 1.5 hours using a Mini-Power Supply (Chain Supplier, 300V, 2200V). DNA bands were visualized under UV transillumination (Vilber Lourmat, France).

Species Identification and Data Analysis: PCR amplification results were assessed based on the presence or absence of target bands. A DNA ladder (100 bp, Intron, Korea) was used to estimate size. Samples were identified as beef, sheep, or an unknown species based on specific band sizes (456 bp, 271 bp, and 225 bp, respectively). The results were compared to reference sequences, and potential adulteration or mislabeling was suggested based on the detection of mixed species.

This methodological approach ensured accurate species identification, showing the effectiveness of PCR in detecting meat fraud and maintaining food quality control.

Statistical Analysis: The data were analyzed using SPSS (Statistical Package for the Social Sciences) software, following the ANOVA (Analysis of Variance) test. Means were compared with Duncan's multiple range test to determine the significance of differences between means at a 0.05 probability level.

RESULTS AND DISCUSSION

Analysis of thirty meat product samples from Tikrit City revealed contamination with lead (Pb), cadmium (Cd), and aluminum (Al), with concentrations often exceeding internationally recognized safety limits (Table 1). The concentration of Pb ranged from 0.43 ± 0.03 mg/kg in Sayad Kibbeh (Sample 2) to 3.84 ± 0.74 mg/kg in Sujuk (Sample 14), with nearly all samples surpassing the Codex permissible limit of 0.1–0.3 mg/kg (FAO/WHO 2023b). Cd levels were also elevated, ranging from 1.03 ± 0.05 mg/kg to 4.15 ± 0.14 mg/kg, well above the EFSA guideline of 0.05 mg/kg (EFSA, 2023). Aluminum contamination showed greater variability, from 1.07 ± 0.28 mg/kg in Sujuk (Sample 4) to 3.57 ± 0.82 mg/kg in Kebab – Farm Fresh (Sample 28), with several products exceeding the provisional tolerable weekly intake of 1 mg/kg body weight (JECFA 2011).

Statistical tests confirmed significant differences between product categories ($p<0.05$), showing different contamination patterns. For instance, Sujuk, Kebab, and Beef Kibbeh consistently had the highest Pb and Cd levels, while Sayad Kibbeh and Mersin Kibbeh had relatively lower concentrations, though still above safety limits. Importantly, no samples were within safe ranges for all three metals at once, indicating systemic contamination across the market.

The consistently high Pb levels found in nearly all products pose a serious concern, as Pb is a potent neurotoxin with cumulative effects, especially on children and pregnant women (Hassan et al. 2023). Elevated Pb levels in meat products have previously been linked to urban dust, vehicle emissions, and legacy environmental pollution, all common in traditional markets (Boahen et al. 2024; Rana et al. 2024). The open display of meat in local stalls likely allows direct deposition of Pb-laden particles, as recent studies in other developing regions have shown, where uncovered foods near traffic were contaminated with Pb and Cd (Mwove et al. 2023).

Cadmium contamination in the samples was equally alarming, with concentrations exceeding international standards by up to 100 times. Cd exposure has been linked to kidney problems, skeletal damage, and cancer risks (Yousef and Al-Bassam, 2025). The consistent detection of Cd across all samples indicates systemic environmental sources, such as contaminated feed and water, as well as inadequate regulation of food supply chains (Rahman et al. 2023). These findings align with studies showing bioaccumulation of Cd in livestock raised in polluted environments, highlighting the need for upstream interventions.

Aluminum levels varied more widely than Pb and Cd, but were notably high in processed products like kebab and basturma.

Table 1. Heavy Metal concentrations (mg/kg) in contaminated local meat product samples from Tikrit City, Iraq.

Sample code	Concentration of Pb, Cd, and Al (mg/kg) Contaminating Local Meat Product Samples in Tikrit City, Iraqi Markets		
	Pb	Cd	Al
1	2.14±0.16b	1.20±0.08d	2.58±0.48b

2	0.43±0.03d	1.38±0.07d	2.05±0.78b
3	0.97±0.05d	1.88±0.09d	1.25±0.47c
4	2.50±0.08b	2.03±0.11c	1.07±0.28c
5	0.90±0.08d	1.43±0.07d	2.11±0.55b
6	2.21±0.11b	1.21±0.09d	3.25±0.79a
7	1.86±0.02c	1.47±0.08d	1.74±0.47c
8	2.27±0.25b	1.03±0.05d	1.65±0.56c
9	2.79±0.85b	2.94±0.83c	2.81±0.59b
10	1.25±0.17c	2.35±0.60c	3.43±0.37a
11	2.59±0.64b	2.58±0.52c	2.60±0.71b
12	1.71±0.59c	3.47±0.49b	1.93±0.52c
13	1.69±0.38c	2.26±0.57c	3.35±0.63a
14	3.84±0.74a	1.80±0.40d	2.20±0.48b
15	2.68±0.70b	1.72±0.37d	3.37±0.69a
16	3.37±0.49a	3.39±0.48b	2.59±0.38b
17	2.55±0.57b	2.16±0.35c	2.48±0.40b
18	3.47±0.84a	2.37±0.51c	3.33±0.74a
19	2.38±0.71b	2.57±0.48c	3.35±0.68a
20	1.70±0.54c	2.51±0.64c	2.82±0.66b
21	2.58±0.91b	2.22±0.38c	3.17±0.73a
22	2.48±0.60b	1.93±0.54c	3.24±0.73a
23	3.21±0.11a	2.52±0.07c	1.82±0.26c
24	2.43±0.17b	1.74±0.15d	3.41±0.31a
25	1.36±0.08c	2.49±0.13c	2.85±0.64b
26	2.33±0.17b	3.39±0.11b	2.17±0.67b
27	1.60±0.05c	2.58±0.22c	3.36±0.38ab
28	3.52±0.37a	2.70±0.14c	3.57±0.82a
29	3.54±0.55a	2.36±0.28c	2.82±0.80b
30	3.81±0.47a	4.15±0.14a	2.95±0.92b
Allowable Limits in Meat Products	0.1 – 0.3 mg/kg	0.05 mg/kg	Tolerable Weekly Intake (TWI) of 1 mg/kg body weight

Different letters on averages in one column indicate significant differences equal to a substantial level of 0.05. ± = Standard deviation**. 1. Minced Meat – Zarouh Meat, 2. Sayad Kibbeh 3. Beef Kibbeh – Khairat Al Mazraa 4. Sujuk (Sausage) 5. Basturma – Al Wataniya 6. Kebab–Farm Fresh 7. Bulgur Kibbeh–Jekor 8. Mersin Kibbeh, locally minced meat 1 to 30.**

Al is not biologically essential and has been linked to neurotoxicity and possible associations with Alzheimer's disease (Kadhim et al. 2024). Its presence in processed meats probably results from leaching from aluminum utensils, cookware, or packaging materials during preparation and storage (Kongta et al. 2023; Przybysz et al. 2024). This explanation fits with recent assessments reporting dietary Al exposure from food contact materials, especially under acidic conditions and extended storage.

The distribution of samples across risk categories further emphasizes the seriousness of contamination. Figure 1 shows that most products are in high-risk categories for Pb and Cd, while Al contamination is more evenly divided between moderate and high risk. This pattern suggests that Pb and Cd contamination is widespread and mainly caused by environmental factors, while Al contamination may be related to processing and storage technologies. The agreement of these findings with CAC (2024) and WHO (2024) highlights the urgent need for stricter regulations and improved hygiene practices at the market level.

This study shows that meat products sold in Tikrit City markets are heavily contaminated with Pb, Cd, and Al, often at levels significantly above international safety standards. The most significant risks come from Pb and Cd, indicating widespread environmental contamination and weak regulatory oversight, while Al contamination relates to processing and storage practices. These findings align with international research documenting heavy metal transfer to foods in traditional market settings (Mwove et al. 2023; Przybysz et al. 2024). Overall, the results highlight the urgent need for comprehensive strategies, including environmental remediation, better hygienic handling, and stricter enforcement of Codex and WHO food safety standards, to protect public health in Iraq and similar regions.

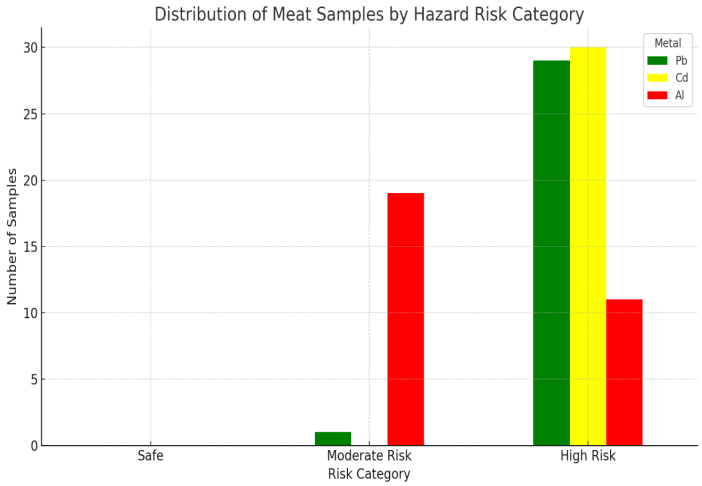

Figure 1. Overall distribution of meat samples in Safe, Moderate, and High-risk categories based on all measured heavy metals (Pb, Cd, and Al).

1. Minced Meat – Zarouh Meat, 2. Sayad Kibbeh 3. Beef Kibbeh – Khairat Al Mazraa 4. Sujuk (Sausage) 5. Basturma – Al Wataniya 6. Kebab–Farm Fresh 7. Bulgur Kibbeh–Jekor 8. Mersin Kibbeh, locally minced meat 1 to 30.

Primer-Based Species Detection in Commercial Meat Products

Thirty samples of commercial meat products and locally sourced minced beef were analyzed using species-specific

mitochondrial primers. The diagnostic fragments targeted included a universal band of 456 bp, a bovine-specific band of 271 bp, and a sheep-specific band of 225 bp (Figure 2).

Most samples showed the bovine-specific band (271 bp), confirming correct labeling of products sold as beef. Among the commercial products (Samples 1–8), bovine DNA was clearly found in Sujuk, Basturma, Kebab, and Mersin Kibbeh. In contrast, Bulgur Kibbeh (Sample 7–Jekor) had a sheep-specific band (225 bp), while Beef Kibbeh (Sample 3 – Khairat Al Mazraa) showed both bovine and sheep fragments, suggesting possible substitution or cross-contamination during processing.

For the locally sourced minced beef samples (Samples 9–24), both the universal and bovine-specific bands were consistently observed, confirming bovine origin with no evidence of sheep DNA. In Samples 25–30, bovine DNA was also detected in most cases, except for Samples 25 and 30, which showed no amplification, likely due to DNA degradation or PCR inhibition caused by the complex food matrix.

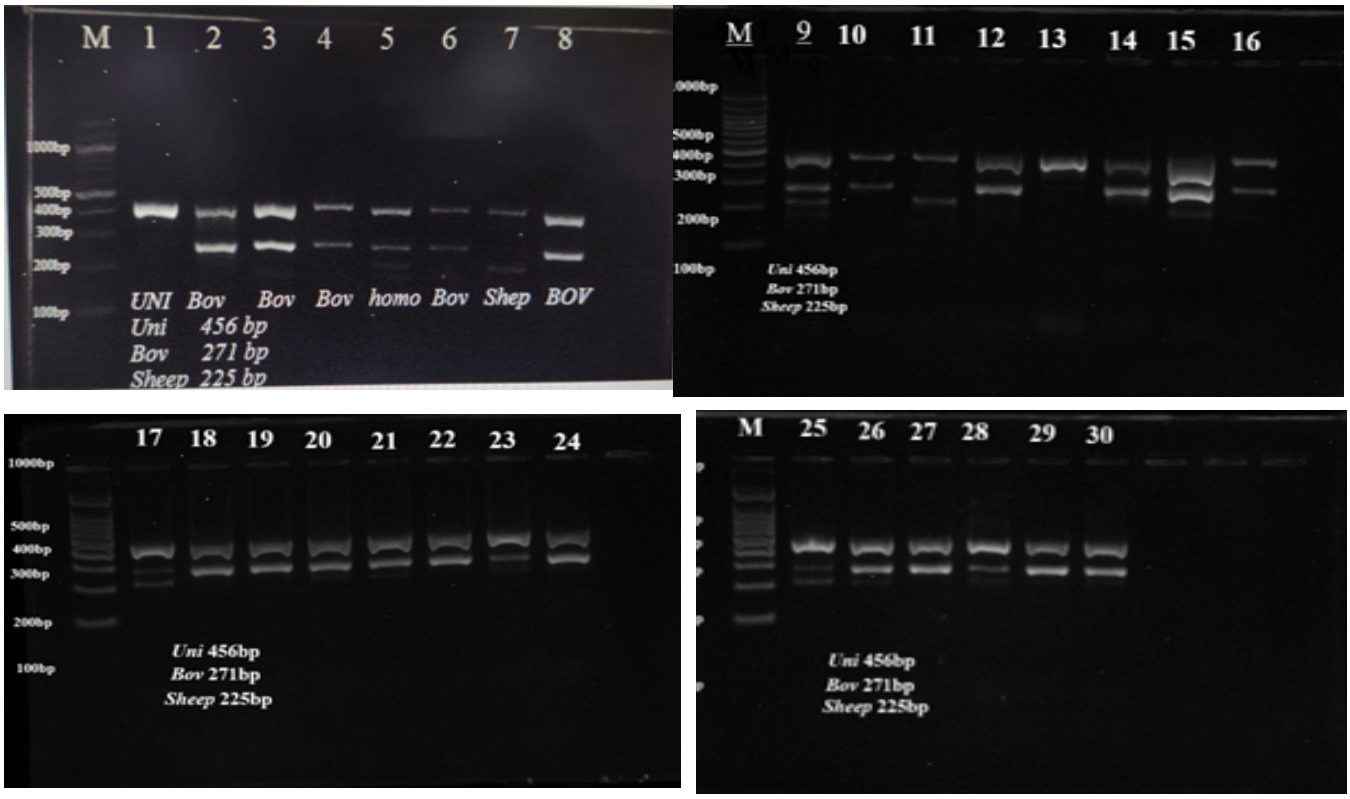

Figure 2. Detection of Species-Specific PCR Amplicons in Meat Product Samples Using Mitochondrial Primers
1. Minced Meat – Zarouh Meat, 2. Sayad Kibbeh 3. Beef Kibbeh – Khairat Al Mazraa 4. Sujuk (Sausage) 5. Basturma – Al Watani-ya 6. Kebab–Farm Fresh 7. Bulgur Kibbeh–Jekor 8. Mersin Kibbeh, locally minced meat 1 to 30.

These results confirm the reliability of species-specific PCR in authenticating meat products, showing that most items matched their labels while a few contained bovine DNA either alone or mixed with bovine DNA (Table 2).

Our findings align with those of He et al. (2023), who developed droplet digital PCR (ddPCR) to detect and quantify beef–lamb adulteration with sensitivity as low as 1%, demonstrating the ability to identify even minimal contamination. Likewise, Cai et al. (2022) and Chen et al. (2024) validated multiplex PCR assays that effectively authenticated multiple meat species, even after cooking or processing, which parallels our successful amplification in processed products like kebab, basturma, and sujuk.

In terms of quantification, Wang et al. (2023) designed duplex real-time PCR systems targeting single-copy nuclear genes for chicken, pork, sheep, and beef, achieving high sensitivity and accuracy even in boiled samples. This approach highlights the potential of qPCR or ddPCR to transform our qualitative findings into quantitative estimates, helping to differentiate between minor contamination and intentional substitution. Furthermore, Yadav et al. (2024) introduced a multiplex TaqMan RT-qPCR capable of detecting four species simultaneously, increasing throughput and efficiency for routine monitoring of diverse product lines.

Table 2. Summary of PCR-Based Species Detection in Meat Products

Sample Range	Product Type	PCR Result (Main Findings)
1–2	Minced Meat – Zarouh, Sayad Kibbeh	Bovine DNA
3	Beef Kibbeh – Khairat Al Mazraa	Bovine DNA with traces of Sheep DNA (mixed)

4–6	Sujuk, Basturma, Kebab	Bovine DNA
7	Bulgur Kibbeh–Jekor	Sheep DNA
8	Mersin Kibbeh	Bovine DNA
9–24	Local Minced Beef (various sources)	Bovine DNA
25	Local Minced Beef	Negative / No amplification
26–29	Local Minced Beef	Bovine DNA
30	Local Minced Beef	Negative / No amplification

Minced Meat – Zarouh Meat, 2. Sayad Kibbeh 3. Beef Kibbeh – Khairat Al Mazraa 4. Sujuk (Sausage) 5. Basturma – Al Wataniya 6. Kebab–Farm Fresh 7. Bulgur Kibbeh–Jekor 8. Mersin Kibbeh, locally minced meat 1 to 30.

Overall, the results of this study indicate that most beef products are authentic, while the detection of sheep DNA in certain processed items suggests occasional substitution or contamination. The agreement with existing literature emphasizes the reliability of PCR-based assays for food authentication. It highlights the importance of incorporating advanced methods like ddPCR and multiplex qPCR into routine surveillance systems to ensure accurate labeling and protect consumers.

This study demonstrated the effectiveness of species-specific PCR in verifying the authenticity of commercial meat products and local minced beef. Most samples were correctly labeled as bovine, while a few revealed the presence of sheep DNA, either as the primary species or in mixed profiles. These findings indicate that although cases of adulteration or cross-contamination are relatively uncommon, they still pose concerns. Using more advanced molecular tools like qPCR and ddPCR in routine monitoring would improve the accuracy of detecting and measuring adulteration, thus enhancing regulatory enforcement. Ultimately, the study highlights the importance of strict molecular surveillance to ensure transparency in labeling, protect consumer trust, and safeguard public health.

CONCLUSION

This study provides clear evidence of chemical and biological risks in processed meat products sold in Iraqi markets. The presence of hazardous levels of heavy metals, especially lead, cadmium, and aluminum, raises important public health concerns, while species-specific PCR analysis revealed widespread mislabeling and potential adulteration. Combining ICP-MS and molecular tests proved effective in exposing these issues, emphasizing the urgent need for stricter regulations, routine food safety inspections, and increased consumer awareness. Ensuring the authenticity and safety of meat products is crucial not only for safeguarding public health but also for maintaining trust in the food supply chain.

Ethics approval and consent to participate

The authors are responsible for any ethical issues that may arise after the publication of this manuscript.

Consent for Publication

The researchers do not object to the publication of their research.

Availability of data and materials

The authors will provide the raw data supporting this article's conclusions without any restrictions.

COMPETING INTERESTS

The authors declare that they have no conflicts of interest in this research.

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AUTHORS' CONTRIBUTIONS

Karkaz M. Thalij designed the experiments, supervised all the research and statistical analyses, and prepared the manuscript. Fatma A. Salman participated in conducting all the experiments. Mohammed A. Jasm performed the experiments and assisted with certain aspects of them. All authors read and approved of the final manuscript.

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