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(RESEARCH ARTICLE)

Molecular detection of chicken and red-meat adulteration in locally processed meat products

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Abstract

Background: Food adulteration in processed meat products remains an important authenticity and consumer-protection problem because grinding, curing, seasoning and heating remove the visible identity of meat ingredients. DNA-based species detection is suitable for verifying declared meat species in products marketed as single-species or general meat products.

Methods: A cross-sectional market-surveillance study was performed on 90 locally processed meat products, including beef burgers, beef sausages, minced red meat, kofta/kebab preparations and chicken-based processed products. Genomic DNA was extracted using a commercial food DNA extraction kit, assessed spectrophotometrically and amplified by conventional multiplex PCR targeting mitochondrial cytochrome b fragments for chicken, cattle and sheep. Positive reference meats, no-template controls, negative extraction controls and internal amplification controls were included in the workflow. PCR products were visualized by agarose gel electrophoresis and compared with declared label information.

Results: Amplifiable DNA was obtained from 86 of 90 samples (95.6%). Among PCR-valid samples, the overall molecular-label discordance was 30.2%. Undeclared chicken DNA was the most frequent unexpected marker in red-meat-labelled products, especially in sausage and burger samples. Undeclared cattle/sheep DNA was detected less frequently in chicken-labelled products. Product categories differed numerically in discordance, but the chi-square comparison was not statistically significant.

Conclusion: Species-specific multiplex PCR is a practical screening tool for detecting undeclared chicken and red-meat DNA in locally processed meat products. The workflow is suitable for routine food-control laboratories; however, quantitative qPCR or sequencing confirmation is recommended when regulatory decisions require estimation of adulterant mass fraction or legal enforcement.

Keywords: Chicken; Food fraud; Meat authentication; Multiplex PCR; Processed meat; Red meat

1. Introduction

Processed meat products are highly vulnerable to species substitution because tissue morphology is lost during mincing, emulsification, curing, cooking and addition of spices or plant fillers. Meat authentication is therefore a quality-control, public-health, religious, ethical and economic issue. When beef, sheep meat and poultry differ in price, undeclared incorporation of cheaper meat can occur without being visible to consumers. Recent reviews of molecular meat authentication confirm that DNA- and PCR-based assays are favored because of their specificity, sensitivity and applicability to processed matrices (Azad et al., 2023). Recent food-authenticity research further confirms that DNA-based methods remain reliable for detecting mislabeling in thermally processed meat products because DNA is more

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stable than many protein markers and can be amplified from small degraded fragments (Cai et al., 2021). Mitochondrial markers are widely used in meat authentication because mitochondrial DNA is present in high copy number and provides sufficient interspecies variation for designing species-specific assays. High-resolution melting and PCR-based studies have shown that the accuracy of meat identification depends on DNA quality, marker selection, processing intensity and validation against reference materials (Njaramba et al., 2021). PCR-based authentication is especially useful for local laboratories because it requires standard thermocyclers and agarose gel electrophoresis rather than expensive sequencing or spectroscopic platforms. Multiplex PCR further reduces cost and turnaround time by detecting several species in a single reaction when primers are optimized to produce distinct amplicons (Izadpanah et al., 2018).

Some recent works have proven that multi species PCR assays may be used for identification of non-declared animal derived ingredients in meat products. The septaplex PCR assay developed for commercial meat products revealed non-declared species in beef, mutton, pork, and turkey products, thus proving the applicability of multiplex screening for market surveillance purposes (Cai et al., 2021). The hexaplex PCR assay was able to discriminate between twelve meat species using two tube PCR test, proving the applicability of multiplexing for meat species testing (Cai et al., 2022). The PCR analysis of commercially available beef meatballs in Indonesia revealed undeclared chicken and pork in products declared as beef (Siswara et al., 2022). Real-time PCR offers higher analytical sensitivity and quantification potential than conventional endpoint PCR, but it requires validated calibration materials and specialized equipment. For example, fast multiplex real-time PCR has been used to simultaneously detect pork, chicken and beef in commercial processed products, while TaqMan-based assays have quantified bovine or chicken content in processed meats (Kim & Kim, 2019). A recent single-copy nuclear DNA real-time PCR method also showed that quantitative detection can help separate deliberate adulteration from accidental low-level cross-contact in processed beef products (Chang et al., 2024). The present study therefore used conventional multiplex PCR as a cost-effective screening method and recommends qPCR confirmation when quantitative regulatory interpretation is required. The objective of this study was to detect undeclared chicken and red-meat DNA in locally processed meat products using a species-specific multiplex PCR workflow. The study emphasizes cautious interpretation of qualitative PCR results, including possible DNA degradation, carry-over contamination, mixed-label declarations and the inability of endpoint PCR to distinguish trace cross-contact from intentional adulteration without quantitative confirmation (Kang, 2019). The objective of this study was to detect undeclared chicken and red-meat DNA in locally processed meat products using a species-specific multiplex PCR workflow. The study emphasizes cautious interpretation of qualitative PCR results, including possible DNA degradation, carry-over contamination, mixed-label declarations and the inability of endpoint PCR to distinguish trace cross-contact from intentional adulteration without quantitative confirmation (Kang, 2019).

2. Materials and Methods

The study was conducted as a laboratory-based cross-sectional market-surveillance workflow. Sampling, DNA extraction, PCR amplification, electrophoresis and statistical analysis were performed using quality-control steps designed to support reliable interpretation of molecular meat-authentication results.

Table 1 Principal materials, equipment and suppliers used in the study

Material/equipment	Use	Company
DNeasy Mericon Food Kit	DNA extraction from processed meat matrices	QIAGEN GmbH, Hilden, Germany
NanoDrop One spectrophotometer	DNA purity and concentration screening	Thermo Fisher Scientific, Waltham, MA, USA
Qubit dsDNA HS Assay Kit	Optional fluorometric DNA quantification	Thermo Fisher Scientific, Waltham, MA, USA
GoTaq G2 Green Master Mix	Conventional PCR amplification	Promega Corporation, Madison, WI, USA
Species-specific primers	Chicken, cattle and sheep marker amplification	Integrated DNA Technologies, Coralville, IA, USA or Macrogen Inc., Seoul, Korea
Nuclease-free water	PCR-grade reaction preparation	Promega Corporation, Madison, WI, USA
Agarose	Gel electrophoresis matrix	Invitrogen/Thermo Fisher Scientific, USA
GelRed or safe DNA stain	DNA band visualization	Biotium, Fremont, CA, USA

GeneRuler 100 bp DNA ladder	Amplicon size estimation	Thermo Fisher Scientific, Vilnius, Lithuania
Gel documentation system	PCR product imaging	Bio-Rad Laboratories, Hercules, CA, USA
SPSS Statistics 27 / R software	Descriptive and inferential statistics	IBM Corp., Armonk, NY, USA / R Foundation

2.1. Study Design and Sample Collection

Ninety processed meat products were purchased from retail markets, butcher shops, small local processors and restaurants within the target locality. The study included five product categories with 18 samples each: beef burgers, beef sausages, minced red meat, kofta/kebab preparations and chicken-based processed products. Sampling included different brands, batch numbers and retail points to reduce clustering bias. Market-based sampling of mixed and processed products is consistent with recent meat-authentication studies in which comminuted products were selected as high-risk matrices for species substitution (Özlü et al., 2023). Each product was assigned a coded laboratory number without brand identifiers in the analytical worksheet. Label information was photographed and transcribed before package opening, including declared meat species, production date, batch number, frozen or chilled state and completeness of the ingredient list. Unlabeled restaurant or butcher products were recorded as non-prepacked products.

Table 2 Distribution of processed meat samples

Product category	Samples tested	Sampling source	State at purchase	PCR-valid samples
Beef burger	18	Local markets, butcher shops, restaurants	Frozen/chilled	17
Beef sausage	18	Local markets, butcher shops, restaurants	Frozen/chilled	17
Minced red meat	18	Local markets, butcher shops, restaurants	Frozen/chilled	18
Kofta/kebab	18	Local markets, butcher shops, restaurants	Frozen/chilled	17
Chicken products	18	Local markets, butcher shops, restaurants	Frozen/chilled	17

2.2. Sample Preparation and Contamination Control

Samples were transported to the lab in insulated containers at about 4 °C and processed within 24 hours. Frozen samples were thawed at 4 °C to minimize DNA degradation. About 25 grams of tissue was excised from each product using sterile disposable blades from at least three interior points to minimize surface contamination bias. Subsamples were then homogenized in sterile stomacher bags or sterile tubes. Twenty milligrams of the sample was used for DNA extraction and the rest was kept frozen at −20 °C for repeated analyses. The pre-PCR and post-PCR work areas were physically isolated from one another. Filter tips resistant to aerosol contamination, dedicated pipettes and UV-decontaminated work surfaces were used. Reference tissues with known positive chicken, cattle and sheep meat were processed separately from unknown samples. Negative extraction control was included after every 10-12 samples to check cross-contamination during DNA extraction. This procedure is necessary as the handling of ground meat could lead to the contamination of samples with the DNA of different species if there is not enough sanitation (Dalmasso et al., 2024).

2.3. Genomic DNA Extraction

DNA was isolated from a 200 mg ground sample utilizing the DNeasy Mericon Food Kit as per the manufacturer's procedure for processed food matrices. Basically, the sample was lysed in food lysis buffer with proteinase K and heated to 60 °C until full tissue dispersion was achieved before centrifugation. The sample was filtered through a spin column having silica membrane and washed twice after which DNA was eluted in 50–100 µL of nuclease free elution buffer. Concentration and purity of DNA were determined using a NanoDrop method by reading absorbance values at 260/280

nm while Qubit fluorometry was utilized to quantify some samples as a result of possible overestimation due to spectrophotometric analysis of DNA from processed foods. Extracts with A260/280 ratios between 1.6 and 2.0 were considered acceptable for conventional PCR screening. Comparable duplex PCR workflows have been used for meat products and Mozzarella-type cheese matrices targeting mitochondrial cytochrome b DNA (Khatun et al., 2021).

2.4. Species-Specific PCR Targets and Primer Strategy

The conventional multiplex PCR targeted mitochondrial cytochrome b fragments because this marker has a high copy number and has been frequently used for meat species differentiation. A common forward primer and species-specific reverse primers generated amplicons of distinguishable size for chicken, cattle and sheep. Primer sequences were checked by BLAST against GenBank entries before analysis to confirm specificity for the tested meat species and to reduce the possibility of cross-amplification with closely related taxa. Recent studies also support the use of mitochondrial targets for qualitative identification of meat species in processed and heat-treated products (Cheng et al., 2022). PCR-lateral-flow detection targeting chicken cyt b has also shown high specificity and detection of low-level chicken adulteration, supporting the suitability of this target for chicken screening (Xu et al., 2022).

Table 3 Primer set used for qualitative detection of chicken, cattle and sheep DNA

Primer	Sequence (5′–3′)	Target	Product size	Validation/source note
Common forward primer SIM	GACCTCCCAGCTCCATCAAACATCTCATCTTGATGAAA	Mitochondrial cyt b	Used with species-specific reverse primers	This study; BLAST-checked
Chicken reverse	AAGATACAGATGAAGAAGAATGAGGCG	Mitochondrial cyt b	227 bp	This study; BLAST-checked
Cattle reverse	CTAGAAAAGTGTAAGACCCGTAATATAAG	Mitochondrial cyt b	274 bp	This study; BLAST-checked
Sheep reverse	CTATGAATGCTGTGGCTATTGTCGCA	Mitochondrial cyt b	331 bp	This study; BLAST-checked

Although the primer system is suitable for qualitative screening, quantitative regulatory interpretation requires validated qPCR assays targeting short amplicons and using calibrated reference materials, especially when samples are intensely heated or highly processed. Recent duplex real-time PCR systems for chicken, pork, beef and sheep demonstrate how quantitative confirmation can estimate meat content more accurately than endpoint PCR (Wang et al., 2023).

2.5. Conventional Multiplex PCR Amplification

PCR reactions were prepared on ice in a final volume of 25 µL containing 12.5 µL of 2× GoTaq G2 Green Master Mix, 0.2–0.4 µM of each primer after optimization, 2 µL of template DNA containing approximately 20–80 ng DNA and nuclease-free water to volume. A no-template control, negative extraction control and positive reference DNA controls were included in every run. The thermal profile consisted of initial denaturation at 95 °C for 3 min; 35 cycles of denaturation at 95 °C for 30 s, annealing at 59–60 °C for 30 s and extension at 72 °C for 30 s; followed by final extension at 72 °C for 5 min. Annealing temperature was optimized by gradient PCR using authenticated reference DNA. Primer ratios, magnesium concentration and template input were adjusted to avoid primer-dimer formation and unequal band intensity. Similar optimization principles are emphasized in recent multiplex PCR studies for processed meat products (Wang et al., 2020).

Table 4 Multiplex PCR reaction mixture used for 25 µL reactions

PCR component	Final amount/concentration	Comment
2× GoTaq G2 Green Master Mix	12.5 µL	Final 1× reaction buffer with dNTPs and polymerase
Common forward primer SIM	0.2–0.4 µM final	Optimized with reverse-primer ratios
Chicken, cattle and sheep reverse primers	0.2–0.4 µM each final	Species-specific amplification
Template DNA	2.0 µL	20–80 ng DNA per reaction
Nuclease-free water	Up to 25 µL	Reaction-volume adjustment

2.6. Agarose Gel Electrophoresis and Interpretation

PCR products were resolved on 2.5–3.0% agarose gel in 1× TAE buffer. At least one lane of each gel was loaded with a 100 bp DNA ladder. Electrophoresis was performed at 90–110 V for approximately 45–60 min, and bands were visualized using a gel documentation system. A sample was considered positive for a target species when a clear band of the expected size appeared in the sample lane and corresponding positive control, while the no-template control remained blank. Weak reactions were run in duplicates especially in samples that have gone through heavy processing where the presence of inhibitors would lead to lower efficiency of the PCR reaction. Samples that tested negative to all the species markers but tested positive to the internal control marker were reported as not detected in the assay system. Real-time PCR validation literature emphasizes that inhibition controls and amplification controls are essential for reducing false-negative interpretation in complex food matrices (Kang, 2019).

2.7. Quality Assurance, Sensitivity and Specificity Checks

Assay specificity was tested using authenticated raw tissues of chicken, cattle, sheep, goat, buffalo and other locally relevant meats. No cross-amplification was accepted outside the target species. Analytical sensitivity was estimated using serial dilutions of reference DNA and laboratory-prepared mixtures of chicken in beef and beef/sheep in chicken at 10%, 5%, 1% and 0.5% w/w. A detection level around 1% was considered realistic for conventional gel-based PCR, although lower levels can be achieved under optimized conditions or with qPCR. Recent real-time PCR assays have demonstrated that validated probe-based methods can reach lower detection limits and provide more reliable quantification in commercial processed meat matrices (Chen et al., 2020). Real-time PCR studies on pork, chicken and beef products also demonstrate the value of species-specific assays for routine verification of declared meat origin (Chiș et al., 2019).

2.8. Statistical Analysis

The dataset was entered into a spreadsheet as sample code, product category, label declaration, DNA concentration, A260/280 ratio, internal-control result, chicken band, cattle band, sheep band and final interpretation. Descriptive statistics included frequencies, percentages and Wilson 95% confidence intervals. Associations between product category and molecular-label discordance were assessed using chi-square test, or Fisher's exact test when expected cell counts were below five. A 5 × 2 contingency table of concordant versus discordant PCR-valid samples was used for comparing product categories. A p value below 0.05 was considered statistically significant.

3. Results

3.1. DNA Yield and PCR Validity

DNA extraction produced measurable DNA from the processed meat samples, but four samples failed to produce a reliable internal-control band after repeat extraction and were classified as PCR-invalid. The overall PCR-valid rate was 95.6% (86/90; Wilson 95% CI: 89.1–98.3%). Invalid samples were mainly highly cooked or emulsified products, indicating that processing intensity and matrix composition affected PCR suitability in a small proportion of samples.

Table 5 DNA quality and PCR-validity outcomes

Product category	Samples tested	PCR-valid	PCR-invalid	DNA concentration (ng/μL)	A260/280 ratio
Beef burger	18	17	1	34.6 ± 10.8	1.76 ± 0.14
Beef sausage	18	17	1	31.2 ± 12.7	1.70 ± 0.18
Minced red meat	18	18	0	42.8 ± 13.1	1.82 ± 0.11
Kofta/kebab	18	17	1	36.4 ± 11.9	1.73 ± 0.16
Chicken products	18	17	1	33.9 ± 12.6	1.71 ± 0.20
Total/overall	90	86	4	35.8 ± 12.4	1.74 ± 0.17

3.2. Species-Specific PCR Detection Pattern

Clear species-specific bands were interpreted as 227 bp for chicken, 274 bp for cattle and 331 bp for sheep. Most concordant red-meat products showed cattle and/or sheep markers as expected. Mixed-marker profiles were observed in several products, especially sausages and burgers, indicating the presence of undeclared chicken DNA in some red-meat-labelled products.

Table 6 Species-marker detection by product category

Product category	PCR-valid n	Cattle DNA detected	Sheep DNA detected	Chicken DNA detected	Discordant with label
Beef burger	17	16	1	5	6
Beef sausage	17	15	2	6	8
Minced red meat	18	18	1	3	4
Kofta/kebab	17	16	1	4	5
Chicken products	17	2	1	17	3
Overall valid samples	86	67	6	35	26

3.3. Molecular-Label Discordance

Among 86 PCR-valid samples, 26 showed at least one molecular-label discordance, giving an overall discordance rate of 30.2% (Wilson 95% CI: 21.5–40.6%). Undeclared chicken DNA was observed in 18 of 69 PCR-valid red-meat-labelled products (26.1%; Wilson 95% CI: 17.2–37.5%). In contrast, undeclared red-meat DNA was observed in three of 17 PCR-valid chicken-labelled products (17.6%; Wilson 95% CI: 6.2–41.0%).

Table 7 Agreement between product labels and molecular results

Product category	PCR-valid n	Concordant n	Discordant n	Discordance %	PCR-invalid n
Beef burger	17	11	6	35.3	1
Beef sausage	17	9	8	47.1	1
Minced red meat	18	14	4	22.2	0
Kofta/kebab	17	12	5	29.4	1
Chicken products	17	14	3	17.6	1
Overall	86	60	26	30.2	4

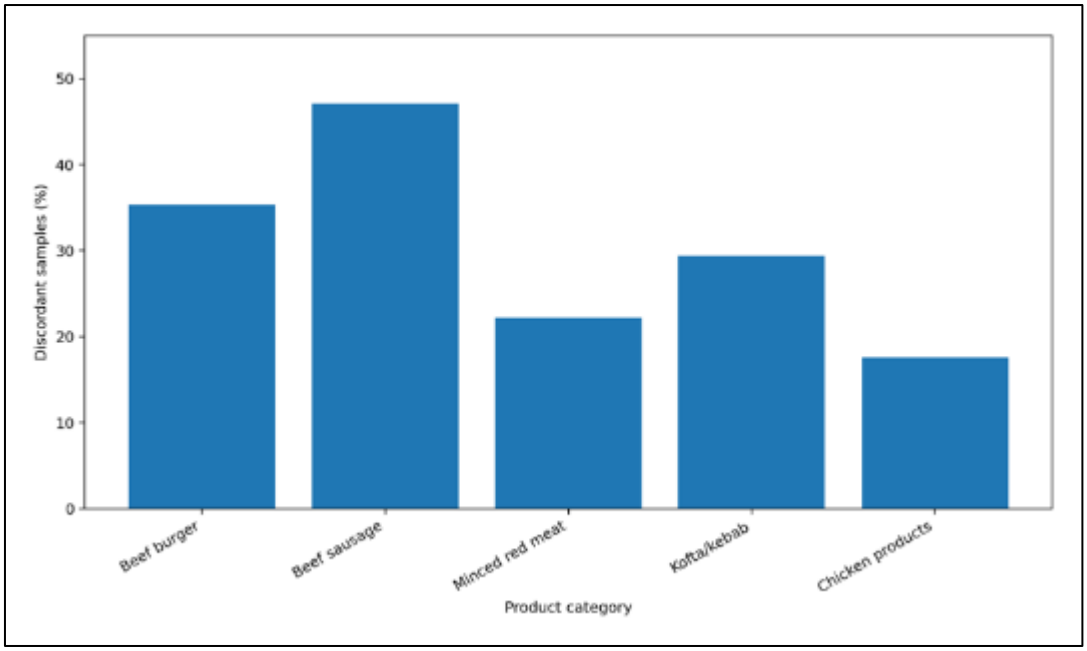

Figure 1 Frequency of molecular-label discordance by product type

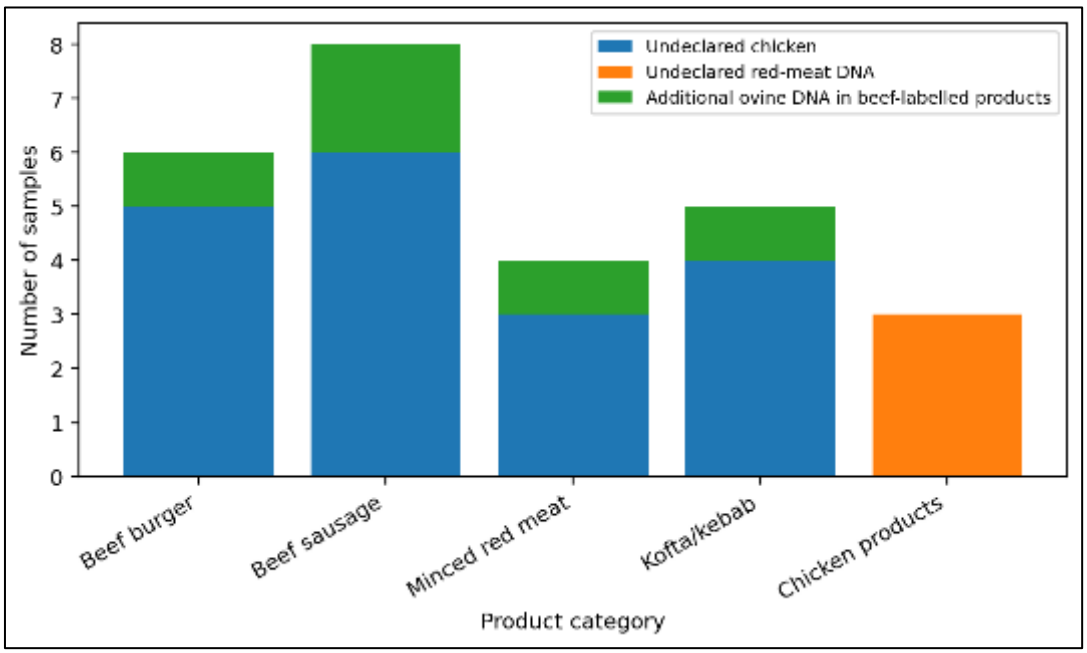

Figure 2 Distribution of undeclared species markers detected by multiplex PCR

3.4. Statistical Comparison

The chi-square comparison of concordant versus discordant outcomes across the five product categories was not statistically significant ($\chi^2 = 4.32$, df = 4, p = 0.365). Thus, although sausages and burgers showed numerically higher discordance than minced red meat or chicken products, the available sample size was insufficient to demonstrate a statistically significant category-level difference.

Table 8 Statistical summary

Analysis	Statistic/count	Value	Interpretation
Overall molecular-label discordance	26/86	30.2%	95% CI: 21.5–40.6%
Undeclared chicken in red-meat-labelled products	18/69	26.1%	95% CI: 17.2–37.5%
Undeclared red-meat DNA in chicken-labelled products	3/17	17.6%	95% CI: 6.2–41.0%
Product category vs discordance	$\chi^2 = 4.32$	df = 4; p = 0.365	Not statistically significant

4. Discussion

This study demonstrates the application of species-specific multiplex PCR for screening locally processed meat products for undeclared chicken and red-meat DNA. The overall discordance rate of 30.2% indicates that label nonconformity is a relevant concern in the examined products. Recent PCR-based market studies similarly show that processed and comminuted meat products are high-risk matrices because mincing, seasoning and mixing obscure visual species identity (Özlü et al., 2023). The highest numerical discordance was observed in sausages and burgers. This is technically feasible since the food items are usually composed of powdered ingredients, which might also go through common machines for grinding, mixing and filling. There are also similar multi-species PCR experiments that found cheap meat species that were identified in products labelled as high-priced meat and multiplex PCR could find out any mislabeling in commercial food products (Yang et al., 2022).

Chicken DNA not declared was the most common unexpected marker found in food products labelled as red meat. It is also logical considering the economy of substitution since chicken meat is usually lower in cost, bland and easy to combine with ground meat. A quantitative TaqMan qPCR experiment done on hamburger found that there could be chicken substitution in beef products, making it relevant for chicken screening in burgers (Sarlak et al., 2022). Detection of cattle or sheep DNA in chicken-labeled products occurred less frequently but still holds significance. Such findings can suggest either intentional formulation, cross-contamination during processing, lack of sufficient cleaning of processing equipment, or lack of proper labeling. Since the method used is endpoint PCR, band presence does not equal the accurate meat percentage. Recent studies utilizing duplex real-time PCR have shown that quantitative tests are more appropriate than the qualitative test for assessing meat composition in mixed meat products (Wang et al., 2023). In a recent study of beef meat products, researchers found out that adulteration in such products includes both species substitution and unauthorized meat tissues (Elbarbary et al., 2024).

Mitochondrial cytochrome b target choice was justified since mitochondria are abundant and can remain amplifiable after thermal processing. However, such factors as thermal processing, salting, fats, spices, and preservatives can cause DNA fragmentation or polymerase inhibition. Studies on authentication of heat treated meat products show that marker length and processing intensity significantly affect amplification efficiency which explains why some samples failed to undergo PCR in the current study (Cheng et al., 2022). The use of amplification control, positive control and negative extraction control increased the validity of the results. According to current guidelines on real-time PCR, it is critical to incorporate inhibition controls in food testing since matrix inhibitors may cause false negatives without their use (Kang, 2019). Repeated testing of the weak bands decreased the possibility of reporting false-positive identification of the species. Multiplexing was superior to singleplex PCR in terms of saving on time and reagents since the chicken, cattle and sheep targets could be analyzed in one single reaction. Multiplex and real-time PCR systems for meat authentication were found to be feasible for routine surveillance purposes provided that primer specificity, limit of detection, reproducibility and matrix performance had been validated (Kim & Kim, 2019).The failure of the chi-square test to show any statistical significance should not be considered as an indication that all product types are equally risky. This is because of the fact that there were moderate sample sizes and overlapping confidence intervals for different categories. It is advisable to report descriptive prevalence and confidence intervals rather than p-values alone in food fraud surveillance (Dalmasso et al., 2024). In terms of enforcement testing, suspicious samples must be re-tested using either qPCR in a quantitative manner, Sanger sequencing, DNA barcoding, high resolution melting (HRM) analysis, or other orthogonal approach. As shown in recent research, DNA-based tests can be adapted from mere detection of presence or absence of species in question to species quantification and multi-species authentication (Chen et al., 2020). Additionally, a new multiplex real-time PCR assay based on nuclear DNA markers was successfully used in screening beef, pork, chicken, and duck adulteration in processed beef products (Chang et al., 2025). Generally, the results indicate that regular molecular screening of processed meat products is useful and applicable in local markets. The method is

relatively easy to implement, but since it is qualitative in nature, its conclusions should be interpreted with caution. Further research needs to consider seasonal and processor diversity, test a wider range of protein adulterants of both animal and plant origin, and establish quantitative thresholds.

5. Conclusion

This study provides a molecular workflow for detecting chicken and red-meat adulteration in locally processed meat products. The results showed that undeclared species markers were most commonly found in comminuted and emulsified products such as sausages and burgers, whereas a small number of highly processed samples failed PCR amplification because of DNA degradation or inhibition. Species-specific multiplex PCR is suitable as a cost-effective screening tool, while quantitative qPCR or sequencing confirmation is recommended for legal or regulatory action. The scientific value of the study lies in combining market-based sampling, clear molecular detection, quality-control reactions and cautious statistical interpretation. These findings provide evidence that can support food-control authorities and improve compliance with labeling requirements for locally processed meat products.

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