MOLECULAR DETECTION OF BOVINE MEAT ADULTERATION IN BANGLADESH USING MULTIPLEX-PCR OF THE MITOCHONDRIAL CYTOCHROME B GENE

Bioresearch Communications
Volume 12, Issue 2, July 2026

DOI:
doi.org/10.3329/brc.v12i2.91458

Md. Aminul Islam[2,3], Anwarul Azim Akhand[2,4] and Gazi Nurun Nahar Sultana[1]*

[1]*Centre for Advanced Research in Sciences, University of Dhaka, Dhaka-1000, Bangladesh.*
[2]*Department of Genetic Engineering and Biotechnology, University of Dhaka, Dhaka-1000, Bangladesh.*
[3]*Department of Genetic Engineering and Biotechnology, East West University, Dhaka-1212, Bangladesh*
[4]*Mawlana Bhashani Science and Technology University, Tangail-1902, Bangladesh.*

ABSTRACT

Meat and meat product fraud poses a serious threat to food safety and public health, as it may lead to metabolic disorders, allergic reactions, and infections. Beyond health concerns, such adulteration also violates market, ethical, and religious principles. To address this issue, multiplex PCR techniques targeting the mitochondrial cytochrome b gene were optimized to evaluate the authenticity of various meat and meat products in Bangladesh. Raw meat samples of bovine (*Bos indicus*), pig (*Sus scrofa domesticus*), and canine (*Canis lupus familiaris*) were collected, with some portions cooked, to establish and optimize multiplex-PCR protocols.

The optimized multiplex-PCR method successfully detected pig and canine meat adulteration in bovine meat, even at varying proportions. Both raw and cooked samples demonstrated high sensitivity, being able to detect adulterants as low as 10%. To validate the PCR results, sequencing and analysis of the amplified products confirmed species-specific identification. Additionally, fried bovine meat samples (n = 5) collected from Bangladeshi restaurants were tested and found to be free of adulteration.

This study highlights the effectiveness of multiplex-PCR as a reliable tool for detecting meat adulteration in both raw and cooked products. The method showed high sensitivity and accuracy, making it suitable for routine monitoring of meat authenticity. While continuous surveillance and strict regulatory enforcement remain essential, the optimized PCR-based techniques can play a vital role in strengthening food safety and ensuring consumer trust in Bangladesh.

KEYWORDS: Meat adulteration; Cytochrome b; Multiplex-PCR; Food safety; Authenticity

RECEIVED: 30 April 2026, ACCEPTED: 07 June 2026

TYPE: Original Article

***CORRESPONDING AUTHORS: Gazi Nurun Nahar Sultana, PhD, Centre for Advanced Research in Sciences, University of Dhaka, Dhaka-1000, Bangladesh.**
Email: nngazi@du.ac.bd

Introduction

Consumer awareness regarding the issue of meat adulteration is rising, and as a result, there is an increasing demand for transparent and reliable information regarding the meat contents of food products (Meira et al., 2017). Meat, as a source of animal protein, has always been an essential foodstuff across the globe, so ensuring its purity and authenticity is paramount (Kua et al., 2022). Meat is edible raw but, in most cases, is typically cooked or processed before consumption. Meat among the other products, are most susceptible to adulteration. Prevalent methods of meat product adulteration are the total replacement of less expensive species for more expensive ones and mixing the meat of less expensive species with more expensive ones (Momtaz et al., 2023). Meat adulteration in Bangladesh has emerged as a significant challenge in food supply chain with serious economic, regulatory and public health implications. In a diverse society where distinct communities following strict religious dietary laws—such as the prohibition of pork in Islam or beef in Hinduism—ensuring food authenticity is critical for maintaining consumer trust and cultural compliance. Beyond ethical and religious concerns, commercial meat adulteration presents critical health risks. The illegal introduction of different meat species can trigger severe food allergies in unaware consumers and bypass veterinary inspection protocols, potentially introducing foodborne pathogens into the market (Werfel et al., 1997; Chao et al., 2005; Takachi et al., 2011; Montowska and Pospiech, 2010). A recent investigation revealed that 22.0% of analyzed meat products contained meat from species not declared on the label (Ayaz et al., 2006). Additionally, a study revealed that 60% of the examined samples included a substitution of a different variety of meat than the officially identified species (Szyłak et al., 2023). Similar instances of mislabeling have been extensively documented by Ballin (2010). Therefore, a reliable

and fast approach for the detection of meat adulteration is necessary.

Several analytical approaches have been used for investigating meat authenticity based on anatomical, histological, chemical, microscopic, organoleptic, chromatographic, or immunological considerations. Most of these methods, however, have been replaced by more modern DNA-based molecular methods because the previous methods have numerous drawbacks (Kumar et al., 2013). Protein-based techniques have some limitations because they may involve challenges such as the denaturation of certain immunogenic proteins due to high cooking temperatures during food processing (Dooley et al., 2004; Kesmen et al., 2009). Conversely, DNA exhibits high thermal stability, is ubiquitous in cells, and contains species-specific sequences. Molecular techniques based on DNA analysis have demonstrated detection of target DNA present at very low levels (Ballin et al., 2009). These techniques, therefore, playing a central role in species identification in both raw and cooked meat blends (Dooley et al., 2004; Laube et al., 2007; López-Andreo et al., 2005; Sawyer et al., 2003). Polymerase chain reaction (PCR) is a very quick, reliable and efficient DNA-based way to detect contamination in both raw and cooked meat samples. This is achieved by amplifying species-specific sequences present in the genome, such as a fragment of the mitochondrial DNA (mt-DNA) Cytochrome b (Cyt b) gene (Girish et al., 2005).

The mitochondrial cytochrome b (cyt b) gene is frequently used for species identification because of its significant sequence variability across species and conserved regions that are suitable for primer design (Farias et al., 2001). Utilizing these features, multiplex PCR enables the simultaneous amplification of multiple distinct loci in a single reaction, thus making it a cost-effective and efficient option for food authentication (Elnifro et al., 2000). While several molecular assays have been developed around the world, there is still a significant gap in standardized, accessible molecular monitoring techniques targeted to the Bangladeshi commercial market. This study aims to address this gap by optimizing and validating a multiplex PCR assay targeting the cyt b gene to detect potential bovine meat adulteration with pig and canine meat. This study establishes the multiplex PCR framework for monitoring commercial meat authenticity in Bangladesh, offering a scalable tool for national food safety regulation.

Materials and Methods

Meat samples collection and processing

Authentic meat samples of bovine (*Bos indicus*), pig (*Sus scrofa*), and canine (*Canis lupus familiaris*) species were collected from different regions in Bangladesh (n = 8 samples per species) to validate the specificity and robustness of the multiplex PCR assay (Table 1). Fresh bovine and pig meat samples were collected from local municipal slaughterhouses under veterinary inspection. Canine tissue samples (n = 8) were safely extracted from a deceased dog following a confirmed road accident in the Rajshahi area, with clear procurement permission issued by the local municipal administration. Bovine fried meat products (n=5) were also collected from some restaurants to observe the real scenario of adulteration. All samples were transported to the laboratory in sterile containers under refrigeration (4° C) and processed within 24 hours of collection. Each biological sample was fully homogenized and divided into separate subgroups for raw and thermal processing procedures.

A standardized heat treatment was used to mimic home and commercial cooking procedures in order to assess the performance of the assay on processed meats. For each species, 50 g of homogenized meat was transferred to separate, closed glass containers. A homogeneous mixture of sterile distilled water and a standardized spice blend consisting of ground pepper, turmeric, and sodium chloride (1.5% w/w each) were added into the meats in order to replicate authentic regional culinary preparation. The sealed containers were subjected to thermal processing at 100° C for 45 minutes utilizing an induction thermal unit monitoring internal core temperatures. The cooked samples were cooled to room temperature (25° C) following thermal treatment and stored at -20° C prior to molecular analysis.

Table 1. Collected meat samples used in this study.

Species	Sample type	Sample size	Collection area
Bovine	Raw meat	8	Dhaka, Bangladesh
Pig	Raw meat	8	Dhaka, Bangladesh
Canine	Raw meat	8	Rajshahi, Bangladesh
Bovine	Fried meat	5	Dhaka, Bangladesh

DNA extraction

Total genomic DNA was isolated from both raw, thermally processed and commercial meat samples (1 g starting material) using a modified organic phenol-chloroform-isoamyl alcohol (25:24:1) extraction protocol based on the standard methodology described Elkins (2012). Cellular lysis was achieved by incubating the homogenized tissues in a high-salt lysis buffer (10 mM Tris-HCl, 100 mM EDTA, and 0.5% SDS, pH 8.0) supplemented with Proteinase K (20 mg/mL) at 4° C overnight. Phase separation was executed via sequential organic extractions, and the genomic DNA was precipitated using ice-cold absolute ethanol. The resulting DNA pellets were air-dried, resuspended in sterile TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The purity (ratio of absorbance at 260 nm

and 280 nm) and concentration of extracted DNA samples were determined using a NanoDrop UV–Vis spectrophotometer (Thermo Fisher Scientific, NanoDrop 2000c, Waltham, MA, USA). DNA was stored at −20 °C before use in PCR amplification.

Statistical analysis

Data obtained from the spectrophotometric quantification of DNA yields were expressed as mean ± standard deviation (s.d.). To evaluate the impact of thermal processing on DNA concentrations, a two-tailed paired Student's t-test was performed to compare the DNA concentrations between the raw and cooked reference groups within each animal species. Statistical significance was set a priori at $p < 0.05$. All statistical computations were conducted utilizing GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA).

Target gene selection and species-specific primers

Cytochrome b gene was selected for the detection of all 3 species because sequence variability of cytochrome b makes it most useful for the comparison of species (Castresana, 2001). To amplify species-specific cytochrome b gene, specific primers for bovine, pig and canine were used. Primer sequences were selected from reference studies (Table 2) and purchased from Integrated DNA Technologies, USA.

Table 2. Species-specific Cyt b gene primer sequences and amplicon sizes used in this study.

Species	Primer	Sequence (5′ to 3′)	Target gene	Amplicon	Reference
Bovine	Uni-F	GACCTCCCAGCTCCATCAAACATCTCATCTTGATGAAA	Cyt b	274 bp	Matsunaga et al. (1999)
	Bov-R	CTAGAAAAGTGTAAGACCCGTAATATAA			
Pig	Uni-F	GACCTCCCAGCTCCATCAAACATCTCATCTTGATGAAA	Cyt b	398 bp	Matsunaga et al. (1999)
	Pig-R	GCTGATAGTAGATTTGTGATGACCGTA			
Canine	Uni-F	GACCTCCCAGCTCCATCAAACATCTCATCTTGATGAAA	Cyt b	146 bp	Matsunaga et al. (1999); Rahman et al. (2014)
	Can-R	TGGGTGACTGATGAAAAAG			

Singleplex-PCR of raw and cooked bovine, pig and canine DNA

The specificity of the three species-specific primer pairs was tested using DNA extracted from raw and cooked bovine, pig and canine. Single PCR reactions to determine the specificity of each primer set were carried out in ProFlex PCR System (Applied Biosystems, USA) in a total volume of 25 µL containing 2.5 µL of 10X PCR buffer, 2 µL of 0.25 mM deoxynucleotide triphosphate (dNTP), 1.5 µL of 25 mM $MgCl_2$, 0.3 µL of 1 U Taq Polymerase (Applied Biosystems, USA), 0.5 µL of each primer (10 µM) and 1 µL of DNA (50 ng/µL). PCR reactions were performed under the following thermal cycling conditions: initial denaturation at 95 ºC for 3 min; followed by 30 cycles of denaturation at 95 ºC for 15 s, annealing at 60 ºC for 30 s, and elongation at 72 ºC for 30 s; and final extension at 72 ºC for 3 min. PCR products were separated and analyzed in a gel electrophoresis system (FB3000, Fisher Scientific, USA) using 1% agarose gel (Promega, Promega Corporation, Madison, USA) containing ethidium bromide (EtBr) stain (Promega, Promega Corporation, Madison, USA) and 1X TAE buffer for 35 minutes at 100V. The agarose gels were visualized under a gel image documentation system (Bio-Print, Vilber Gel Documentation, USA).

Optimization of multiplex-PCR for detecting raw and cooked bovine, pig and canine DNA in different proportions

Optimization of the multiplex system was achieved by systematically adjusting the ratios of individual primer pairs to eliminate primer-dimer formation and prevent preferential amplification of high-abundance targets. The optimized working concentration (10 µM) was balanced in a final 25 µL reaction volume by incorporating 0.9 µL Uni-F, 0.4 µL Bov-R, 0.3 µL Pig-R, and 0.2 µL Can-R. The finalized reaction mixture consisted of 2.5 µL of 10X PCR buffer, 2.0 µL of 0.25 µL

dNTPs, 1.5 µL of 25 mM $MgCl_2$, and 0.3 µL of 1 U Taq DNA Polymerase (Applied Biosystems, Foster City, CA, USA). To validate the assay's development and determine its detection sensitivity in adulterated mixture, separate multiplex reactions were performed for both raw and cooked DNA templates mixed in varying proportions. Tube 1 served as a non-template negative control (NTC) containing no genomic DNA. Tube 2 evaluated equal ratios (50:50) of target and adulterant DNA. To evaluate the limit of detection (LOD) for low-level contamination, subsequent reactions (Tubes 3, 4, 5, and 6) were prepared with descending proportions of adulterant DNA (75%, 50%, 20%, and 10%, respectively) relative to the bovine DNA. Thermal cycling was performed using a ProFlex PCR System (Applied Biosystems, USA) under optimized profile conditions: initial denaturation at 95 ºC for 3 min; followed by 30 cycles of denaturation at 95 ºC for 15 s, annealing at 60 ºC for 30 s, and elongation at 72 ºC for 30 s; and final extension at 72 ºC for 3 min. PCR products were separated and analyzed in a gel electrophoresis system (FB3000, Fisher Scientific, USA) using 2% agarose gel (Promega, Promega Corporation, Madison, USA) containing ethidium bromide (EtBr) stain (Promega, Promega Corporation, Madison, USA) and 1X TAE buffer for 60 minutes at 90V. The agarose gels were visualized under a gel image documentation system (Bio-Print, Vilber Gel Documentation, USA).

Sequencing of the PCR-amplified products

PCR products were purified by ExoSAP-IT™ (Applied Biosystems, USA) following PCR. The purified PCR products were used as the templates for the cycle sequencing reaction with BigDye® Terminator v3.1 sequencing kit (Applied Biosystems, USA). Cycling condition of the sequencing PCR involved initial denaturation at 95 ºC for 3 min; followed by 30 cycles of 94 ºC for 10 s, 55 ºC for 5 s, and 60 ºC for 4 min. After that, cycle sequencing products were purified by ethanol precipitation. Then the capillary electrophoresis was performed in the SeqStudio™ Genetic Analyzer (Applied Biosystems, USA). The chromatograms were visualized and fasta sequences were retrieved from Chromas v2.6.6 and DNA Baser Assembler v5.15.0 softwares. NCBI-Blast (https://blast.ncbi.nlm.nih.gov/) was performed to find out and cross check the desired species. Alignment with the reference sequence (Bovine Accession ID: OR576225, Pig Accession ID: KT965278, Canine Accession ID: MN699618) was also observed from the blast results for each species.

Authenticity testing of bovine meat products of restaurants

Meat products of restaurants have been frequently adulterated with other low-cost species. So, authenticity of some bovine meat products, such as fried meat of different restaurants of Dhaka city were evaluated using our optimized multiplex PCR protocol.

Results and Discussion

Quantitative Evaluation and Yield Analysis of Extracted Genomic DNA

Genomic DNA extracted from both the reference validation cohorts (n = 8 per group) and commercial restaurant samples (n = 5) was quantitatively evaluated via spectrophotometry (Table 3). The average DNA concentrations for raw samples ranged from 714.00 ng/µL to 940.20 ng/µL, whereas cooked samples resulted in relatively lower concentrations, ranging from 88.44 ng/µL to 252.13 ng/µL. To evaluate the impact of thermal processing on DNA recovery, a student's t-test was performed comparing raw and cooked cohorts within each species. The statistical analysis revealed a highly significant reduction in DNA concentration following cooking treatments across all three species ($p < 0.001$). This reduction in DNA yield following thermal processing is consistent with previous findings indicating that excessive heat degrades and denatures genomic DNA, leading to fragment cleavage and reduced template recovery (Musto, 2011; Buntjer et al., 1999; Camma et al., 2012). For the commercial field assessment, the 5 fried bovine meat samples collected from local restaurants yielded a mean DNA concentration of 280.96 ± 23.5. Despite the observed thermal degradation, the final DNA yields were adequate to support downstream multiplex PCR amplification.

Table 3. Quantitative analysis and statistical comparison of extracted DNA from reference and commercial meat samples.

Sample Type	Sample Size	Average DNA Concentration (ng/µL)	p-value (Raw vs. Cooked)
Bovine meat			
Raw bovine meat sample	8	765.98 ± 44.56	< 0.001***
Cooked bovine meat sample	8	177.25 ± 22.79	
Pig meat			
Raw pig meat sample	8	714 ± 41.74	< 0.001***

Cooked pig meat sample	8	88.44 ± 15.43	
Canine meat			
Raw canine meat sample	8	940.2 ± 45.61	< 0.001***
Cooked canine meat sample	8	252.13 ± 26.71	
Commercial Field Samples			
Fried Bovine Restaurant Meat	5	280.96 ± 23.5	N/A

*** *Indicates a highly statistically significant difference ($p < 0.001$) based on a two-tailed paired Student's t-test.*

Specificity results of singleplex PCR of raw and cooked bovine, pig and canine DNA

Species-specific primer pairs targeted mitochondrial genes that varied in sequence between closely related species, resulting in amplification of PCR products of 274 bp (Fig. 1A), 398 bp (Fig. 1B) and 146 bp (Fig. 1C) in bovine, pig and canine respectively. Both raw and cooked samples showed specific amplification. Multiple raw and cooked samples were analyzed for each species to increase the specificity. PCR products amplified by specific primer sets were sequenced according to the procedure of Kim & Kim (2017) to verify the specific species and the specific species were confirmed (S1, S2 and S3).

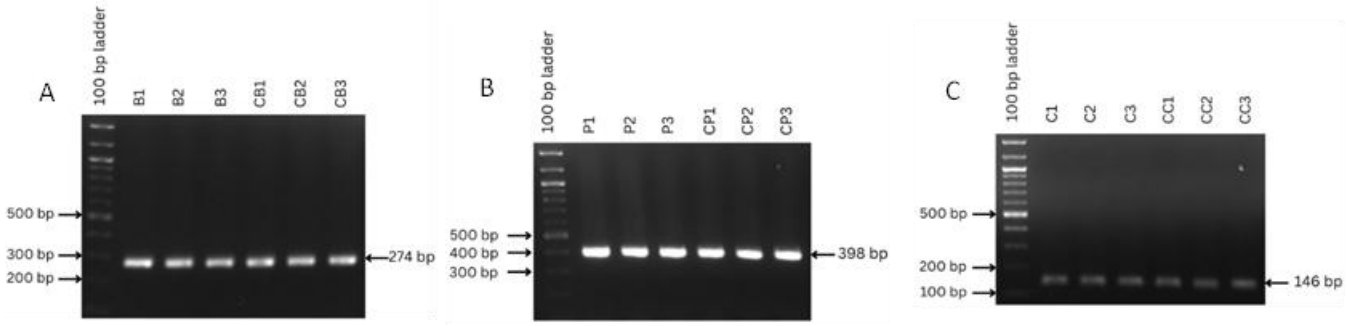

Figure 1. Gel electrophoresis of the singleplex PCR products of the cytochrome b gene of bovine (A), pig (B), and canine (C) DNA samples. Here, B1, B2, B3 are raw bovine DNA samples and CB1, CB2, CB3 are cooked bovine DNA samples; P1, P2, P3 are raw pig DNA samples and CP1, CP2, CP3 are cooked pig DNA samples; C1, C2, C3 are raw canine DNA samples and CC1, CC2, CC3 are cooked canine DNA samples. Clear bands were observed for all the singleplex products.

Identification of raw and cooked bovine, pig and canine DNA by multiplex PCR when mixed in different proportions

Adulteration of bovine meat or beef has been a serious concern. A prior investigation employing the PCR-RFLP technology found 12% adulteration in Egyptian meat products labeled as 100% beef (Dalia & Sanaa, 2015).

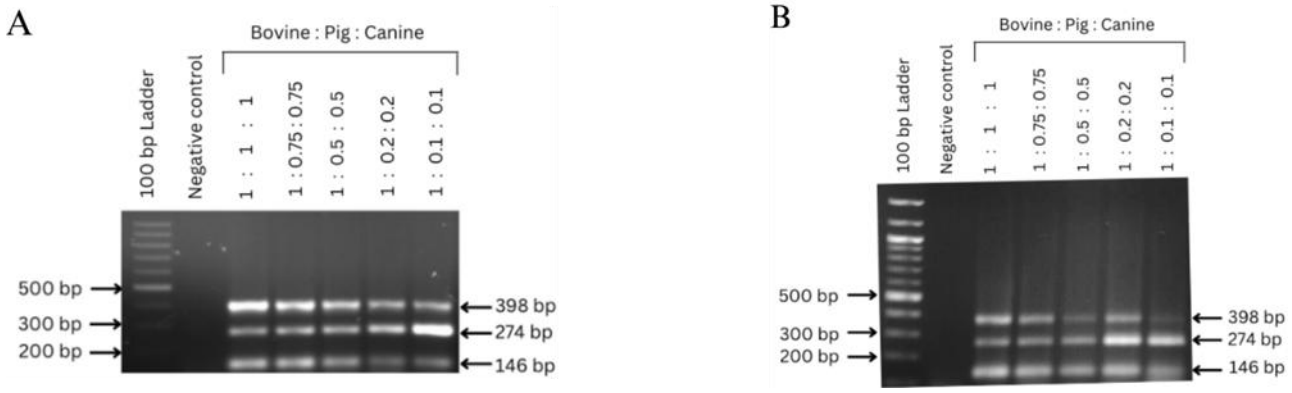

Figure 2. Gel electrophoresis of the multiplex PCR products of the cytochrome b gene of raw (A) and cooked (B) bovine, pig and canine DNA samples in different proportions. Here, products of multiplex PCR with different proportions of the raw and cooked mixed DNA of three species were run for gel electrophoresis and three separate bands were observed in each case.

The optimized multiplex PCR assay was performed using varying genomic DNA proportions to determine its specificity and establish the limit of detection (LOD) across both raw and cooked meat samples (Fig. 2). On the 2% agarose gel, the PCR products showed very clear and reproducible bands for each target animal. Specifically, Lane 1 contained the DNA ladder to confirm the exact sizes of our bands, while Lane 2 was the negative control with no DNA and showed no bands at all, confirming there was no contamination in our reaction. Lane 3 contained equal amounts of DNA from all three species (bovine, pig, and canine), producing three sharp, distinct bands that matched the expected size for each animal. Subsequently, Lanes 4 through 7 tested the sensitivity of the assay using decreasing amounts of pig and canine DNA at 75%, 50%, 20%, and 10%, respectively, mixed with the bovine DNA baseline. Importantly, both the raw and cooked DNA samples consistently showed all three bands across all the tested mixing ratios. The specific band sizes for the cytochrome b (*cyt b*) gene were easy to identify at 274 bp for bovine, 398 bp for pig, and 146 bp for canine.

To identify bovine (*Bos indicus*) meat, a species-specific singleplex PCR was used in a study, focusing on a 381 bp region of the mitochondrial D-loop (Karabasanavar et al., 2017). In Bangladesh, a duplex PCR based study was performed to differentiate between cattle and buffalo (Elsheikh et al., 2022). But our optimized multiplex PCR protocol can be used to detect both raw and cooked pig and canine meat adulterants in raw and cooked bovine meat samples in a single PCR, even if they are mixed in different proportions.

Assessment of the PCR amplified products by sequencing

To further validate our findings, we conducted sequencing of the PCR products, focusing on the mitochondrial cytochrome b gene of bovine, pig and canine (supplementary). This additional step was crucial in confirming that the gel bands observed were specific to the intended species, reinforcing the accuracy of our results. The results of NCBI-Blast and alignment showed that our declared gel bands were species-specific (S1, S2 and S3).

Multiple studies have reported that meat species could be accurately identified by sequencing (Cottenet et al., 2020; Chaora. et al., 2022; Mane et al., 2013). But meat species identification by sequencing would be expensive and it would also be very difficult to implement in a developing country like Bangladesh. So, we focused on easier and low-cost PCR based techniques which were also validated by sequencing.

Authenticity testing of meat products of restaurants

Our aims were to test whether the bovine meat samples of restaurants were adulterated by pig or canine meat samples using our optimized multiplex PCR techniques. After multiplex PCR of the restaurant bovine meat samples (fried meat) according to our optimized protocol, only bovine-specific clear bands were observed at 274 bp for all the samples (Fig. 3). It indicated that those bovine meat samples collected from restaurants were not adulterated with pig or canine meat samples, as there were no pig-specific 398 bp bands and canine-specific 146 bp bands.

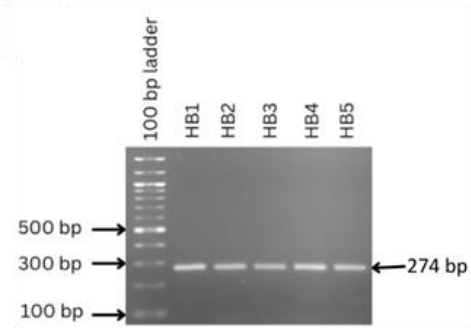

Figure 3. Gel electrophoresis of the PCR products of the cytochrome b gene of restaurant bovine samples. PCR products of all the restaurant bovine DNA samples (HB1, HB2, HB3, HB4 and HB5) showed bovine specific 274 bp bands and no adulteration was observed.

Authenticity assessment is an extremely vital step in the current world, where meat adulteration has been running rampant. Many reports have been published regarding meat adulteration worldwide (Kane & Hellberg, 2016; X. Li et al., 2023). Research conducted in Bangladesh also revealed that approximately 43.75% of slaughterhouses mixed low-quality meat with high-quality meat (M. Rahman & Hasibul, 2018). So, these incidents should be stopped. Some PCR protocols were suggested for the detection of canine meat, but those were not tested on commercially available meat products (Gao et al., 2004; Ilhak & Arslan, 2007).

Conclusion

In recent years, the rising incidence of meat adulteration and deliberate mislabeling in Bangladesh has drawn attention of both consumers and regulatory authorities. Because meat fraud directly impacts religious compliance, public health, and general food safety, developing reliable verification methods is a critical priority. This study successfully optimized a multiplex PCR assay capable of accurately identifying target animal species, with species-specific amplification further validated through sequencing analysis. Ultimately, this multiplex molecular approach provides a rapid, efficient, and highly sensitive screening platform for detecting commercial meat substitution based on genomic DNA. The assay has the capability of simultaneous detection of multiple species and can be a practical and scalable tool towards improvement of market surveillance, protection of consumer rights and strengthening of food safety enforcement across Bangladesh.

Statements and Declarations

Declaration of competing of Interest

The authors affirm that they have no financial or personal conflicts of interest that could be perceived as influencing the work presented in this paper.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical Statement

The authors declare that no live animals were used, handled, or sacrificed for the purposes of this study. Tissue samples of bovine (*Bos indicus*) and pig (*Sus scrofa*) species were procured from market under standard commercial food supply chain regulatory guidelines. Canine (*Canis lupus familiaris*) tissues were collected from a deceased animal following a verified road traffic accident, with official procurement clearance granted by the local municipal authority in Rajshahi.

CRediT authorship contribution statement

Md Aminul Islam: Writing - original draft, Writing - review and editing, Methodology, Conceptualization, **Anwarul Azim Akhand:** Writing – review and editing, Supervision. Visualization, Validation, **Gazi Nurun Nahar Sultana**: Formal analysis. Writing - review and editing, Supervision, Methodology, Conceptualization.

Data Availability

The data will be made available on request.

Acknowledgements

We gratefully acknowledge the Centre for Advanced Research in Sciences at the University of Dhaka for their support in the research.

References

1. Meira L, Costa J, Villa C, Ramos F, Oliveira MBP, Mafra I. EvaGreen real-time PCR to determine horse meat adulteration in processed foods. LWT. 2017;75:408–416. https://doi.org/10.1016/j.lwt.2016.08.061
2. Kua JM, Azizi MMF, Talib MAA, Lau HY. Adoption of analytical technologies for verification of authenticity of halal foods – a review. Food Addit Contam Part A. 2022;39(12):1906–1932. https://doi.org/10.1080/19440049.2022.2134591
3. Momtaz M, Bubli SY, Khan MS. Mechanisms and health aspects of food adulteration: a comprehensive review. Foods. 2023;12(1):199. https://doi.org/10.3390/foods12010199
4. Werfel SJ, Cooke SK, Sampson HA. Clinical reactivity to beef in children allergic to cow's milk. J Allergy Clin Immunol. 1997;99(3):293–300. https://doi.org/10.1016/S0091-6749(97)70045-9
5. Chao A, Thun MJ, Connell CJ, McCullough ML, Jacobs EJ, Flanders WD, et al. Meat consumption and risk of colorectal cancer. JAMA. 2005;293(2):172.
6. https://doi.org/10.1001/jama.293.2.172
7. Takachi R, Tsubono Y, Baba K, Inoue M, Sasazuki S, Iwasaki M, Tsugane S. Red meat intake may increase the risk of colon cancer in Japanese, a population with relatively low red meat consumption. PubMed. 2011;20(4):603–612. https://pubmed.ncbi.nlm.nih.gov/22094846
8. Montowska M, Pospiech E. Authenticity determination of meat and meat products on the protein and DNA basis. Food Rev Int. 2010;27(1):84–100. https://doi.org/10.1080/87559129.2010.518297
9. Ayaz Y, Ayaz N, Erol I. Detection of species in meat and meat products using enzyme-linked immunosorbent assay. J Muscle Foods. 2006;17(2):214–220. https://doi.org/10.1111/j.1745-4573.2006.00046.x
10. Szyłak A, Kostrzewa W, Bania J, Tabiś A. Do you know what you eat? Kebab adulteration in Poland. Foods. 2023;12(18):3380. https://doi.org/10.3390/foods12183380
11. Ballin NZ. Authentication of meat and meat products. Meat Sci. 2010;86(3):577–587. https://doi.org/10.1016/j.meatsci.2010.06.001
12. Kumar A, Kumar RR, Sharma BD, Gokulakrishnan P, Mendiratta SK, Sharma D. Identification of species origin of meat and meat products on the DNA basis: a review. Crit Rev Food Sci Nutr. 2013;55(10):1340–1351. https://doi.org/10.1080/10408398.2012.693978
13. Dooley JJ, Paine KE, Garrett SD, Brown HM. Detection of meat species using TaqMan real-time PCR assays. Meat Sci. 2004;68(3):431–438. https://doi.org/10.1016/j.meatsci.2004.04.010
14. Kesmen Z, Gulluce A, Sahin F, Yetim H. Identification of meat species by TaqMan-based real-time PCR assay. Meat Sci. 2009;82(4):444–449. https://doi.org/10.1016/j.meatsci.2009.02.019

15. Ballin NZ, Vogensen FK, Karlsson AH. Species determination – can we detect and quantify meat adulteration? Meat Sci. 2009;83(2):165–174. https://doi.org/10.1016/j.meatsci.2009.06.003
16. Laube I, Zagon J, Broll H. Quantitative determination of commercially relevant species in foods by real-time PCR. Int J Food Sci Technol. 2007;42(3):336–341. https://doi.org/10.1111/j.1365-2621.2006.01249.x
17. López-Andreo M, Lugo L, Garrido-Pertierra A, Prieto MI, Puyet A. Identification and quantitation of species in complex DNA mixtures by real-time polymerase chain reaction. Anal Biochem. 2005;339(1):73–82. https://doi.org/10.1016/j.ab.2004.11.045
18. Sawyer J, Wood C, Shanahan D, Gout S, McDowell D. Real-time PCR for quantitative meat species testing. Food Control. 2003;14(8):579–583. https://doi.org/10.1016/S0956-7135(02)00148-2
19. Girish P, Anjaneyulu A, Viswas K, Shivakumar B, Anand M, Patel M, Sharma B. Meat species identification by PCR-RFLP of mitochondrial 12S rRNA gene. Meat Sci. 2005;70(1):107–112. https://doi.org/10.1016/j.meatsci.2004.12.004
20. Farias IP, Ortí G, Sampaio I, Schneider H, Meyer A. The cytochrome b gene as a phylogenetic marker: the limits of resolution for analyzing relationships among cichlid fishes. J Mol Evol. 2001;53(2):89–103. https://doi.org/10.1007/s002390010197
21. Elnifro EM, Ashshi AM, Cooper RJ, Klapper PE. Multiplex PCR: optimization and application in diagnostic virology. Clin Microbiol Rev. 2000;13(4):559–570. https://doi.org/10.1128/CMR.13.4.559-570.2000
22. Elkins KM. Forensic DNA biology: a laboratory manual. Academic Press; 2012.
23. Castresana J. Cytochrome b phylogeny and the taxonomy of great apes and mammals. Mol Biol Evol. 2001;18(4):465–471. https://doi.org/10.1093/oxfordjournals.molbev.a003825
24. Matsunaga T, Chikuni K, Tanabe R, Muroya S, Shibata K, Yamada J, Shinmura Y. A quick and simple method for the identification of meat species and meat products by PCR assay. Meat Sci. 1999;51(2):143–148. https://doi.org/10.1016/S0309-1740(98)00112-0
25. Rahman MM, Ali ME, Hamid SBA, Mustafa S, Hashim U, Hanapi UK. Polymerase chain reaction assay targeting cytochrome b gene for the detection of dog meat adulteration in meatball formulation. Meat Sci. 2014;97(4):404–409. https://doi.org/10.1016/j.meatsci.2014.03.011
26. Da Silva ANB, De Cássia Moreira De Souza R, Honorato NRM, Martins RR, Da Câmara ACJ, Da Cunha Galvão LM, Chiari E. Comparison of phenol-chloroform and a commercial DNA extraction kit for identification of bloodmeal sources from triatomines. Rev Soc Bras Med Trop. 2020;53. https://doi.org/10.1590/0037-8682-0189-2020
27. Musto M. DNA quality and integrity of nuclear and mitochondrial sequences from beef meat as affected by different cooking methods. Food Technol Biotechnol. 2011;49(4):523–528. https://hrcak.srce.hr/75193
28. Buntjer JB, Lamine A, Haagsma N, Lenstra JA. Species identification by oligonucleotide hybridisation: the influence of processing of meat products. J Sci Food Agric. 1999;79(1):53–57. https://doi.org/10.1002/(SICI)1097-0010(199901)79:1
29. Cammà C, Di Domenico M, Monaco F. Development and validation of fast real-time PCR assays for species identification in raw and cooked meat mixtures. Food Control. 2012;23(2):400–404. https://doi.org/10.1016/j.foodcont.2011.08.007
30. Kim M, Kim H. Species identification of commercial jerky products in food and feed using direct pentaplex PCR assay. Food Control. 2017;78:1–6. https://doi.org/10.1016/j.foodcont.2017.02.027
31. Dalia Z, Sanaa H. Use of molecular biology techniques in the detection of fraud meat in the Egyptian market. Afr J Biotechnol. 2015;14(5):360–364. https://doi.org/10.5897/AJB2014.14297
32. Karabasanavar N, Girish PS, Kumar D, Singh SP. Detection of beef adulteration by mitochondrial D-loop based species-specific PCR. Int J Food Prop. 2017;1–8. https://doi.org/10.1080/10942912.2017.1369103
33. Elsheikh BH, Shawish RR, Elbayoumi ZH. Detection of meat species substitution in some ready to eat beef-based meat products in Egypt. Adv Anim Vet Sci. 2021;10(1). https://doi.org/10.17582/journal.aavs/2022/10.1.68.77
34. Cottenet G, Blancpain C, Chuah PF, Cavin C. Evaluation and application of a next generation sequencing approach for meat species identification. Food Control. 2020;110:107003. https://doi.org/10.1016/j.foodcont.2019.107003
35. Chaora NS, Khanyile KS, Magwedere K, Pierneef R, Tabit FT, Muchadeyi FC. A 16S next generation sequencing based molecular and bioinformatics pipeline to identify processed meat products contamination and mislabelling. Animals. 2022;12(4):416. https://doi.org/10.3390/ani12040416
36. Mane B, Mendiratta S, Tiwari A, Narayan R. Sequence analysis of mitochondrial 16S rRNA gene to identify meat species. J Appl Anim Res. 2013;41(1):77–81. https://doi.org/10.1080/09712119.2012.738213
37. Kane DE, Hellberg RS. Identification of species in ground meat products sold on the U.S. commercial market using DNA-based methods. Food Control. 2016;59:158–163. https://doi.org/10.1016/j.foodcont.2015.05.020
38. Li X, Zang M, Li D, Zhang K, Zhang Z, Wang S. Meat food fraud risk in Chinese markets 2012–2021. NPJ Sci Food. 2023;7(1). https://doi.org/10.1038/s41538-023-00189-z
39. Rahman M, Hasibul H. Meat adulteration at Khulna City of Bangladesh: a criminological analysis. Int J Humanit Soc Sci Educ. 2018;5(4). https://doi.org/10.20431/2349-0381.0504013
40. Gao H, Xu B, Liang C, Zhang Y, Zhu L. Polymerase chain reaction method to detect Canis materials by amplification of species-specific DNA fragment. J AOAC Int. 2004;87(5):1195–1199. https://doi.org/10.1093/jaoac/87.5.1195
41. İlhak Oİ, Arslan A. Identification of meat species by polymerase chain reaction (PCR) technique. Turk J Vet Anim Sci. 2007;31.