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Canine-Specific PCR Assay Targeting Cytochrome b Gene for the Detection of Dog Meat Adulteration in Commercial Frankfurters

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Abstract This report described a cytochrome b (cytb)-based polymerase chain reaction (PCR) assay for the detection of canine tissues in commercial frankfurters. Discriminating detection of canine derivatives in processed food products has important application in halal authentication as well as in health, religions, and fare trades. The assay based on a pair of canine-specific primers that targeted a 100 bp region of canine mithochondrial-cytb gene which is present in multiple copies and highly conserved within the same species. The specificity of the assay was tested against dog and eight most common animal meat species as well as five plant species commonly found in frankfurter formulation. The stability and specificity of the assay were verified under different thermal processing conditions under pure and complex matrices. Three commercial brands of chicken and beef frankfurters were tested in triplicate, and specific PCR products were obtained only from deliberately contaminated formulations. The detection limit of the assay was 0.1 % (0.02 ng DNA) of canine meat spiked with other meats in a typical frankfurter formulation. Shorter amplicon length, superior stability, and higher sensitivity of the assay suggested its potential application in the screening of canine-origin biomaterials in processed food products.

Keywords Canine-origin biomaterials · Halal authentication · Commercial frankfurters

Introduction

The authentication of meat species is an increasingly concern and a vital part to ensure quality foods in compliance with health, religions, and fair prices (Ali et al. 2012a, b; Rohman et al. 2011). Furthermore, the newly emerging "zoonotic threat which is an animal origin infectious disease capable of being transmitted to human and other animals" has tremendously added drive toward meat species identification and meat quality authentication (Karabasanavar et al. 2011). According to European law, food manufacturers must declare and clearly label ingredients used in the preparation of both raw and finished foods (Commission 2002). Including Malaysia, many countries in the world either already have or are being having regulatory bodies to ensure proper labelling as well as Halal status of the processed foods (Ali et al. 2012a, b, c; Musa and Jalil 2012).

"Halal" logo on food products is trusted by 1.8 billion Muslims of the globe, and it signifies that the products are prepared following the Shariah law of Islam for hygienic foods, and Muslims have no religious obstructions to consume those

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(Ali et al. 2012a). However, since the prices of halal foods are higher than those of non-halal brands because of its inherent nature of specialized preparation, fraudulent labelling of Halal logos have been prevalently taken place (Ali et al. 2012a; Yusop et al. 2012).

Dog meats have been used for human consumption for decades in many parts of the world including South Korea, China, and Vietnam (Podberscek 2009). Since stray dogs are available in many parts of the world without any offered prices (Totton et al. 2010), fraudulent mixing of dog meats with costly halal meats would offer additional profit. In Malaysia, reports have been made that dog meat have been being eaten by foreign workers, especially from Myanmar and Vietnam (Nagpal 2008). However, no systematic studies have been conducted for the detection of dog meats in commercial food products in Malaysia or elsewhere. For the detection of dog meats or its derivatives in food products, several polymerase chain reaction (PCR)-based assays have been proposed (Abdel-Rahman et al. 2009; Abdulmawjood et al. 2003; Gao et al. 2004; İlhak and Arslan 2007; Martín et al. 2007). However, most of them are having long amplicon targets and thus may not be stable under extensive food processing conditions which often breakdown the longer DNA targets. Additionally, these assays were not tested under the excessive pool of heterogenous DNA often found in complex commercial food matrices.

Several reports have been published highlighting the need of shorter amplicon-length PCR assays, preferably of <150 bp product size, for the analysis of highly processed foods, since longer targets are fragmented under harsh conditions (Ali et al. 2012a). Therefore, the objective of this paper was to develop a short amplicon-size PCR assay suitable for the detection of adultered dog meats in commercial meat products.

Frankfurter is an emulsion-type cooked sausage, very popular in most parts of the World including Malaysia. Frankfurter was originated in Germany and was named according to the German state, Frankfurt (Srinivassane 2011). Nowadays, it has been achieved popularity and appeal in all over the world. In the United States, the annual sale of frankfurters and sausages was $1.6 billion in 2009 (Srinivassane 2011). In Malaysia, it is a highly consumed meat product and has gained popularity from school children to grown up individuals at home and in work places (Huda et al. 2010). The manufacturing ingredients of frankfurters may vary from country to country, depending on the consumer choices and availability of the meats which is its major constituent.

In this paper, we have selectively amplified a 100-bp region of canine cytb gene through a pair of canine-specific primers. The canine specificity of the primer pairs was proven against nine animals and five plants species. The stability of the assay was tested under various food processing conditions and in the backgrounds of common frankfurter formulations.

Materials and Methods

Collection of Samples

Meat samples of eight commonly used meat species, namely chicken, duck, turkey, goat, sheep, cattle, buffalo, and pig; the most consumed four fish species, namely tilapia, rohu, tuna, and shrimp; and the five most common plant materials including rice flour, wheat flour, maize flour, garlic, and nutmeg were purchased from the various supermarkets in Kuala Lumpur and Selangor in Malaysia. The dog meat was collected from the Faculty of Veterinary Science, University Putra Malaysia and Dewan Bandaraya Kuala Lumpur, Malaysia. All meat samples were transported under ice-chilled condition (4 °C) and were stored at −20 °C for future work and DNA extraction.

DNA Extraction

DNA was extracted from 25 mg of raw and processed meat samples using NucleoSpin® Tissue extraction kit (Macherey-Nagel, Germany) following the manufacturer's instructions. DNA from plant sources such as rice flour, wheat flour, maize flour, garlic, and nutmeg powder was extracted using cetyltrimethylammonium bromide (CTAB) buffer as described by Ma et al. (2000). For admixed and commercial samples, a modified CTAB method was used for DNA extraction and was purified using Promega Wizard™ DNA isolation kit (Promega Corporation, Madison, USA). Qualitative DNA analysis was done by visualizing the bands after running the total DNA in 1 % agarose gel (Promega, Madison, USA) in 1× lithium borate (LB) buffer of pH 8.0 at a constant voltage of 170 V for 20 min. Concentration and purity of the DNA was analyzed by Eppendorf UV–vis Biophotometer (Eppendorf, Germany).

Canine-Specific Primer Design

A pair of canine-specific primers (forward: 5′ CCTTACTAG GAGTATGCTTG 3′ and reverse: 5′ TGGGTGACTG ATGA AAAAG 3′) defining a 100-bp region of dog cytb gene was developed using a publicly available primer3plus Software (www.bioinformatics.nl/cgi-bin/primer3plus/primer3 plus.cgi) following Ali et al. (2012a). The primers were screened for unique canine specificity to eliminate potential non-specific primer binding to the DNAs of other animal or plant species using online BLAST local alignment tool in NCBI data base (http://www.ncbi.nlm.nih.gov/blast). The primers were purchased from the First Base Laboratories, Pte. Ltd., Selangor, Malaysia.

PCR Assay Optimization

The polymerase chain reaction was performed in a 20 μl of total reaction volume composed of 1× PCR master mix

(Promega Corporation, Madison, USA) with 50 units/ml of Taq DNA polymerase supplied in a proprietary reaction buffer of pH 8 containing 400 μM each of dATP, dGTP, dCTP, dTTP, and 3 mM MgCl2, 100 nM of each primer, and 20 ng of total DNA. PCR was performed in a gradient thermocycler (Eppendorf, Germany) using an initial denaturation at 94 °C for 3 min followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 1 min. The final extension was performed at 72 °C for 5 min. The PCR products were stored at −20 °C for further analysis. The separation of PCR products, pre-stained with 6× loading dye, was performed in 1 % agarose gel (Promega, Madison, USA) in 1× LB buffer of pH 8.0 at a constant voltage of 170 V for 20 min using a 100 bp DNA ladder (Fermentas, USA). DNA visualization was performed with ethidium bromide staining under a gel documentation system (AlphaImager HP; Alpha Innotech Corp., San Leandro, CA, USA).

Species Specificity Testing

To assess the canine specificity of the PCR assay, three methods were followed. Firstly, theoretical specificity was analyzed using bioinformatic softwares. The retrieved cytb gene sequences of animal and cob gene sequence of plant were aligned with the primer sequences using BioEdit version 7.0 (Hall 2004) and ClustalW sequence alignment tool (Thompson et al. 1994). Secondly, experimental analysis was performed in a real PCR amplification reaction against nine animal, four fish, and five plant species. Finally, the purified PCR product was sequenced in triplicate, and the sequences were matched through BLAST analysis in NCBI data base. The PCR product was purified using QIAquick PCR purification kit (Qiagen, USA), and the sequencing was done by the First Base Laboratories Pte. Ltd., Selangor, Malaysia. The sequencing results were used to construct a dendogram showing the genetic closeness and distance among the studied species using molecular evolutionary and phylogenetic analysis software, MEGA version 5 (Tamura et al. 2011).

Meat Processing

The meat samples were cut into small pieces and were subjected to various processing treatments. The raw cut meat samples were boiled for 90 min at 100 °C to simulate traditional cooking. The pasteurization was performed according to European legislation (Commission 2002) by heating the meats at 120 °C for 50 min, 110 °C for 120 min, and 133 °C at 43.51 psi for 20 min. Finally, extensive autoclaving which breaks down the target DNA was executed at 120 °C under 45 psi for 2.5 h (Ali et al. 2011). All the treated samples were stored at −20 °C.

Specificity Under Mixed Background

A complex pool of heterogeneous DNA mixture was prepared by spiking variable amount of dog meat (0.01–10 %) into 1:1 mixture of chicken and beef. Each specimen of the ternary admixtures contained a total of 100 g meats of dog, chicken, and beef in the ratio of 10:45:45, 5:47.5:47.5, 1:49.5:49.5, 0.1:49.95:49.95, and 0.01:49.995:49.995 to realize 10 %, 5 %, 1 %, 0.1 %, and 0.01 % of dog meat contaminated ternary meat mixtures. All admixtures were vigorously blended in replicates on three different days and were autoclaved at 120 °C under 45 psi pressure for 2.5 h.

Frankfurter Preparation

Dummy chicken and beef frankfurters were prepared according to Savic (1985). The negative controls were prepared using pure beef and chicken meats along with fats and seasoning (Table 1), and the positive controls were prepared by spiking into 10 %, 5 %, 1 %, 0.1 %, and 0.01 % of dog meat into the chicken and beef meats. The specified amount of chicken, beef, and dog meats (Table 1) were thawed, cut into small stripes, grounded with a manual grinder of 3 mm plate (Hobart, USA), and mixed with one third of ice and seasoning (Table 1) for 5 min. Subsequently, trimmed fat and rest of ice were added and mixed well for 10 min. From this admixture, individual frankfurter was made by stuffing with commercial halal cellulose casings (Zxchem, China) and hanged for 1 h to dry in room temperature. To get hot smoke effect, all frankfurters were kept in hot oven at 70 °C for 60 min. The traditional cooking was simulated through cooking the frankfurter in hot water for 15 min at 75° C until the internal meat temperature was reached to 68–70ºC. Thus, prepared frankfurters were showered in cold water for 5 min and stored at −20° C for subsequent DNA extraction. For commercial food product analysis, chicken and beef frankfurters from three different brands (labelled as A–C) were purchased in triplicate from Malaysian supermarkets located in Selangor, Petaling Jaya, and Kuala Lumpur in Malaysia. All dummy and dog meat-contaminated frankfurters were prepared in three replicates on three different days.

Results and Discussion

DNA Extraction and PCR Amplification

Extractions of DNA from raw and thermally treated meat samples were performed using a commercial kit (Macherey-Nagel, Germany) that is known to reduce the loss of DNA during aqueous and organic phase separation and give a high

Table 1 Ingredients level for 100 g frankfurters using chicken and beef meat

Ingredients	Chicken frankfurter	Beef frankfurter
Chicken meat	80 g	
Beef meat		50 g
Chicken fat	10 g	
Beef fat		35 g
Wheat flour	2 g	
Ice	8 g	15 g
Seasoning		
Nitrite salt	1.80 g	2.30 g
Dextrose	0.20 g	0.03 g
Black pepper	0.15 g	
White pepper		0.23 g
Red pepper	0.12 g	
Coriander	0.05 g	0.02 g
Mace		0.03 g
Monosodium glutamate	0.02	
Thyme	0.05	
Phosphate	0.04	
Sodium nitrite		0.04 g
Chili		0.01 g
Sage		0.01 g
Nutmeg		0.04 g
Garlic		0.03 g
Sugar		0.3 g
Sodium ascorbate		0.04 g

0.01 %, 0.1 %, 1 %, 5 %, and 10 % of dog meat was mixed in these formulations for the preparation of canine-positive frankfurters.

yield of DNA (Karabasanavar et al. 2011). The highest DNA yield was obtained from the raw dog meat (180–230 ng/mg) and the lowest from the extensively autoclaved meat samples (90–102 ng/mg). This might be due to the degradation of genomic DNA to a certain extent under prolonged heat and pressure processing (Ali et al. 2011). A higher sample size (100 mg) was used to extract DNA from admixed meat and frankfurters using a modified CTAB method, popularly known to give higher yield of good quality DNA. The yield of DNA was higher (430–456 ng/mg) in admixed samples than those of frankfurters (322–360 ng/mg). This might be due to the presence of plant materials and higher content of fat in frankfurter formulations (Table 1). The purity (A260/A280) of all DNA samples was 1.90–2.0 in triplicates. Primer annealing at a higher temperature increases specificity and eliminates nonspecific hybridization (Ali et al. 2012a). Therefore, an optimized high annealing temperature (58 °C), as determined through repeated run of gradient PCR, was used for the amplification of the selected region of cytb gene from raw, treated, admix, and commercial samples.

Canine Specificity

To determine the canine specificity of the developed primers, cytb and cob gene sequences of dog and other 17 animal, fish, and plant species were retrieved from NCBI database (dog: JF489119.1, chicken: EU839454.1, beef: EU807948.1, water buffalo: D32193, domestic duck: HQ122601.1, turkey HQ122602.1, sheep: EU365990.1, goat: EU130780.1, pig: GU135837.1, tilapia: AF015020.1, rohu: JQ346135.1, tuna: AM989973.1, shrimp: EU069446.1, rice: X17064.1, maize X00789.1, wheat: X02352.1, nutmeg: DQ916628.1, garlic: AF356823.1). The canine-specific primer pairs designed from the retrieved mithochondrial (mt)-cytb sequences showed perfect match with the canine cytb gene and more than seven nucleotide variations with other species. In addition, the blast results showed 100 % identity with the canine species and eliminate the probability of primer binding with non-target species DNAs. In a real PCR run using DNAs from total 18 species including dog and other animals, fish, and plant species, the assay successfully amplified only 100 bp of canine target (Fig. 1) The sequencing result of the 100 bp PCR product also confirmed 100 % similarity only with the canine (*Canis lupus familiaris*) cytb gene.

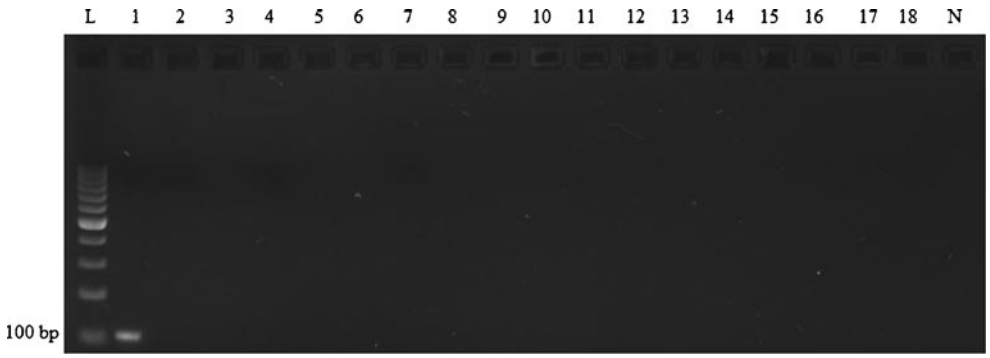

Fig. 1 Species specificity of the mitochondrial cytb-based canine-specific PCR assay. A 100-bp product was obtained only from DNA extracted from dog meat and from not any other 12 animals and 5 plants species. *Lane N*: negative control and *Lane L*: 100 bp Ladder. *Lanes 1–9*: dog, chicken, duck, turkey, sheep, goat, beef, buffalo, and pig, respectively; *Lanes 10–14*: DNA from rice, maize, wheat, nutmeg, and garlic, respectively; *Lanes 15–18*: tilapia, rohu, tuna, and shrimp, respectively

Table 2 Pairwise distances between 100 bp cytb canine-specific site and common meat, fish, and plant species potentially found in frankfurter formulations

	Dog	Chicken	Turkey	Duck	Goat	Sheep	Beef	Buffalo	Pig	Rice	Maize	Wheat	Nutmeg	Garlic	Tilapia	Rohu	Tuna	Shrimp
Dog	0.00																	
Chicken	0.45																	
Turkey	1.20	2.00																
Duck	1.03	1.83	0.14															
Goat	0.27	0.42	1.22	1.20														
Sheep	0.26	0.52	1.25	1.28	0.10													
Beef	0.30	0.37	1.37	1.34	0.07	0.18												
Buffalo	0.35	0.37	1.46	1.48	0.14	0.15	0.09											
Pig	0.36	0.42	1.28	1.25	0.13	2.0	0.15	0.15										
Rice	0.75	0.82	1.77	1.77	0.79	0.68	0.83	0.77	0.72									
Maize	0.69	0.75	1.91	1.91	0.72	0.68	0.73	0.77	0.66	0.06								
Wheat	0.72	0.79	1.64	1.65	0.76	0.68	0.80	0.77	0.69	0.03	0.04							
Nutmeg	0.80	0.88	1.71	1.65	0.85	0.77	0.86	0.87	0.78	0.06	0.04	0.03						
Garlic	2.29	1.60	1.61	1.27	2.27	2.24	2.20	2.29	2.30	2.01	1.96	1.91	1.83					
Tilapia	0.51	0.52	1.49	1.69	0.39	0.51	0.35	0.41	0.43	0.68	0.55	0.65	0.65	1.83				
Rohu	0.42	0.47	1.14	1.57	0.36	0.41	0.38	0.38	0.38	0.83	0.76	0.08	0.90	1.92	0.31			
Tuna	0.61	0.69	2.01	2.00	0.57	0.51	0.57	0.53	0.55	0.83	0.73	0.08	0.86	1.84	0.48	0.41		
Shrimp	0.93	1.97	0.38	0.36	1.03	1.05	1.17	1.28	1.03	1.37	1.42	1.30	1.30	1.84	1.33	1.28	1.83	0.00

Species-specific PCR techniques are widely used for the detection of meat species from raw and admixed meat product such as sheep (Karabasanavar et al. 2011), beef (Mane et al. 2012), pork (Ali et al. 2012a), etc. It has been implicated in several reports that the efficiency of the PCR assay might reduce or end up with failure in amplification in presence of single mismatches in the primer binding region (Ali et al. 2012a). The proposed mt–cytb-based primer pair had multiple mismatches in the primer binding sites with the other tested species and, in a real PCR run, amplified only the canine species. Previously, four different species-specific PCR assays using mitochondrial whole genome (322 bp) (İlhak and Arslan 2007), mitochondrial cytb (808 bp) (Abdel-Rahman et al. 2009), D-loop (213 bp) (Gao et al. 2004), and 12S rRNA (101 bp) (Martín et al. 2007) have been proposed for dog meat detection. However, three of these assays used longer-sized amplicon (≥213 bp) (Abdel-Rahman et al. 2009; Gao et al. 2004; İlhak and Arslan 2007), which may not be stable under extreme physio-chemical conditions of food processing. The latest studies appreciated short-amplicon-length PCR assay

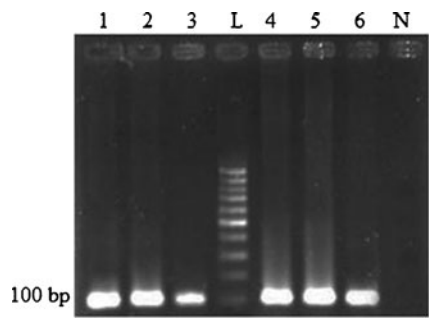

Fig. 2 Stability of the mitochondrial cytb-based canine-specific PCR assay under thermal processing conditions. Clear 100-bp PCR product was obtained from different thermally treated dog meat samples. *Lane 1*: Raw dog meat, *Lane 2*: dog meat boiled for 90 min; *Lane 3*: dog meat autoclaved at 120 °C under 45 psi for 2.5 h.; *Lane L*: 100 bp ladder; *Lanes 4–7*: dog meat autoclaved at120°C for 50 min; 110 °C for 12 min, and 133 °C for 20 min under 43.51 psi, respectively, and *Lane N*: negative control

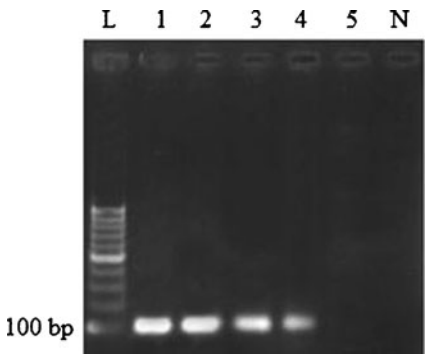

Fig. 3 Performance of the mitochondrial cytb-based canine-specific PCR assay under admixed background. *Lane L*: 100 bp ladder; *Lanes 1* to *5*: 10 %, 5 %, 1 %, 0.1 %, and 0.01 % of dog meat spiked in 1:1 chicken and beef meat admixtures, respectively; *Lane N*: negative control. Clear PCR products were obtained only from ternary admixtures containing of 10 % to 0.1 % of dog meat (*Lanes 1–4*) spiked with chicken and beef

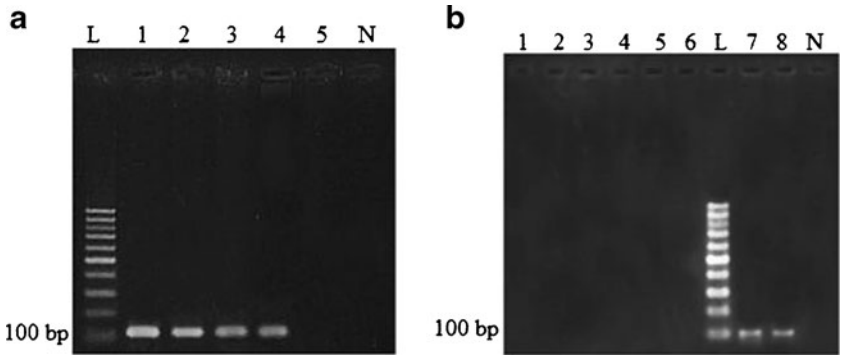

Fig. 4 Frankfurter analysis using mitochondrial cytb-based (100 bp) canine-specific PCR assay. **a** 100 bp PCR products obtained from 10 %, 5 %, 1 %, and 0.1 % (*Lanes 1–5*) of dog meat-spiked dummy chicken frankfurters. **b** No PCR product was obtained from commercial frankfurters (*Lanes 1–3*: commercial chicken frankfurters and *Lanes 4–6*: commercial beef frankfurters) except from 0.1 % dog meat-spiked dummy chicken (*Lane 7*) and beef (*Lane 8*) frankfurters. *Lanes L* and *N* represent 100 bp ladder and negative control, respectively

(<150 bp) for the detection of species in highly processed foods (Ali et al. 2012a). Martín et al. (2007) proposed a short-amplicon-length PCR assay targeting 101 bp region of canine 12S rRNA gene which should be suitable for the analysis of processed foods. However, recent species identification schemes have showed that cytb-based PCR assays are more accurate to reconstruct the mammalian phylogeny along with higher resolution over other mitochondrial genes (Nicolas et al. 2012). Therefore, we expected that a mt–cytb-based short-amplicon-length (100 bp) PCR assay would detect canine-derived materials under raw as well in highly processed commercial foods.

Pairwise distances among the studied species computed using the maximum composite likelihood method (Tamura et al. 2011) were in the range of 0.26 to 2.29 (Table 2). The lowest distance was observed between dog and sheep, and the highest was found between dog and garlic. The mismatch bases in primer binding sites of the studied species were between 42 % and 55 %. The dendogram constructed by neighbor-joining method (Saitou and Nei 1987) showed clear discrimination of the dog from other animals and plants species (data not shown).

Target Stability Under Thermal Processing

The efficiency of the PCR assay under different thermal processing was tested, and clear PCR products were obtained from all samples (Fig. 2), reflecting the stability of this assay. Martín et al. (2007) have proposed a PCR assay targeting a 101 bp region of 12S rRNA gene of canine species and tested it under various heat treatments in accordance with the European legislation (Commission 2002). However, Martín et al. (2007) did not test the stability and performance of their PCR assay under extensive autoclaving conditions which is known to breakdown target DNA (Ali et al. 2011). In the present assay, the 100-bp target of the canine cytb gene was obtained from the extensively autoclaved (2.5 h) dog meats, showing an extraordinary stability of the developed PCR assay. This was not surprising since Ali et al. (2012a) also documented an extraordinarily stable short-length (109 bp) PCR assay targeting cytb gene for pork authentication in halal foods.

Performance at Mixed-Meat Background

Mt–cytb-based canine-specific PCR assay developed here successfully detected canine-specific target from as low as 0.1 % (0.02 ng DNA) of dog meat-contaminated admixtures (Fig. 3) under the excessive pool of chicken and beef DNAs, reflecting a high sensitivity and specificity of the assay under complex matrices.

The sensitivity of the dog-specific PCR assay documented by Martín et al. (2007) targeting a 101 bp fragment of 12S

Table 3 Analysis of frankfurters using canine mt–cytb (100 bp)-based PCR assay

Frankfurter samples	Day 1	Day 2	Day 3	% Dog meat-positive samples	Detection probability
Dummy pure chicken frankfurter	3	3	3	0/9	100
Dummy pure beef frankfurter	3	3	3	0/9	100
Dummy dog spiked chicken frankfurter	3	3	3	9/9	100
Dummy dog spiked beef frankfurter	3	3	3	9/9	100
Commercial chicken frankfurter					100
A	3	3	3	0/9	
B	3	3	3	0/9	100
C	3	3	3	0/9	100
Commercial beef frankfurter					
D	3	3	3	0/9	100
E	3	3	3	0/9	100
F	3	3	3	0/9	100

rRNA gene in meat-oats binary admixtures under normal autoclaving condition was 0.1 % (0.125 ng). Compared with this assay, our assay was 6.25 times more sensitive in terms of the amount of template DNA used (125 ng versus 20 ng) and in terms of mixed-meat matrices (ternary meat mixtures) under extensive autoclaving. To the best of our knowledge, the highest detection limit (0.01 %) for the detection of dog meat was reported by Abdulmawjood et al. (2003) in a PCR-RFLP assay where the target was a 808 bp fragment of mitochondrial cytb gene. However, Abdulmawjood et al. (2003) did not describe the amount of DNA template they used in their analysis. A number of literatures reported that longer DNA-based PCR assays are less sensitive and less stable over the shorter ones under extensive food processing conditions (Ali et al. 2011). However, the percentage composition, which is an undefined method for concentration expression, can be manipulated through the use of higher amount of template DNA. Thus, the sensitivity of the 808-bp-length PCR assay cannot be higher than that of 101-bp PCR assay.

Evaluation in Frankfurters

Finally, the performance of the assay was tested in dummy chicken and beef frankfurters deliberately spiked with 0.01 to 10 % dog meat. Clear PCR products were obtained from 0.1 % to 10 % dog-meat-spiked dummy chicken (Fig. 4a) and beef frankfurters (data not shown). Thus, in a blind experiment, 0.1 % dog meat-spiked dummy frankfurters were used as a positive control for screening three halal (A–C) logo containing commercial chicken and beef frankfurters obtained from various super markets across Malaysia. No commercial samples were found to be positive for dog meat adulteration (Table 3 and Fig. 4b).

Although different PCR assays were previously proposed for canine meat detection (Abdel-Rahman et al. 2009; Abdulmawjood et al. 2003; Gao et al. 2004; İlhak and Arslan 2007; Martín et al. 2007), none of them have been tested for commercial meat products. But presence of various additives and inhibitors in commercial meat and food products might prevent the primer binding at specific sites and reduce the amplification efficiency. (Di Pinto et al. 2005). Therefore, we analyzed our assay performance under dummy chicken and beef frankfurter formulation. A constant detection limit of 0.1 % (0.02 ng DNA) was obtained in all positive control showing high performance under complex background of frankfurter and may be due to the shorter size (100 bp) of the cytb gene target, which is known to have better sensitivity and stability under harsh conditions (Ali et al. 2012a). The Malaysian government has a strong commitment to build a halal hub in local and international arenas (Talib et al. 2008). Therefore, our study was in line with the government policy, and we found the validity and applicability of our assay for the detection of canine tissues both in raw and processed commercial products.

Conclusion

A cytochrome b-based short-amplicon-length species-specific PCR assay was developed and utilized to detect canine meat tissues in raw and processed meats as well as in commercial chicken and beef frankfurters. The assay has utilized a very short length (100 bp) target of the canine cytb gene and thus was very stable and sensitive under all potential food processing conditions. The specificity of the assay was tested against nine meat providing animal species and five plant species commonly utilized in commercial food and meat products. The detection limit of the assay was 0.1 % of spiked dog meat in ternary meat mixtures and frankfurter formulations using 0.02 ng template DNA. We believe that this assay would find application in food industry for the authentication of canine origin materials in halal foods.

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Shuhaimi Mustafa declares that he has no conflict of interest to publish this paper.

Subha Bhassu declares that she has no conflict of interest to publish this paper.

Sharifah Bee Abd Hamid declares that she has no conflict of interest to publish this paper.

Uda Hashim declares that he has no conflict of interest to publish this paper.

Compliance with Ethics Requirements This study was conducted following all institutional and national guidelines for the handling of dog and other animal meats.

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