HALALSPHERE
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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

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 (Merck, 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 quantitated 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):

$$\text{dsDNA Concentration} = 50\ \mu g/mL \times OD_{260} \times \text{Dilution Factor}$$

where:
50 µg/mL is equivalent to 1.0 at OD_{260} for a 1 cm pathlength cuvette;
OD_{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	√(Foreign)
4	C4	Chicken lyoner, Singapore	Chicken	N.A.
5	C5	Chicken luncheon meat 2, Singapore	Chicken	N.A.
6	C6	Chicken liver mousse, France	Chicken liver	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 claw	N.A.
13	C13	Chicken luncheon meat 7, Denmark	Chicken	√(Foreign)
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	CP	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	√(Foreign)
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.

51	SF3	Fried sardine salted black beans in soy sauce, China	Sardines	N.A.
52	SF4	Fried sardines in chilli sauce, China	Sardines	N.A.
53	SF5	Sardines in tomato sauce 1, China	Sardines	√ (Foreign)
54	SF6	Sardines in tomato sauce 2, China	Sardines	√ (Foreign)
55	SF7	Sardines in tomato sauce 3, China	Sardines	√ (Foreign)
56	SF8	Sardines in tomato sauce 4, China	Sardines	N.A.
57	SF9	Wild sardines in tomato sauce 5, Poland	Sardines	N.A.
58	TUF1	Tuna light standard, South Korea	Tuna	N.A.
59	TUF2	Fried curry sauce light tuna, South Korea	Tuna	N.A.
60	TUF3	Tuna steak, UK	Tuna	N.A.
61	MF2	Mackerel	Mackerel	N.A.
62	MF3	Mackerel fillets in korma style sauce, UK	Mackerel fillets	N.A.
63	DF1	Fried dace with salted black beans 1, China	Dace	√ (Foreign)
64	DF2	Fried dace with salted black bean 2, China	Dace	N.A.
65	DF3	Fried dace with salted black bean 3, China	Dace	√ (Foreign)
66	DF4	Fried dace with bean curd stick, China	Dace	N.A.
67	DF5	Fried dace with salted black beans 4, China	Dace	N.A.
68	DF6	Fried dace with salted black beans 5, China	Dace	√ (Foreign)
69	CF1	Yellow croaker, China	Croaker	N.A.
70	TF1	Fried trigger fishes, China	Trigger fishes	N.A.
71	AF1	Long tailed anchovy, China	Anchovy	√ (Foreign)
72	F1	Fried fish with salted black beans, China	Fried fish	√ (Foreign)
73	SQ2	Seasoned squid with soya bean sauce, China	Squid	N.A.
74	SQ3	Pepus squid in ink, Spain	Squid	N.A.
75	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℃ 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. Smeared 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.

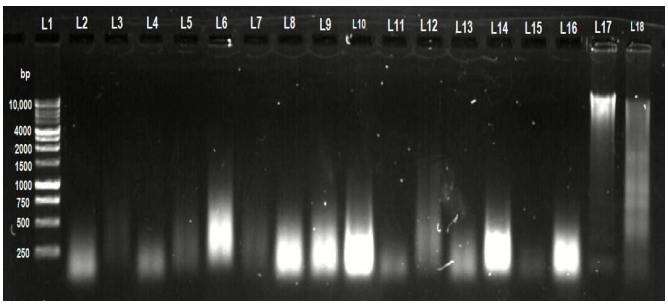

Figure 1: Representative analysis of DNA integrity electrophoretic gel image for poultry samples from C1 to CD. **L1:** Ladder; **L2:** Chicken curry with potatoes (C1); **L3:** Chicken luncheon meat premium (C2); **L4**: Chicken luncheon meat 1 (C3); **L5**: Chicken lyoner (C4); **L6**: Chicken luncheon 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 (C.P.); **L18:** Chicken dumpling (CD)

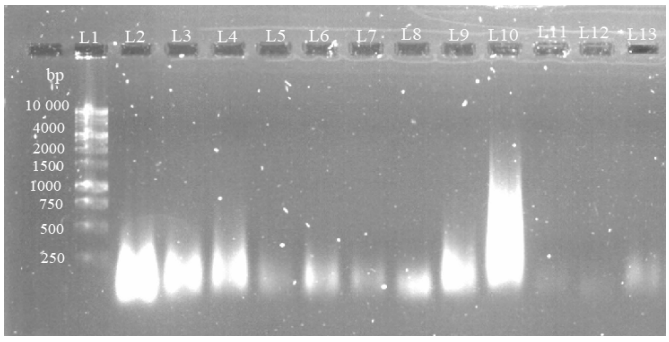

Figure 2: Representative analysis of DNA integrity electrophoretic gel image for seafood samples from SF1 to DF2. **L1**: Ladder; **L2**: Spicy sardines (SF1); **L3**: Sardines in brine (SF2); **L4**: Fried sardine salted black beans in soy sauce (SF3); **L5**: Fried sardines in chilli sauce (SF4); **L6**: 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)

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.

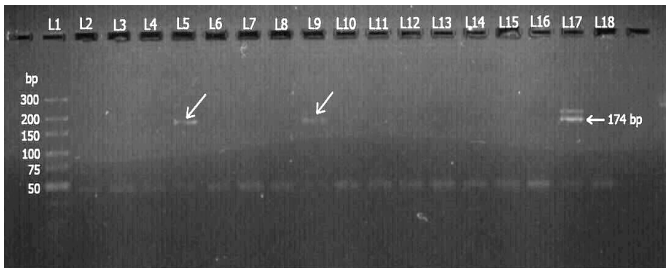

Figure 3(a): Representative analysis of electrophoretic gel image for amplified *D-loop* of poultry samples from C1 to C17. **L1**: Ladder; **L2**: Chicken curry with potatoes (C1); **L3**: Chicken luncheon meat premium (C2); **L4**: Chicken luncheon meat 1 (C3); **L5**: Chicken lyoner (C4); **L6**: 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)

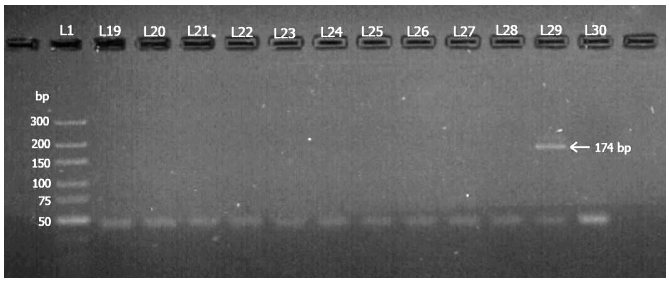

Figure 3(b): Representative analysis of electrophoretic gel image for amplified *D-loop* of poultry samples showing negative detection compared with positive control and negative control. **L1**: Ladder; **L29**: Positive control; **L30**: Negative control

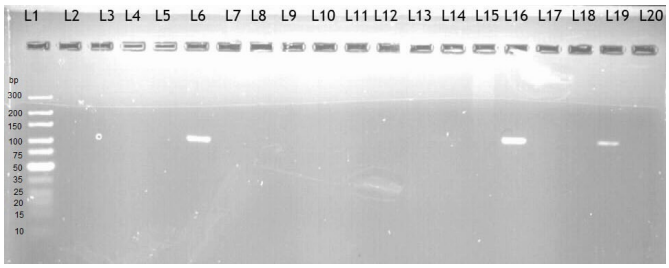

Figure 4: Representative analysis of electrophoretic gel image for amplified *Cytb* gene of beef samples from B1 to B17. **L1**: Ladder, **L2**: Beef luncheon meat (B1), **L3**: Corned beef 1 (B2), **L4**: Beef curry with potatoes premium (B3), **L5:** Corned beef 2 (B4), **L6**: Corned beef 3 (B5), **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

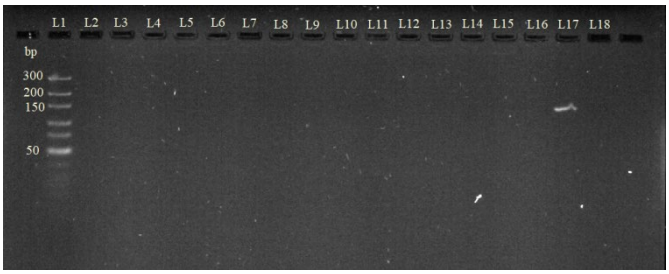

Figure 5: Representative gel image of PCR products for processed seafood products from SF1 to MF3. **L1**: Ladder; **L2**: Spicy sardines (SF1); **L3**: Sardines in brine (SF2); **L4**: Fried sardine salted black beans in soy sauce (SF3); **L5**: Fried sardines in chilli sauce (SF4); **L6**: Sardines 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

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
Positive control	*Sus scrofa*	99.29 - 100.0

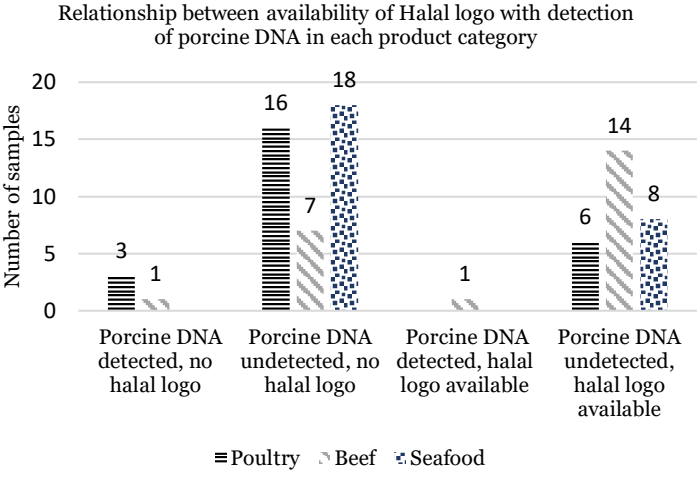

Figure 7: Relationship between the availability of *Halal* logo with porcine DNA detection in each commercial meat products category

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 was labelled 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 (Nakyinsige *et al.*, 2012).

Figure 6(a): China Islamic Association *Halal* logo recognised by JAKIM.

Figure 6(b): China Islamic Association *Halal* logo on a corned beef sample (B15) product labelling.

JAKIM is a certified authority safeguarding the *Halal* status of the imported food products Marketed in Malaysia. According to Trade Descriptions (Certification and Marking of '*Halal*') Order 2011, Section 4(1) under "Certification of "*Halal*" for Food and Imported Goods", only food products that are certified as *Halal* by the FHCB recognised by JAKIM can be marketed in Malaysia. Violation of order by the corporate organisation and non-corporate organisation makes them liable to punishment with a fine or imprisonment according to Section 8(a) and (b). Therefore, the appointment of credible foreign *Halal* certification bodies (FHCB) is vital that permit monitoring of all raw materials and ingredients used at every step and process involved in making the products. The onsite audit process through a visit to the operation office of FHCB, food processing plants and certified abattoirs ensures strict conformance to the *Halal* certification system (Department of Islamic Development Malaysia, 2017). JAKIM has made the list of the recognised foreign Halal certification bodies and authorities available to the public with their respective *Halal* logos from respective foreign countries (Department of Islamic Development Malaysia, 2020).

Inaccurate information on product labelling also affect 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 under 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.

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