Detection of Porcine DNA in Commercially Processed Meat Products Sold in Sarawak

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Abstract

Porcine DNA authentication in commercial products is critical due to improper or non-certified Halal logo on its packaging. This study detected the presence of porcine DNA in particularly processed meat products sold in Sarawak. A total of 75 samples were collected, consisting of poultry, beef, and seafood products. DNA was isolated and amplified in a polymerase chain reaction that targeted cytochrome B (cytb) and mitochondrial D-loop. PCR products were analysed via gel electrophoresis and viewed through gel documentation. The positive result was observed in 5 samples: 3 from poultry and 2 from beef. The DNA band were detected at an amplicon size of 174 base pairs for poultry products whereas 100 base pairs for beef products. Porcine DNA was absent in seafood products. Positive samples were validated through DNA sequencing. Nucleotide sequences from DNA sequencing were compared with the database using the Basic Local Alignment Search Tool (BLAST). BLAST analysis indicated percentages ranging from 96.83% to 100%, demonstrating high similarity with porcine DNA; one of the samples was labelled with a foreign Halal logo. In summary, this study provides the groundwork for further inspection of the Halal status in commercially processed meat products available in Sarawak.

1. Introduction

Over the years, food fraud cases involving adulteration and mislabelling have escalated globally. Food fraud is intentionally committed by deceiving manufacturers for economic or financial gain (Bouzembrak et al., 2018). Food is considered adulterated when it is added or substituted with other cheaper or inferior substances, which reduces the quality of the food products (Faizunisa et al., 2016). For instance, the lower pork meat prices were substituted for expensive meats (Doosti et al., 2014), thus, offering profit to manufacturers. In addition, whilst seafood consumption is increasing, fraud cases involving the fish-processing industry are also escalating leading to extensive fish species authentication (Piskata et al., 2017). Surimi, a fish product, has been reported to contain non-Halal plasma transglutaminase that enhances its gelling characteristics (Alina et al., 2012 and Huda et al., 2010).

Jeopardising food quality also indirectly threatens consumers’ rights to their religion, belief and health (Barakat et al., 2014). In some developed countries, superfluous attention by food safety authorities has been given to mislabelling and food substitution cases (Chin et al., 2016) which emphasise the importance of food authentication. However, meat species identification in processed food products through visual observation is difficult due to changes in appearance, colour and texture following intensive processing techniques (Chuah et al., 2016). Therefore, the DNA-based method through the application of the polymerase chain reaction (PCR) technique is a suitable option because of the rapidity, high specificity and ability to detect heat-treated samples attributed to the DNA thermal stability in the canned meat products (Lo & Shaw, 2018). Few stages are involved in identifying meat species using PCR, such as the DNA detection of desired meat species by target gene, analysis of PCR products through agarose gel electrophoresis, visualisation of DNA band and comparison with standard DNA marker.

Demand for Halal food is gradually increasing to sustain the...
A growing number of Muslim populations. This has allowed some irresponsible manufacturers to commit fraud through bogus Halal logos. Improper labelling or the bogus Halal logo in certain meat products has triggered insecurity among consumers, especially Muslims. Individuals predisposed to allergic reactions due to porcine-derived food product consumption have also been victims of the manufacturer’s malicious act. Hence, porcine DNA detection in these products using simple, specific, and reliable detection methods is critical to validate their Halal status.

Furthermore, the scarcity of data associated with porcine DNA detection in commercial meat products sold in Sarawak warrants further investigation. In this regard, this study aims to pinpoint the porcine contamination in potentially commercial processed meat products sold in Sarawak that further enables the correlation of the main ingredient with the Halal logo on the product’s labelling.

2. Materials and methods

2.1 Sample collection

Meat processed products (n=75) were bought from 6 local supermarkets. Samples included beef (n = 23), poultry (n = 25), seafood (n = 26), and pork meat (n = 1). Canned pork was used as a positive control. Criteria for sample collection were based on the presence of Halal logos from foreign countries and the absence of a certified Halal logo by the Department of Islamic Development Malaysia (JAKIM). The samples’ details are listed in Table 1.

2.2 In-silico primer specificity validation

Primer sequences for cytb and mitochondrial D-loop specific to porcine species were adapted from López-Andreo et al. (2005) and Che Man et al. (2012). In silico verification of the primer specificity was conducted using the Basic Local Alignment Search Tool (BLAST) and synthesised at Integrated DNA Technologies (IDT), Singapore.

2.3 DNA isolation

DNA was isolated using DNeasy Mericon Food Kit (Qiagen, Germany) under a Small Fragment Protocol (200 mg) for processed food products. The DNA extraction was done according to the manufacturer’s instruction, with a slight modification in buffer volume. Two hundred milligrams of samples were weighed into a 1.5 mL tube. One millilitre of Food Lysis Buffer was added to lyse and disrupt the cell membrane, whereas 2.5 µL of 20 mg/mL Proteinase K was added to degrade the protein. The mixture was vortexed to ensure homogenisation before being incubated for 30 minutes at 60°C with constant shaking at 1000 rpm. The samples were then allowed to cool on the ice at room temperature, followed by centrifugation at 2,500 x g for 5 minutes. Subsequently, 700 µL of clear supernatant was transferred into a tube containing 500 µL of chloroform (Merek, Germany). The mixture was vortex for 15 seconds before centrifugation at 14,000 x g for 15 minutes. Centrifugation formed three layers: upper aqueous DNA layer, interphase layer, and organic phase layer. Then, 250 µL of the upper aqueous DNA layer was added into 1 mL Buffer PB to facilitate DNA binding on the spin column. The mixture was transferred to a QIAquick spin column placed in a 2 mL collection tube, centrifuged at 17,900 x g for 1 minute, and the flow through was discarded the flow-through. The step was repeated with the remaining mixture. Then, 500 µL of Buffer AW2 was added and centrifuged at 17,900 x g for 1 minute. The step was repeated to ensure the drying of the membrane on the spin column. Buffer AW2 or washing buffer ensured the removal of contaminants from DNA. The QIAquick spin column was then transferred to a new 1.5 mL tube. Fifty microlitres of Buffer EB or elution buffer was added to elute DNA. The isolated DNA was quantified spectrophotometrically and stored at -20°C before use in the downstream application.

2.3.1 DNA quantitation and integrity

Isolated DNA was quantified spectrophotometrically using Cary 60 (Agilent, USA). The DNA was diluted 100x through the addition of 5 µL DNA into 495 µL nuclease-free water. The absorbance at 260 nm/280 nm of ~1.8 indicates the DNA purity. DNA concentration was calculated according to the standard formula below (Barbas III et al., 2007):

\[
\text{dsDNA Concentration} = 50 \mu g/mL \times \frac{A_{260}}{A_{260}} \times \text{Dilution Factor}
\]

where:
- 50 µg/mL is equivalent to 1.0 at A_{260} for a 1 cm pathlength cuvette;
- A_{260} represent absorbance reading at 260 nm

In addition, the integrity of DNA was determined via 1% agarose gel electrophoresis. Agarose gel was prepared by weighing 0.3 g agarose power (Vivantis, Malaysia), dissolved in 30 mL 1x TBE buffer (Vivantis, Malaysia) and heated in the microwave oven. One microlitre or SYBR™ Safe DNA Gel Stain (Thermo Fisher Scientific, USA) was added to the molten agar before pouring into the gel cast. The agarose gel was allowed to solidify for 15 minutes. Three microlitres of DNA sample were mixed with 1 µL of loading dye before loading into the agarose well. The gel electrophoresis was run at 120 V for 30 minutes. The DNA integrity was viewed using E-box gel documentation (Vilber, France).

2.4 Detection of porcine DNA by PCR

The master mix for each gene was dispensed into each PCR tube with a total volume of 10 µl containing the following reagents: final concentration of 1× DreamTaq Green PCR Master Mix (Thermofisher Scientific, USA), 0.4 µM of both forward and reverse primers of mitochondrial D-loop and nuclease-free water. Another master mix reaction was prepared in a single tube by adding 0.5 µM of both forward and reverse primers of the cytb instead of the mitochondrial D-loop. Then, DNA for each sample (10 ng) was added to each 0.2 mL tube which makes a total volume of 10 µl, except for positive control containing porcine DNA and negative control without DNA (substituted with nuclease-free water). The repeatability and validity of the result were ensured through two independent studies.
Table 1: List of samples used in this study

| No | ID  | Product description / country origin                                      | Labelling information | Main ingredient          | Halal logo |
|----|-----|---------------------------------------------------------------------------|-----------------------|--------------------------|------------|
| 1  | C1  | Chicken curry with potatoes, China                                        | Chicken               | N.A.                     |            |
| 2  | C2  | Chicken luncheon meat premium, China                                      | Chicken               | N.A.                     |            |
| 3  | C3  | Chicken luncheon meat 1, Brazil                                           | Chicken               | N.A.                     |            |
| 4  | C4  | Chicken liver mousse, France                                             | Chicken               | N.A.                     |            |
| 5  | C5  | Chicken luncheon meat 2, Singapore                                        | Chicken               | N.A.                     |            |
| 6  | C6  | Chicken liver mousse, France                                             | Chicken               | N.A.                     |            |
| 7  | C7  | Chicken luncheon meat 3, China                                           | Chicken               | N.A.                     |            |
| 8  | C8  | Chicken luncheon meat 4, China                                           | Chicken               | N.A.                     |            |
| 9  | C9  | Chicken luncheon meat 5, Denmark                                         | Chicken               | N.A.                     |            |
| 10 | C10 | Grilled chicken (Maruha grilled pieces of chicken teriyaki), Japan        | Chicken               | N.A.                     |            |
| 11 | C11 | Chicken luncheon meat 6, China                                           | Chicken               | N.A.                     |            |
| 12 | C12 | Fried chicken claws with salted black bean, China                         | Chicken               | N.A.                     |            |
| 13 | C13 | Chicken luncheon meat 7, Denmark                                         | Chicken               | N.A.                     |            |
| 14 | C14 | Chicken cheese meat loaf, Singapore                                       | Chicken               | √ (Foreign)              |            |
| 15 | C15 | Chicken black pepper meat loaf, Singapore                                 | Chicken               | √ (Foreign)              |            |
| 16 | C16 | Meat loaf, Philippines                                                   | Chicken               | N.A.                     |            |
| 17 | C17 | Chicken spread, USA                                                      | White chicken meat    | N.A.                     |            |
| 18 | C18 | Fried young chicken with bone, China                                      | Chicken               | N.A.                     |            |
| 19 | CF  | Chicken paste with seasoning, Sibu, Sarawak                              | Chicken               | N.A.                     |            |
| 20 | CD  | Chicken dumpling, Sibu, Sarawak                                          | Chicken               | N.A.                     |            |
| 21 | D1  | Peking duck with preserved vegetable, China                               | Peking duck           | N.A.                     |            |
| 22 | D2  | Duck with preserved vegetable 1, China                                    | Peking duck           | N.A.                     |            |
| 23 | D3  | Duck with preserved vegetable 2, China                                    | Preserved vegetable   | N.A.                     |            |
| 24 | T1  | Oven roasted turkey, USA                                                 | White turkey          | N.A.                     |            |
| 25 | T2  | Turkey luncheon meat with chicken, Denmark                               | Turkey meat           | √ (Foreign)              |            |
| 26 | B1  | Beef luncheon meat, China                                                | Beef                  | N.A.                     |            |
| 27 | B2  | Corned beef 1, China                                                     | Cooked beef           | N.A.                     |            |
| 28 | B3  | Beef curry with potatoes premium, China                                   | Beef                  | √ (Foreign)              |            |
| 29 | B4  | Corned beef 2, China                                                     | Cooked beef           | N.A.                     |            |
| 30 | B5  | Corned beef 3, China                                                     | Cooked beef           | N.A.                     |            |
| 31 | B6  | Corned beef 4, Brazil                                                    | Cooked beef           | √ (Foreign)              |            |
| 32 | B7  | Corned beef 5, Brazil                                                    | Cooked beef           | √ (Foreign)              |            |
| 33 | B8  | Corned beef with onions, Brazil                                          | Cooked beef           | √ (Foreign)              |            |
| 34 | B9  | Beef luncheon meat, Brazil                                               | Beef                  | √ (Foreign)              |            |
| 35 | B10 | Corned beef 6, South Africa                                              | Meat (mechanically deboned) | √ (Foreign)              |            |
| 36 | B11 | Beef meat loaf, Singapore                                                | Beef                  | √ (Foreign)              |            |
| 37 | B12 | Corned beef 7, China                                                     | Cooked beef           | N.A.                     |            |
| 38 | B13 | Corned beef 8, China                                                     | Cooked beef           | N.A.                     |            |
| 39 | B14 | Luncheon meat with beef, Netherland                                      | Mechanically separated chicken (48%), beef lungs (19%) | N.A. |            |
| 40 | B15 | Corned beef 9, China                                                     | Cooked beef           | √ (Foreign)              |            |
| 41 | B16 | Beef luncheon meat with chicken, Denmark                                  | Beef 48%              | √ (Foreign)              |            |
| 42 | B17 | Corned beef premium quality, Brazil                                       | Cooked beef           | √ (Foreign)              |            |
| 43 | B18 | Corned beef 10, Brazil                                                   | Cooked beef           | √ (Foreign)              |            |
| 44 | B19 | Corned beef 11, Philippines                                              | Cooked beef           | √ (Foreign)              |            |
| 45 | B20 | Corned beef 12, Philippines                                              | Cooked beef           | √ (Foreign)              |            |
| 46 | B21 | Beef jerky, New Zealand                                                  | Beef                  | N.A.                     |            |
| 47 | B24 | Corned beef 13, Brazil                                                   | Beef                  | √ (Foreign)              |            |
| 48 | B25 | Corned beef 14, Brazil                                                   | Cooked beef           | √ (Foreign)              |            |
| 49 | SF1 | Spicy sardines, China                                                    | Sardines              | N.A.                     |            |
| 50 | SF2 | Sardines in brine, UK                                                    | Sardines              | N.A.                     |            |
and reverse primers of the cytb gene. Preparation was done by mixing 0.5 µM of both forward and reverse primers of the mitochondrial D-loop gene, except for the positive control containing porcine DNA and negative control without DNA (substituted with nuclease-free water). Another master mix reaction was performed to obtain a high yield of DNA before sequencing. The master mix was dispensed into each PCR tube with a total volume of 40 µl containing the following reagents: final concentration of 1× DreamTaq Green PCR master mix (Thermofisher Scientific, USA), 0.4 µM of both forward and reverse primers of mitochondrial D-loop, and 0.4 µM of both forward and reverse primers of the cytb instead of the mitochondrial D-loop gene. Then, DNA for each sample (10 ng) was added to each 0.2 mL tube which makes a total volume of 40 µl, except for positive control containing porcine DNA and negative control without DNA (substituted with nuclease-free water).

Due to multiple DNA bands associated with the D-loop gene, the specific DNA band amplified at 174 bp was incised and chosen for further validation. DNA sequencing employed the Sanger Sequencing method, which involved the chain-terminator cycle sequencing chemistry. DNA sequencing was done by Apical Scientific (Selangor, Malaysia) through a 96-capillary system (Applied Biosystems, USA). DNA sequencing results were analysed via nucleotide BLAST software in the National Centre for Biotechnology Information (NCBI) database.

| Sample ID | Description                                      | Species       | Result |
|-----------|--------------------------------------------------|---------------|--------|
| SF3       | Fried sardine salted black beans in soy sauce, China | Sardines      | N.A.   |
| SF4       | Fried sardines in chilli sauce, China             | Sardines      | N.A.   |
| SF5       | Sardines in tomato sauce 1, China                 | Sardines      | N.A.   |
| SF6       | Sardines in tomato sauce 2, China                 | Sardines      | N.A.   |
| SF7       | Sardines in tomato sauce 3, China                 | Sardines      | N.A.   |
| SF8       | Sardines in tomato sauce 4, China                 | Sardines      | N.A.   |
| SF9       | Wild sardines in tomato sauce 5, Poland           | Sardines      | N.A.   |
| TUF1      | Tuna light standard, South Korea                  | Tuna          | N.A.   |
| TUF2      | Fried curry sauce light tuna, South Korea         | Tuna          | N.A.   |
| TUF3      | Tuna steak, UK                                    | Tuna          | N.A.   |
| MF2       | Mackerel                                          | Mackerel      | N.A.   |
| MF3       | Mackerel fillets in korma style sauce, UK         | Mackerel fillets | N.A.   |
| DF1       | Fried dace with salted black beans 1, China       | Dace          | N.A.   |
| DF2       | Fried dace with salted black beans 2, China       | Dace          | N.A.   |
| DF3       | Fried dace with salted black bean 3, China        | Dace          | N.A.   |
| DF4       | Fried dace with bean curd stick, China            | Dace          | N.A.   |
| DF5       | Fried dace with salted black beans 4, China       | Dace          | N.A.   |
| DF6       | Fried dace with salted black beans 5, China       | Dace          | N.A.   |
| CF1       | Yellow croaker, China                             | Croaker       | N.A.   |
| TF1       | Fried trigger fishes, China                       | Trigger fishes | N.A.   |
| AF1       | Long tailed anchovy, China                        | Anchovy       | N.A.   |
| F1        | Fried fish with salted black beans, China         | Fried fish    | N.A.   |
| SQ2       | Seasoned squid with soya bean sauce, China        | Squid         | N.A.   |
| SQ3       | Pepus squid in ink, Spain                         | Squid         | N.A.   |
| PC        | Pork luncheon meat, China                         | Pork          | N.A.   |

Note: N.A., not available; U.K., United Kingdom; USA, United States of America

2.5 Sequencing and verification of positive results

PCR reaction for positive samples containing porcine DNA was performed to obtain a high yield of DNA before sequencing. The master mix was dispensed into each PCR tube with a total volume of 40 µl containing the following reagents: final concentration of 1× DreamTaq Green PCR Master Mix (Thermofisher Scientific, USA), 0.4 µM of both forward and reverse primers of mitochondrial D-loop and nuclease-free water. Another master mix reaction was prepared in a single tube by adding 0.5 µM of both forward and reverse primers of the cytb instead of the mitochondrial D-loop gene. Then, DNA for each sample (10 ng) was added to each 0.2 mL tube which makes a total volume of 40 µl, except for positive control containing porcine DNA and negative control without DNA (substituted with nuclease-free water).

Due to multiple DNA bands associated with the D-loop gene, the specific DNA band amplified at 174 bp was incised and chosen for further validation. DNA sequencing employed the Sanger Sequencing method, which involved the chain-terminator cycle sequencing chemistry. DNA sequencing was done by Apical Scientific (Selangor, Malaysia) through a 96-capillary system (Applied Biosystems, USA). DNA sequencing results were analysed via nucleotide BLAST software in the National Centre for Biotechnology Information (NCBI) database.

3. Results and discussion

3.1 In-silico primer specificity validation

Cytb sequence is available in the GenBank for many species, enabling additional verification steps of meat species through sequencing and BLAST analysis (Kitpipit et al., 2014). The sequence is highly variable among inter-species or between species and less variable among intra-species or within similar species, offering a better output in meat species differentiation (Tobe et al., 2010 and Linacre & Tobe, 2011). Besides, the D-loop is the most rapidly evolving mitochondrial DNA (mtDNA) region suitable for species discrimination resulting from indels and collection of tandemly repeated sequences at the hypervariable region (Haunshi et al., 2009 and Fajardo et al., 2008). In silico primer specificity validation for porcine species-specific mitochondrial D-loop and cytb were verified using nucleotide BLAST software on the NCBI database to ensure its specificity to Sus scrofa. Based on the BLAST analysis, mitochondrial D-loop have a high per cent identity (99.29% - 100%), whereas cytb was 100% identical with the target sequence in BLAST for Sus scrofa. Hence, these primer sequences demonstrated specificity with porcine species, which permits porcine DNA detection.

3.2 Assessment of isolated DNA

The yield and quality of isolated DNA are vital for downstream application. DNA molecules in canned meat products are liable to degradation when subjected to extensive thermal processing at 115°C for 75-90 minutes.
(Featherstone et al., 2016) and exposure to chemical or physical treatment (Piskata et al., 2019). This was evidenced by the reduced intensity of DNA bands at higher thermal temperatures following technological food processing. Smeread DNA band was also demonstrated in food products following excessive processing (Schrader et al., 2012). In this study, significant DNA degradation was shown in Figure 1, exhibited by expanded smear and fragmented DNA. Furthermore, the reduced yield of genomic DNA due to extensive heating of meat products could affect the DNA amplification (López-Andreo et al., 2012). However, Şakalar et al. (2012) compared the duration of heating meat samples with DNA fragment size. The study concluded that the PCR amplification efficiency is higher in a shorter DNA fragment than in a longer DNA fragment.

Figure 1: Representative analysis of DNA integrity electrophoretic gel image for poultry samples from C1 to CD. L1: Ladder; L2: Chicken curry with potatoes (C1); L3: Chicken luncheon meat premium (C2); L4: Chicken luncheon meat 1 (C3); L5: Chicken lyoner (C4); L6: Chicken luncheon meat 2 (C5); L7: Chicken liver mousse (C6); L8: Chicken luncheon meat 3 (C7); L9: Chicken luncheon meat 4 (C8); L10: Chicken luncheon meat 5 (C9); L11: Grilled chicken (C10); L12: Chicken luncheon meat 6 (C11); L13: Fried chicken claws with salted black bean (C12); L14: Chicken luncheon meat 7 (C13); L15: Oven-roasted turkey (T1); L16: Turkey luncheon meat with chicken (T2); L17: Chicken paste (T.P.); L18: Chicken dumpling (CD)

Figure 2 shows a faint DNA band after isolating DNA from seafood products containing chilli and tomato sauce. The isolated DNA was different in yield, and quality as observed in brine, oil, vinegar, and tomato seafood products. This observation was previously described by Chapela et al. (2007), who suggested that the type of preservatives used in the canning of food products could affect the DNA quantity. The quality and traceability of DNA were also compromised, observed notably in canned tuna (Pecoraro et al., 2020). The low yield of DNA could also be due to the incomplete lysis phase, causing failure to break the food sample's cell membrane, resulting in reduced DNA yield and purity (Sirakov, 2016). Nevertheless, simple, rapid and affordable DNA isolation techniques are mandatory to reduce the presence of inhibitors to harvest high-quality DNA for the downstream process (Sajib et al., 2017).

3.3 Detection of porcine DNA by PCR and validation of positive sample

PCR amplification targeting cytb and D-loop successfully detected porcine DNA in certain meat-pro processed products. Despite the food products being subjected to high temperatures and DNA fragmenting, the DNA band was still detected in the PCR assay. This is because the shorter amplicon size targeted in this study demonstrated PCR capability following extensive thermal treatment, while a more extended amplicon size is vulnerable to breakage (Rashid et al., 2015). Moreover, mtDNA is abundant and enclosed in a stable circular structure that is less prone to degradation (Gefrides & Welch, 2011). Thus, these circumstances confer higher survival chances of mtDNA in processed food following intense food processing conditions (Mohamad et al., 2013). Based on the finding, amplicon size of DNA band for the respective gene was observed in comparison with positive control. Both positive and negative control in the study serves different purposes. Positive control validates the result as an internal control, whereas negative controls indicate potential contamination from the reagent used in the amplification process.

In this study, 3 poultry samples contained porcine DNA, as demonstrated by the DNA band at 174 bp targeting the D-loop on agarose gel image shown in Figures 3(a) and 3(b). In addition, 2 beef samples contained porcine DNA, as indicated by the DNA band at 100 bp targeting the cytb, indicated in Figure 4. However, the absence of porcine DNA was reported in all seafood samples, indicated by the absence of a DNA band following gel electrophoresis, as shown in Figure 5. This outcome implied the absence of contamination, addition, or substitution of porcine elements in seafood samples. Positive porcine DNA samples were verified through DNA sequencing. BLAST analysis demonstrated a high per cent identity (96.83%-100%) of the query sequence with the target sequence of Sus scrofa. Thus, the positive samples were validated to contain the porcine elements. Table 2 shows the BLAST per cent identity of the positive sample with Sus scrofa. The correlation between ingredients declared on food labelling with the presence of the Halal logo is shown in Figure 7.
Figure 3(a): Representative analysis of electrophoretic gel image for amplified D-loop of poultry samples from C1 to C17. L1: Ladder; L2: Chicken curry with potatoes (C1); L3: Chicken luncheon meat premium (C2); L4: Chicken luncheon meat 1 (C3); L5: Chicken lyoner (C4); L6: Chicken luncheon meat 2 (C5); L7: Chicken liver mousse (C6); L8: Chicken luncheon meat 3 (C7); L9: Chicken luncheon meat 4 (C8); L10: Chicken luncheon meat 5 (C9); L11: Grilled chicken (C10); L12: Chicken luncheon meat 6 (C11); L13: Fried chicken claws with salted black bean (C12); L14: Chicken luncheon meat 7 (C13); L15: Chicken cheese meatloaf (C14); L16: Chicken black pepper meatloaf (C15); L17: Meatloaf (C16); L18: Chicken spread (C17).

Figure 3(b): Representative analysis of electrophoretic gel image for amplified D-loop of poultry samples showing negative detection compared with positive control and negative control. L1: Ladder; L29: Positive control; L30: Negative control.

Figure 4: Representative analysis of electrophoretic gel image for amplified Cytb gene of beef samples from B1 to B17. L1: Ladder; L2: Beef luncheon meat (B1); L3: Corned beef 1 (B2); L4: Beef curry with potatoes premium (B3); L5: Corned beef 2 (B4); L6: Corned beef 3 (B5); L7: Corned beef 4 (B6); L8: Corned beef 5 (B7); L9: Corned beef with onion (B8); L10: Beef luncheon meat (B9); L11: Corned beef 6 (B10); L12: Beef meatloaf (B11); L13: Corned beef 7 (B12); L14: Corned beef 8 (B13); L15: Luncheon meat with beef (B14); L16: Corned beef 9 (B15); L17: Beef luncheon meat with chicken (B16); L18: Corned beef premium quality (B17); L19: Positive control; L20: Negative control.

Table 2: DNA sequencing analysis

| Sample | Common species | BLAST percent identity (%) |
|--------|----------------|-----------------------------|
| C4     | Sus scrofa     | 98.6 - 100                  |
| C8     | Sus scrofa     | 97.26 – 98.55               |
| C16    | Sus scrofa     | 98.55 – 98.63               |
| B5     | Sus scrofa     | 96.83                       |
| B15    | Sus scrofa     | 98.44 - 100                 |

Out of all meat processed food samples, corned beef (B5) from China, chicken lyoner (C4) from Singapore, chicken luncheon meat (C8) from China, and meatloaf (C16) from the Philippines contained porcine DNA. These products were not labelled with a Halal logo on product labelling; thus, contamination with porcine meat could be intentional.

Figure 5: Representative gel image of PCR products for processed seafood products from SF1 to MF3. L1: Ladder; L2: Spicy sardines (SF1); L3: Sardines in brine (SF2); L4: Fried sardine salted black beans in soy sauce (SF3); L5: Fried sardines in chilli sauce (SF4); L6: Sardines in tomato sauce 1 (SF5); L7: Sardines in tomato sauce 2 (SF6); L8: Sardines in tomato sauce 3 (SF7); L9: Sardines in tomato sauce 4 (SF8); L10: Wild sardines in tomato sauce 5 (SF9); L11: Tuna light standard (TUF1); L12: Fried curry sauce light tuna (TUF2); L13: Tuna steak (TUF3); L14: Mackerel fillets in tomato sauce (MF1); L15: Mackerel (MF2); L16: Mackerel fillets in korma style sauce (MF3); L17: Positive Control; L18: Negative control.

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| C8     | Sus scrofa     | 97.26 – 98.55               |
| C16    | Sus scrofa     | 98.55 – 98.63               |
| B5     | Sus scrofa     | 96.83                       |
| B15    | Sus scrofa     | 98.44 - 100                 |

Number of samples

Porcine DNA detected, no halal logo | Porcine DNA undetected, no halal logo | Porcine DNA detected, halal logo available | Porcine DNA undetected, halal logo available

- Poultry
- Beef
- Seafood

Figure 7: Relationship between availability of Halal logo with porcine DNA detection in each commercial meat products category

Out of all meat processed food samples, corned beef (B5) from China, chicken lyoner (C4) from Singapore, chicken luncheon meat (C8) from China, and meatloaf (C16) from the Philippines contained porcine DNA. These products were not labelled with a Halal logo on product labelling; thus, contamination with porcine meat could be intentional.
or unintentional, as implied by the detection of porcine DNA. The manufacturers that processed chicken, beef, and pork simultaneously at the same factory could cause cross-contamination or unintentional adulteration among the mixed species due to improper handling using shared equipment for processing the meat species. This could also be due to the staff who might not adhere to good manufacturing practices (GMP) and unhygienic equipment handling during food processing.

Besides, porcine DNA was detected in a corned beef sample (B15) from China that waslabelled by a foreign Halal certification body (FHCB) recognised by JAKIM. The product labelling indicated chicken and beef as the main ingredients, without indicating pork meat. A comparison between the certified Halal logo shown in Figure 6(a) with the Halal logo used in the corned beef sample (B15) shown in Figure 6(b) indicates a subtle difference in the spelling of “supervised”, “association” and the Arabic spelling of Halal. Therefore, this is not the actual logo as the recognized logo by JAKIM, and detection of porcine in this product implied misconduct. In some instances, the Halal logo has been misused by traders, such as using it on food products that have not been certified as Halal (Asa, 2019). The false representation of the products and services through any name, sign, words or letter has been stipulated under Section 16 of the Trade Description Act 2011. Violation of this section by a corporate and non-corporate organisation is considered an offence and shall be penalised according to Section 21(a) and (b). Similarly, Section 29 highlights the importance of competent authority and the use of approved information marking on the products, in which violation of this section shall be penalised accordingly. Thus, this provision strictly prohibits the misuse of the Halal logo, including imitation of the Halal logo and certification. Preventive measure such as labelling food products precisely assists in adhering to Halal standards (Erwanto et al., 2018). Likewise, knowledge and awareness of recognising the certified Halal logo are essential (Nakynisige et al., 2012).

Inaccurate information on product labelling also affects individuals who have a dietary limitation on the consumption of pork due to health problem such as allergic reaction. Food Act 1983 (Act 281) and Food Regulations 1985 are the two significant laws that provide legal authority for relevant agencies and to protect consumers’ rights. Food Act 1983 (Act 281) stipulates penalties of not exceeding three years imprisonment or fine or both for individuals who commit offences due to false labelling. Section 16, Food Regulations 1985 serve as a reference standard for regulation related to general food labelling, labelling of various product products and guidelines for food packaging. Penalties of fine not exceeding five thousand ringgit or imprisonment for a term not exceeding two years shall be imposed on individuals, including manufacturers who fail to comply with these provisions.

4. Conclusion

Accurate information about food ingredients, certified Halal logo usage on product labelling and verification during the manufacturing process are essential to ensure the consumption of the genuine products. Detection of porcine DNA in a product that misused the Halal logo results in financial gain by the irresponsible manufacturer but affects the consumers in various aspects. Hence, strict compliance with Halal standards should have been adhered to in the factories or facilities that produce certified Halal products. Strict penalties imposed by the legal authority are essential to curb the issue of abusing the Halal logo and mislabelling food products. This study is a groundwork that demonstrates the practicability of both mitochondrial D-loop and cytb using conventional PCR to screen meat products containing or contaminated with porcine DNA in Sarawak. Further investigation through real-time PCR assay, encompassing specificity and establishing the limit of detection for method validation, are warranted.

Acknowledgement

The authors thank the University of Technology Sarawak for providing financial support through Project No. UCTS/RESEARCH/1/2019/02 and research equipment for sustaining this research project.
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