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# Investigating Pork and Chicken Adulteration in Beef-Meatball Products from Small Food Vendors Using Porcine Detection Kit and PCR Analysis

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**Abstract**

Food adulteration compromises consumer health and violates dietary and religious principles by introducing undeclared or prohibited ingredients. Economic pressures and fluctuating beef prices are key drivers behind the substitution of beef with cheaper alternatives such as chicken or pork. These meats can be easily blended into comminuted products, such as meatballs, making adulteration difficult to detect visually. Pork adulteration is of particular concern in Muslim communities, where its consumption is strictly prohibited under Islamic dietary law. This study investigated pork and chicken adulteration in 28 beef meatball products (BMPs) obtained from small vendors. Initial porcine detection was conducted using a Porcine Detection Kit (PDK), with results confirmed through Polymerase Chain Reaction (PCR). Simplex PCR was applied to detect pork and chicken adulteration and to verify the presence of beef, while multiplex PCR enabled the simultaneous identification of all three species, offering a more efficient diagnostic approach. The results revealed that most BMP samples contained chicken, while two samples tested positive for pork contamination. PCR also detected undeclared chicken in products marketed as beef, indicating potential mislabeling and food fraud. These findings highlight the complementary role of PDK as a rapid field screening tool and PCR as a confirmatory method. Together, they provide an effective framework for routine halal authentication and food integrity monitoring in processed meat products.



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## Introduction

Halal authentication in processed meat products is a critical issue for Muslim consumers, particularly concerning the potential adulteration of beef with pork, which is strictly prohibited in Islam. In addition, the undeclared addition of chicken to beef products constitutes food fraud, misleading consumers and violating labeling regulations. Detecting both pork and chicken adulteration is therefore essential for consumer protection, the enforcement of religious dietary laws, and the preservation of market integrity. Food adulteration—whether intentional or unintentional—remains a major global concern, threatening public health, violating consumer trust, and undermining cultural and religious values. This issue is especially critical in regions with strict dietary restrictions, such as Islamic communities, where the consumption of pork is forbidden (haram). Due to its high commercial value, meat has long been a common target for adulteration, with numerous reports documented worldwide (Zdenkova et al., 2018). Processed meat products, such as meatballs, are particularly vulnerable to adulteration with haram substances, including dog meat and porcine derivatives, highlighting the importance of ensuring halal integrity (Manalu et al., 2019; Rahman et al., 2014; Razzak et al., 2015; Windarsih et al., 2024). This concern carries heightened significance in Indonesia, where the halal industry is projected to make a substantial contribution to the national GDP by 2030 (Fischer & Nisa, 2025). Halal standards adopt a strict zero-tolerance policy toward pork adulteration, whereby even trace amounts of non-halal substances are unacceptable. Given that over 85% of Indonesia's population identifies as Muslim, halal certification strongly influences consumer purchasing behavior and confidence (Alfaini et al., 2024; Azam, 2016; Riaz & Riaz, 2024). Ensuring halal authenticity in food products is therefore indispensable to safeguarding consumer rights, religious observance, and economic development.

Meatballs are a widely consumed dish in Indonesia, available across diverse settings ranging from street vendors to upscale restaurants (Sujarwanta et al., 2021). Traditionally, they are prepared from a mixture of beef and chicken, with the proportion of beef determining both product quality and price; meatballs with a higher beef content are generally more expensive. However, fluctuations in beef prices, a primary ingredient in beef meatball products (BMPs), often lead producers to substitute beef with chicken or flour to reduce costs. Although chicken is permissible under Islamic dietary laws, vendors marketing their products as 100% beef must disclose the presence of chicken, as such substitution alters both the value and perceived authenticity of the product. When undeclared, this practice constitutes mislabeling and may be regarded as food fraud. In some cases, beef has even been illicitly replaced with pork, which is considerably cheaper (Khasanah et al., 2021). This fraudulent substitution not only violates halal requirements but also undermines consumer trust and raises serious concerns regarding food authenticity and integrity (van Ruth et al., 2017).

A range of analytical methods has been developed to detect meat adulteration, including protein- and fat-based techniques. These, however, face limitations in processed products, where protein denaturation and lipid oxidation often occur during heating. In contrast, DNA-based approaches, such as porcine detection kits (PDKs) and polymerase chain reaction (PCR) assays, provide greater stability and specificity for species identification, making them effective in detecting adulteration even in thermally processed foods. Immunochromatographic assays, such as PDKs, are rapid, user-friendly, and widely available, making them suitable for on-site screening by regulatory authorities (Yusop et al., 2022). Nonetheless, their limitations include reduced sensitivity and lower reliability in heavily processed or diluted samples (Li et al., 2023; Magiati et al., 2019; Zvereva et al., 2020). Consequently, PCR is often employed to confirm PDK results, as it enables precise detection of specific DNA sequences and offers high sensitivity and accuracy (Doroudian et al., 2023; Liberty et al., 2025; Wang et al., 2023). PCR has become a standard method for verifying the halal status and authenticity of food products derived from animals. Because PCR amplification targets specific DNA sequences, it can detect trace amounts of species-specific DNA, ensuring reliability even in complex or processed samples. Numerous studies have demonstrated the superiority of PCR over protein-based methods in terms of sensitivity, effectiveness, and accuracy (Besbes et al., 2012; Fajardo et al., 2010; Mutalib et al., 2015). Multiplex PCR further enhances efficiency by enabling the simultaneous detection of multiple species within a single assay (Cahyadi et al., 2020; Chaudhary & Kumar, 2022).

Accordingly, the present study investigated pork adulteration in meatballs sold by small vendors who marketed their products as halal. Initial screening was

conducted using the rapid PDK assay, followed by PCR confirmation for species-specific DNA detection. The analysis encompassed beef, chicken, and pork identification within the meatball samples. The findings highlight the necessity of stringent food safety and halal authentication measures, as well as accurate and transparent labeling practices. Such measures are particularly crucial in regions with large Muslim populations, where protecting consumer trust and public health is paramount.

## Materials and methods

### 1. Materials

Fresh beef, pork, and chicken used as positive controls were purchased from a supermarket in Yogyakarta, Indonesia. Fresh pork was used for both PDK and PCR analysis (both simplex and multiplex PCR), while fresh chicken and fresh beef were used for positive control in simplex and multiplex PCR analysis. A total of 28 meatball samples were randomly bought from various small street vendors across different areas in Yogyakarta, Indonesia. The vendors were chosen specifically from those with permanent or semi-permanent sales locations that appeared crowded based on researcher observations. During the purchasing process, the researcher inquired whether the meatballs contained pork, but did not disclose that the meatballs were intended for research purposes. The meatballs were subjected to separation, with only the meatballs being retained and stored in a refrigerator. The accompanying side dishes were discarded and meatballs samples were then stored at -18°C until further analysis. The subsequent analysis was conducted at the Center for Food and Nutrition Studies, Universitas Gadjah Mada (UGM) and the Biochemistry Laboratory of the Faculty of Veterinary Medicine at UGM.

### 2. Porcine detection KIT

Prior to analysis samples were thawed and subsequently ground. After grounding, 200 mg of each sample was diluted in 200 µL of phosphate-buffered saline (PBS) to a concentration of 10% (0.2 g/2 mL). The diluted samples were then centrifuged at 2,000 rpm for 20 min. Positive controls of the 10% and 50% (w/v) fresh pork meat were treated the same as the samples. The supernatants obtained from the centrifugation process for all samples and positive controls were subjected to an immunochromatographic strip test using the PDK (XEMAtest PORK, XEMATest, Findland). The test strip was removed from the package just prior to use to ensure no moisture was present, which can potentially reduce the sensitivity. The test strip was immersed in the supernatant to the first white line under the arrow and held briefly for 15 sec until the solution had migrated halfway through the white center of the strip. The strip was placed horizontally and allowed to air dry. The appearance of a second red line at the test position indicates a positive result, meaning the sample contains pork, while if only one red line appears at the control position, the sample does not contain pork. Documentation was then performed by capturing images of the strips with a camera within 15 min post-immersion. The analysis was done twice on each supernatant.

### 3. DNA extraction

DNA extraction was conducted as a preparatory step for PCR analysis to obtain DNA templates from the meatball samples. Total metagenomic DNA was isolated from the beef meatball product (BMP) samples, encompassing all DNA within the complex food matrix without prior species separation. Positive controls from fresh pork, chicken, and beef were extracted using the same procedure. DNA was isolated using the Genomic DNA Mini Kit (Geneaid, Qiagen, Hilden, Germany), which includes GT buffer, GBT buffer, RNase, absolute ethanol, W1 buffer, wash buffer, elution buffer, and proteinase-K (Invitrogen, USA). Extractions were performed with minor modifications to the manufacturer's protocol. The quality and success of DNA isolation were verified by agarose gel electrophoresis. A 1.5% (w/v) agarose gel was prepared in 1× TBE buffer and submerged in the same buffer. DNA samples were mixed with GoodView™ Nucleic Acid Stain loading dye and loaded into the gel. Electrophoresis was carried out at 100 V for 45 min, enabling DNA migration from the negative to the positive pole. DNA bands were visualized under a UV transilluminator at 260 nm, and images were recorded for documentation. Samples showing clear DNA bands were deemed suitable for downstream analysis. The extracted DNA was subsequently subjected to PCR amplification using species-specific primers, following the method described by Dalmasso et al. (2004).

### 4. Simplex and multiplex PCR

DNA amplification was performed using the PCR method. The preparation of the Master Mix for PCR was carried out using the following formulation: 3 µL of one set of primers (forward and reverse), 4.5 µL of PCR-grade water ($H_2O$-PCR), 1 µL of $MgCl_2$, and 4 µL of template DNA for each sample. There were three sets of different

primers: beef (*Bos taurus*), pork (*Sus scrofa*) and chicken (*Gallus gallus*) used in both analysis of simplex and multiplex PCR. Simplex PCR utilized only one set of primers during DNA amplification, while in multiplex PCR, all three sets of primers were used at the same time. The primers were specific to each species and were designed from various regions of mitochondrial DNA, including 12S rRNA, tRNA Val, and 16S rRNA and were published by Dalmasso et al., 2004. Sequence of oligonucleotide primer used in both simplex and multiplex PCR are presented in Table 1.

**Table 1** Sequence of oligonucleotide primer used in both simplex and multiplex PCR

| Species | Primer | Oligonucleotide primer | Amplicon (bp) |
|---|---|---|---|
| Beef | forward | 5'GAA AGG ACA AGA GAA ATA AGG 3' | 104 |
| *(Bos taurus)* | reverse | 5' TAG GCC CTT TTC TAG GGC A 3' | |
| Chicken | forward | 5' TGA GAA CTA CGA GCA CAA AC 3' | 183 |
| (*Gallus gallus*) | reverse | 5' GGG CTA TTG AGC TCA CTG TT 3' | |
| Pork | forward | 5' CTA CAT AAG AAT ATC CAC CAC A 3' | 290 |
| (*Sus scrofa*) | reverse | 5' ACA TTG TGG GAT CTT CTA GGT 3' | |

PCR reactions were carried out using an INFINIGEN PCR Thermocycler. Amplification conditions were adapted from Ghovvati et al. (2009), with modifications shown in Table 2. For each PCR assay, different annealing temperatures were tested to optimize amplification efficiency. The temperature yielding the clearest DNA bands was selected for subsequent analyses, which were performed in triplicate to ensure reproducibility. Multiplex PCR was developed using primer sets originally designed for simplex PCR. Amplification was performed in a 25 µL final reaction volume, consisting of 12.5 µL Master mix, 3 µL primer, 4.5 µL PCR-grade water, 1 µL $MgCl_2$, and 4 µL template DNA. Template DNA concentrations were adjusted according to sample characteristics. PCR cycling conditions were based on the optimized parameters and repeated three times to confirm consistency of results.

The PCR products were analyzed by electrophoresis on a 1.5% agarose gel run in 1X TBE buffer for 55 min at 100 V. A DNA ladder BIORONTM was used as a marker to determine the base pair sizes of the DNA fragments from 100 to 1000 bp. The amplified DNA sample was mixed with BluejuiceTM that contain blue dye bromophenol as coloring agent and glycine which acts as a weight to ensure that the DNA bands migrate within the wells of the agarose gel submerged in 1X TBE buffer. The results of the gel electrophoresis were visualized under UV light, allowing for the assessment of the band sizes, which were then compared to the expected sizes based on the primers used in the PCR mix.

## Results and discussion

### 1. Porcine detection KIT

The Porcine detection Kit (PDK) was employed as an initial screening tool to detect pork contamination in the meatball samples. The assay is specifically designed for the rapid identification of porcine content in food products. Among the 28 beef meatball product (BMP) samples tested, only Sample 7 yielded a clear positive result. Sample 9 exhibited a faint line at the test position; however, in accordance with the manufacturer's interpretation guidelines, this was classified as negative. The remaining 27 samples showed a single red line at the control position, confirming negative results for porcine content. The PDK functions on the principle of lateral flow immunochromatography, whereby antigens present in the sample bind to specific antibodies immobilized on the test strip, forming antigen–antibody complexes (Hendrickson et al., 2023). As the sample migrates along the strip, these complexes are captured at the test line, which contains labeled conjugates (e.g., latex beads) that bind to the antigen. When the antigen is present, a visible red line develops at the test position, indicating a positive result (Kim et al., 2023).

**Table 2** PCR conditions for both simplex and multiplex PCR

| Type of PCR | Primer | Initial denaturation | Denaturation (35 cycles) | Annealing | Extention | Final extention |
|---|---|---|---|---|---|---|
| **Simplex** | Beef | 94°C for 5 min | 94°C for 30 sec | 55°C for 1 min<br>57°C for 1 min | 72°C for 1 min | 72°C for 5 min |
| **Simplex** | Chicken | 94°C for 5 min<br>94°C for 5 min | 94°C for 30 sec<br>94°C for 30 sec | 57°C for 1 min<br>55°C for 1 min | 72°C for 1 min<br>72°C for 1 min | 72°C for 5 min<br>72°C for 5 min |
| **Simplex** | Pork | 94°C for 5 min | 94°C for 30 sec | 57°C for 1 min<br>59°C for 1 min | 72°C for 1 min | 72°C for 5 min |
| **Multiplex** | Beef, chicken, pork | 94°C for 5 min | 94°C for 30 sec | 56°C for 1 min<br>57°C for 1 min | 72°C for 1 min | 72°C for 5 min |

Immunoassay-based methodologies, such as lateral flow immunochromatography, have demonstrated high sensitivity for detecting pork in complex food products. Previous studies reported that PDK strips successfully detected trace levels of pork adulteration in meatballs (Kuswandi et al., 2017; Yusop et al., 2022) and as little as 0.01% in raw pork (Masiri et al., 2016). Kuswandi et al. (2017) further showed that test line intensity increased proportionally with higher adulteration levels, achieving excellent reproducibility (100%) in most cases, though slightly reduced at the lowest concentration tested (0.05% pork-to-beef ratio). The PDK offers several advantages for field inspections, including rapid turnaround (approximately 35 minutes), cost-effectiveness, and user-friendliness, requiring no specialized equipment or expertise (Li et al., 2023; Magiati et al., 2019). These features make it suitable for on-site testing in restaurants, supermarkets, or household settings, where quick screening of potentially non-halal products is necessary (Kim et al., 2023; Li et al., 2023). By enabling early identification of suspect samples, the PDK streamlines quality control, halal authentication, and market surveillance workflows, allowing resources to be focused on confirmatory testing with PCR.

Nevertheless, the accuracy of PDK testing can be compromised by complex food matrices such as meatballs, which often contain spices and undergo extensive heat treatment. High-temperature processing may denature proteins, impairing antigen–antibody recognition and leading to false negatives. In this study, such an effect was observed in Sample No. 9, where pork DNA was present but the PDK result was faint and inconclusive. For this reason, PDK is best applied as a preliminary screening tool, while molecular-based methods such as PCR are essential for confirmatory analysis. PCR detection of species-specific DNA provides the sensitivity required to detect trace amounts of pork, ensuring the reliability of halal authentication in complex food systems.

## 2. DNA extraction and PCR optimization

DNA extraction was conducted to all samples and positive control to produce template for PCR amplification. DNA extraction is the crucial first step in the PCR process, as it involves isolating the DNA from meatball to ensure that a clean, pure DNA template from each species, whether beef, chicken or pork is available for amplification. This step is essential because contaminants like proteins, lipids, or other cellular components can inhibit the PCR reaction and affect the quality of the amplified product. The goal of DNA extraction is to break open the cells, release the DNA, and then purify it from other cellular debris (Dopheide et al., 2019).

The results of DNA extraction were visualized using a UV transilluminator, and the images are shown in Fig. 2. All of DNA bands did not align with any of the DNA size markers because they represent the isolated of total DNA from the protein in the sample, rather than specific DNA fragments of defined sizes. Samples 18, 21, and 28 showed faint or almost invisible bands while the other samples , including the positive controls (raw chicken, raw beef, and raw pork), showed a clearly visible band, indicating the presence of DNA and its readiness for amplification.

Based on the visualization of isolated DNA shown in Fig. 1, sample numbers 18, 21 and 28 were not distinctly observable, suggesting that DNA degradation may have occurred during the cooking process of the meatballs or due to repeated heating. Previous studies have shown that even highly fragmented DNA can still serve as a PCR template. For example, Bottero and Dalmasso (2011) demonstrated successful amplification from degraded DNA, while Siswara et al. (2022) reported that DNA concentrations as low as 320 ng/µL remain suitable for PCR. Low DNA yields below the detection threshold of gel electrophoresis may also account for the weak or absent bands observed in some samples. It is

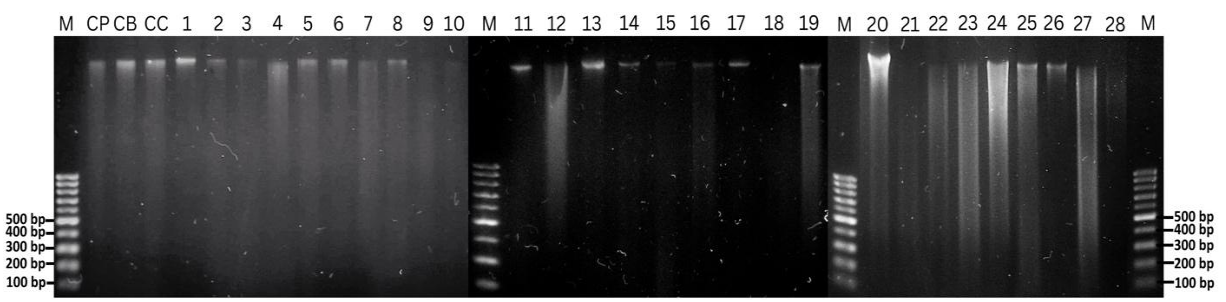


**Fig. 1** Visualization of isolated DNA, M: Marker, CP: Control positive from fresh pork, CB: Control positive from fresh beef, CC: Control positive from fresh chicken, 1-28: meatball samples

also acknowledged that the absence of an internal amplification control (IAC) represents a limitation of this study. The use of an IAC in future work would enable more reliable detection of PCR inhibition. Despite these challenges, DNA is generally more thermally stable than protein, and previous research has confirmed its persistence under high heat (Shahimi et al., 2021). Accordingly, the extracted DNA in this study was considered suitable for downstream PCR amplification.

Optimization of simplex PCR conditions indicated that chicken and beef primers yielded optimal amplification at an annealing temperature of 57°C, while porcine primers produced clearer bands at 59°C compared to 57°C. For multiplex PCR, an annealing temperature of 57°C provided specific and distinct DNA bands for pig, bovine, and chicken primers. Optimization is a critical step in PCR as it ensures reaction conditions are suitable for efficient and specific amplification of target DNA. In particular, annealing temperature plays a key role in primer specificity, reducing the risk of nonspecific binding (Shen et al., 2007). Reported annealing temperatures vary across studies depending on the primers and target sequences used, ranging from 53.3°C to 64°C in meatball analysis (Orbayinah et al., 2020; Wu et al., 2020).

**3. Simplex and multiplex PCR**

The presence of beef DNA in all meatball samples was confirmed by a 105 bp fragment (Fig. 2). This result aligns with vendor claims and is consistent with the requirement that beef serves as the primary ingredient in beef meatball products (BMPs). However, beef's fluctuating market price, particularly during festive periods such as Eid Mubarak and Christmas as well as Indonesia's reliance on Australian beef imports (Bindon & Jones, 2001) exert economic pressure on small vendors, which may drive adulteration with cheaper meats such as pork. Chicken DNA was detected in all samples except Sample 21, producing a 183 bp band (Fig. 3). This finding is consistent with previous studies reporting the frequent detection of chicken in BMPs (Kusnadi & Harfiyanti, 2023; Sari et al., 2017). The addition of chicken is a common practice among Indonesian street vendors to reduce costs and impart distinctive flavors to their products. While chicken itself is permissible and widely consumed, labeling a product containing chicken as "100% beef" constitutes food fraud. Such misrepresentation deceives consumers, who pay a higher price for what they believe to be a pure beef product, thereby undermining consumer trust and food supply chain integrity (van Ruth et al., 2017). Porcine DNA was detected in Samples 7 and 9, with amplification of a 290 bp fragment (Fig. 4). Notably, Sample 9 tested negative using the PDK immunoassay but was confirmed positive by PCR. This discrepancy underscores the higher sensitivity of PCR compared to protein-based assays such as PDK. Whereas PDK requires a sufficient concentration of porcine proteins for detection, PCR can amplify trace DNA quantities, with a detection limit as low as 0.001 ng/µL (Doroudian et al., 2023; Liberty et al., 2025). The presence of porcine DNA in Sample 9 may be attributed to contamination from lard in the soup used to cook meatballs. Although lard itself contains little or no DNA, residual proteins or fat particles may carry trace DNA, which can be detected by PCR but not by PDK. This highlights the unique advantage of PCR in detecting minute contamination levels that may evade immunoassay-based methods. The detection of chicken and pork in BMPs raises significant concerns from both economic and religious perspectives. While undeclared chicken adulteration constitutes mislabeling and economic fraud, the presence of pork—irrespective of concentration—is prohibited (haram) under Islamic law. According to the Indonesian Minister of Religion Regulation No. 26 (2019), Chapter 25, paragraph 2, pork is classified as haram, while the Halal Product Assurance Law (Law No. 33/2014) explicitly prohibits its inclusion in halal-certified food. Thus, pork adulteration in meatballs not only constitutes food fraud but also represents a legal violation with religious and ethical implications (Riaz & Riaz, 2024; Siswara et al., 2022). Despite these clear regulations, pork adulteration continues to be reported in various studies (Aina et al., 2020; Kusnadi & Harfiyanti, 2023; Orbayinah et al., 2020). Multiplex PCR analysis yielded results consistent with simplex PCR, confirming species identification of beef, chicken, and pork in a single assay. DNA fragments of 290 bp (pork, *Sus scrofa*), 104 bp (beef, *Bos taurus*), and 183 bp (chicken, *Gallus gallus*) were simultaneously amplified. The use of multiplex PCR significantly reduced analysis time while maintaining accuracy. Although positive controls were included for each species, No Template Controls (NTCs) and internal amplification controls (IACs) were not employed, which is recognized as a limitation of this study. Future work should incorporate such controls to further strengthen reliability and detect potential contamination or PCR inhibition. Despite challenges such as DNA fragmentation caused by heat treatments, which can

reduce amplification efficiency (Bottero & Dalmasso, 2011; Dalmasso et al., 2004), this study demonstrates that multiplex PCR is a powerful and efficient method for species detection. Its ability to simultaneously detect pork and chicken adulteration in beef meatball products aligns with findings from other studies (Cahyadi et al., 2021; Chaudhary & Kumar, 2022; Kusnadi & Harfiyanti, 2023; Rosyid et al., 2023; W. Wang et al., 2019) and highlights its value as a tool for safeguarding food authenticity and halal compliance.

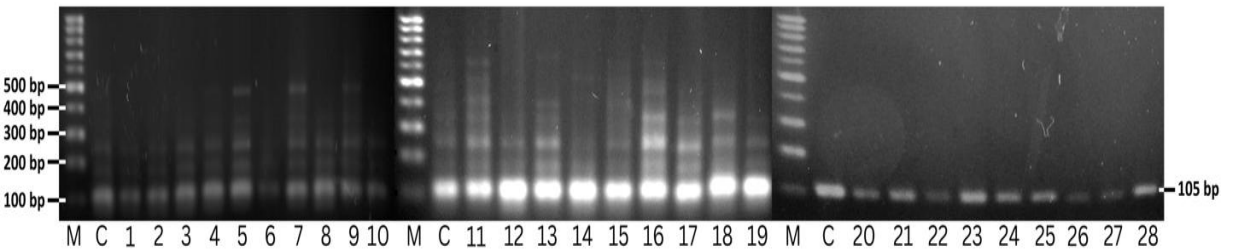


**Fig. 2** Visualization of isolated DNA under UV light resulted from simplex PCR with beef primer (annealing at 57°C) with the length 183 bp, M: Mark,,er, C: Control positive from fresh beef, 1-28: meatball samples.

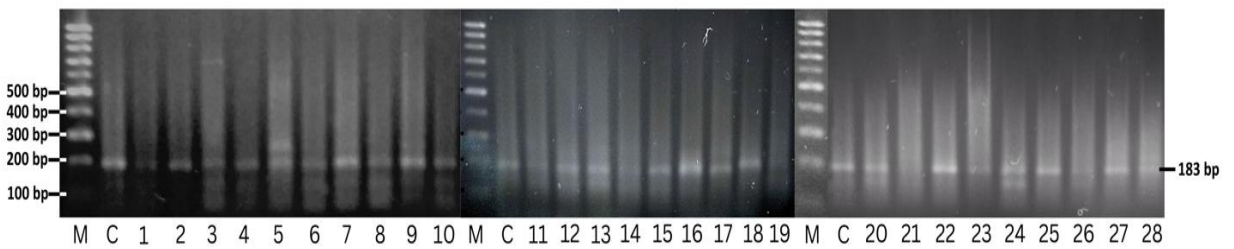


**Fig. 3** Visualization of isolated DNA under UV light resulted from simplex PCR with chicken primer (annealing at 57°C) with the length 183 bp, M: Marker, C: Control positive from fresh chicken, 1-28: meatball samples.

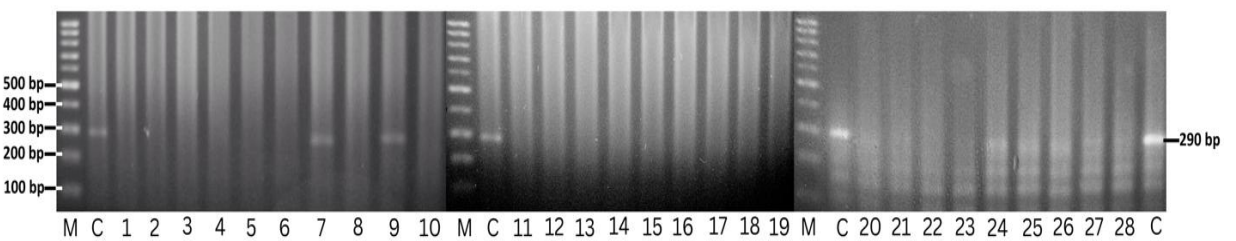


**Fig. 4** Visualization of isolated DNA under UV light resulted from simplex PCR with pork primer (annealing at 59°C) with the length 290 bp, M: Marker, C: Control positive from fresh chicken, 1-28: meatball samples.

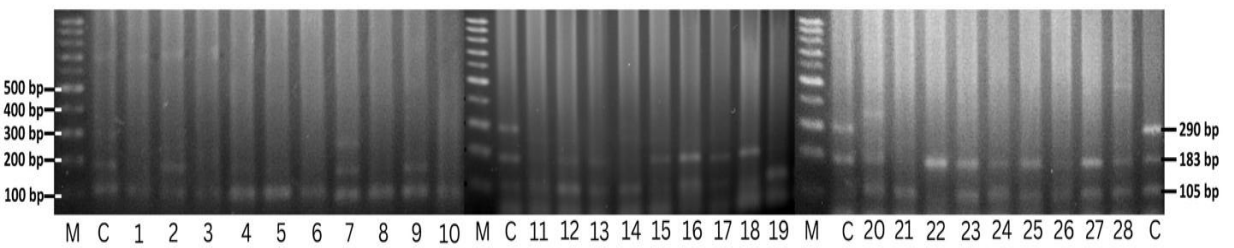


**Fig. 5** Visualization of isolated DNA under UV light resulted from multiplex PCR with pork, beef and chicken primer (annealing at 57°C) with the length 290, 105 and 183 bp, respectively, M: Marker, C: Control positive from fresh pork, beef and chicken, 1-28: meatball samples.

The complete results of all methods described are summarized in Table 3. Overall, this study demonstrates that pork adulteration poses a serious concern for Muslim consumers due to religious dietary restrictions, while chicken adulteration constitutes food fraud that affects all consumers. The detection of both pork and chicken in beef meatball products (BMPs) from small vendors underscores the need for more stringent control measures. Adulteration—whether intentional or unintentional—in comminuted products such as meatballs not only results in economic losses for consumers but also compromises food integrity. In particular, the presence of pork in products marketed as halal directly violates religious principles, raising significant ethical, cultural, and legal concerns.

To address these issues, stakeholders should implement routine inspections and monitoring programs to reduce adulteration practices. The Porcine Detection Kit (PDK) offers a practical screening tool for field inspections, as it is rapid, inexpensive, and requires no specialized expertise. However, its limitations necessitate confirmatory testing. Polymerase Chain Reaction (PCR), while more time-consuming and technically demanding, provides reliable and highly accurate detection. This study further highlights the value of multiplex PCR as a model approach, as it allows simultaneous detection of multiple species in a single assay, thereby improving efficiency and reducing analysis time in food authentication.

**Table 3** Result of BMP detection using PDK and PCR

| Sample no | PDK result | Simplex PCR Result | | | Multiplex PCR Result | | |
|---|---|---|---|---|---|---|---|
| | | Beef | Pork | Chicken | Beef | Pork | Chicken |
| 1 | - | + | - | + | + | - | + |
| 2 | - | + | - | + | + | - | + |
| 3 | - | + | - | + | + | - | + |
| 4 | - | + | - | + | + | - | + |
| 5 | - | + | - | + | + | - | + |
| 6 | - | + | - | + | + | - | + |
| 7 | + | + | + | + | + | + | + |
| 8 | - | + | - | + | + | - | + |
| 9* | - | + | + | + | + | + | + |
| 10 | - | + | - | + | + | - | + |
| 11 | - | + | - | + | + | - | + |
| 12 | - | + | - | + | + | - | + |
| 13 | - | + | - | + | + | - | + |
| 14 | - | + | - | + | + | - | + |
| 15 | - | + | - | + | + | - | + |
| 16 | - | + | - | + | + | - | + |
| 17 | - | + | - | + | + | - | + |
| 18 | - | + | - | + | + | - | + |
| 19 | - | + | - | + | + | - | + |
| 20 | - | + | - | + | + | - | + |
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| 24 | - | + | - | + | + | - | + |
| 25 | - | + | - | + | + | - | + |
| 26 | - | + | - | + | + | - | + |
| 27 | - | + | - | + | + | - | + |
| 28 | - | + | - | + | + | - | + |

**Remark:** *sample number 9 showed different result on the detection of pork, using PDK (-) while using both simplex and multiplex PCR (+)

## Conclusion

Testing with the rapid Porcine Detection Kit (PDK) identified one positive sample; however, molecular analysis using PCR revealed that two of the 28 samples (approximately 7%) were contaminated with pork, while the remaining samples were negative. In addition, chicken DNA was detected in nearly all meatball samples. The presence of chicken is expected, as it is commonly incorporated into meatballs for economic reasons. In contrast, the detection of pork indicates intentional adulteration, which is particularly concerning as it undermines consumer trust and suggests potential violations of halal certification regulations. These findings highlight the need for regulatory authorities to strengthen oversight and conduct routine inspections of meatball vendors, given the widespread consumption of this product in Indonesia. While the PDK provides a rapid and user-friendly tool for field screening, confirmatory testing with PCR remains essential for ensuring accuracy and reliability. Together, these approaches offer a practical framework for safeguarding food authenticity, protecting consumer rights, and upholding halal standards.

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