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Screening of commercial meat products from supermarket chains for feline derivatives using SP-PCR-RFLP and lab-on-a-chip

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ABSTRACT

Determination of feline meat in food products is an important issue for social, health, economic and religious concern. Hence this paper documented the application of species specific polymerase chain reaction-restriction fragment length polymorphism (SP-PCR-RFLP) assay targeting a short-fragments (69 bp) of mitochondrial cytochrome b (cytb) gene to screen feline meat in commercial meat products using lab-on-a-chip. The SP-PCR assay proved its specificity theoretically and experimentally while testing with different common animal, aquatic and plant species of DNA. The feline specific (69 bp, 43- and 26-bp) characteristic molecular DNA pattern was observed by SP-PCR and RFLP analysis. For assay performance, it was tested in three different types of commercial dummy meat products such as frankfurters, nuggets and meatballs and digested with AluI-restriction enzyme. The highest sensitivity of the assay using lab-on-a-chip was as low as 0.1 pg or 0.01 % (w/w) in commercial dummy meat products. We have also applied this assay to screen three important commercial meat products of six different brand from six supermarket chains located at three different states of Malaysia. Thus total 378 samples were tested to validate the specificity, sensitivity, stability of the assay and utilization of it for commercial meat product screening.

1. Introduction

Currently, food safety is an important issue due to increasing consumer’s awareness relate to the foods either from the restaurant or from different supermarket chains. Numerous features such as lifestyles (organic food and vegetarianism), health-hazardous problems (microbial risk and allergens), diet (nutritional balance and calories), economic situation (fair trade) and religious taboo (cat, pork, dog is banned according to religious dietary rules in Islam) are the major factors to verify the ingredients in various commercial food products. Furthermore, reports of fake labelling and stating false ingredients on food products have made a negative impact on customers’ confidence. To gain economic benefits use of lower-priced meats in the higher-priced meat products has become a common exercise in the meat industry (Hsieh et al., 1995). Therefore, usage of proper meats is a prerequisite to maintain fair trade and consumer’s trust for commercial meat products in the market chain.

The estimated value of the Halal market is $2.3 trillion and expected to reach 10 trillion US Dollars by 2030 (Amin et al., 2016). The price of halal foods is higher compared to conventional foods due to the requirement of particular processing and supply chain. For more profit mislabeling of ‘Halal’ symbols on food products has been reported (Ali et al., 2015d) Furthermore, the meat of certain sources are a prospective carrier of hepatitis (chimpanzee, gorilla and orangutan species) (Sa-Nguanmoo et al., 2008), influenza (pig and other species) (Mubareka et al., 2009), avian influenza-H5N1 virus (avian species) (Beiguel et al., 2005), anthrax (horse, cattle and other species), plague (cat and dog species), severe acute respiratory syndrome (SARS) (cat and other species) (Ali et al., 2015) and human immunodeficiency virus (HIV) (chimps species) (Girish et al., 2004). Therefore, it becomes important to screen the commercial meat products from different supermarket chains to maintain sound health, fair trade, consumer trust and religious faith. Frankfurters, nuggets and meatballs are very popular meat products.
almost every part of the world including Malaysia, India, China, Indonesia, Vietnam, Europe and the USA (Rahman et al., 2014). Recently, reports have been published about fraudulent use of horse meat in commercial beef meatball (Rahman et al., 2016), frozen meatballs (Ali et al., 2015a) and frankfurter sausages (Amin et al., 2016). According to the religious faith of Islam consumption of feline meat is forbidden. However, it has been consumed as exotic meals in South Korea, Vietnam, Thailand, Cambodia, and China. No legal market has been allotted to sell feline meats and roaming of feline species across Malaysia, Cheras, Kuala Lumpur; rat (Rattus norvegicus) and turtle (Cuora amboinensis) was bought from Chinese wet market at Selangor, Malaysia. For commercial sample analysis commonly available meat product’s samples of total six different brands of chicken and beef frankfurters, nuggets and meatballs were collected from six different superstore/supermarket chains located across three different states of Malaysia. The samples were collected in triplicate and carried by using the ice-cold box (4 °C) and refrigerated at −20 °C for further use and extraction of DNA.

2.2. Preparation of dummy meat products

To identify meat adulteration, dummy meat products of beef and chicken frankfurters, nuggets and meatballs were prepared (Table 1) by adding 1, 0.5, 0.1 and 0.01 % (w/w) of cat meat into beef and chicken meats according to Rahman et al. (2016). The raw frankfurters and nuggets were autoclaved for 2.5 h under 45-psi pressure at 120 °C and the meatballs were boiled for 90 min at 100 °C (Rahman et al., 2016) to check its consistency under meat processing steps. All the prepared dummy meat products were refrigerated at −20 °C for further use and DNA extraction.

2.3. DNA extraction

Meat and fish samples were sliced into small pieces using sterilized surgical scalpels. Total DNA was extracted from 25 mg of raw meat samples from each specimen using commercial genomic DNA extraction kit (Yeastern Biotech Co. Ltd, Taipei, Taiwan). For the extraction of DNA from plants, commercial and lab-made beef and chicken frankfurters, nuggets and meatballs; 1 g of each sample was used by applying cetyl trimethylammonium bromide (CTAB) method according to Amin et al. (2016). To measure the purity and concentration of extracted DNAs UV–vis NanoPhotometer® (Nano Life Quest Sdn. Bhd; Selangor, Malaysia) were used.

2.4. Feline-specific primer

A pair of feline specific primers based on mitochondrial cytochrome b gene (GenBank: AB194817.1) was developed using an online bioinformatics tool Primer3plus (www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi), which possessed a 22 bp AluI cut-site as an internal oligo (Ali et al., 2015a; Amin et al., 2016). In the in-silico analysis, primer specificity was confirmed by extracting the nucleic acid sequences of more species by using the BLAST (Basic Local Alignment Tool) of NCBI database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Retrieved sequences of 14 most important animal species such as goat, beef, sheep, lamb, buffalo, chicken, duck, pigeon, turkey, pork, dog, Malaysia box turtle, monkey and rat; 5 fish samples of Atlantic shrimp, tilapia, cuttle, carp and cod and 5 plant sources of wheat, tomato, cucumber, potato and onion were aligned by ClustalW (Clustal Omega) sequence alignment tool (https://www.ebi.ac.uk/Tools/msa/clustalo/) to verify the degree of interspecies polymorphism of primer binding site. The total number of mismatch for both primers binding sites were calculated by using MEGAS software. Another set of primer designed by

2. Materials and methods

2.1. Samples collection

Fresh meat samples of goat (Capra hircus), beef (Bos taurus), sheep (Ovis aries), lamb (Ovis aries), buffalo (Bubalus bubalis), pork (Sus scrofa), chicken (Gallus gallus), duck (Anas platyrhynchos), pigeon (Columba livia), turkey (Meleagris gallopavo), shrimp (Lolothaenus vannamei), tilapia (Oreochromis aureus), carp (Cyprinus carpio), cod (Gadus morhua), potato (Solanum tuberosum), cuttle (Sepia officinalis), wheat (Triticum aestivum), cucumber (Cucumis sativus), tomato (Solanum lycopersicum) and onion (Allium cepa) were procured in triplicates from various supermarkets and wet markets in Malaysia. Cat (Felis catus) and dog (Canis familiaris) meat samples were obtained in triplicates from euthanized animals according to animal welfare protocol by authorized personnel from Kuala Lumpur City Hall, Health Ministry, Malaysia. Monkey (Macaca fascicularis sp.) sample was collected from Wildlife Malaysia, Cheras, Kuala Lumpur; rat (Rattus norvegicus) and turtle (Cuora amboinensis) has been allotted to sell feline meats and roaming of feline species across Malaysia. No legal market has
Nealing at 58 °C for 20 s, elongation at 72 °C for 30 s and for 3 min followed by 35 cycles of denaturation at 95 °C for 20 s, an-

Rojas et al. (2010) targeting a conserved region of eukaryotic 18S rRNA gene (141-bp) was employed as an internal control for experimental analysis. The consensus feline specific 69 bp cytb gene sequences were used to determine the pairwise genetic distance and dendrogram was constructed with molecular evolution and phylogenetic analysis software, MEGA version 5. 3D plot was created based on pairwise distances restriction endonuclease in 30 μL reaction volume composed of 1 μL of Alul restriction enzyme (New England Biolab, CA, USA), 10 μL of PCR product, 2 μL of 10× digestion buffer and appropriate volume of nuclease free water. Restriction digestion was performed by using water shaking bath at 37 °C for 30 min. To inactivate the restriction digestion enzyme reheating of the reaction mixture was done for 25 min at 65 °C. For the final RFLP analysis and separation of specific DNA fragments, 1 μL of digested PCR-products were loaded in the lab-on-a-chip-well of 1 K DNA kit and run by using Experion Automated Electrophoresis Station (Bio-Rad Laboratories, USA).

2.6. Enzymatic digestion and RFLP analysis

We successfully digested the PCR products of the dummy and commercial meat products of frankfurters, nuggets and meatballs by restriction endonuclease in 30 μL reaction volume composed of 1 μL of Alul restriction enzyme (New England Biolab, CA, USA), 10 μL of PCR product, 2 μL of 10× digestion buffer and appropriate volume of nuclease free water. Restriction digestion was performed by using water shaking bath at 37 °C for 30 min. To inactivate the restriction digestion enzyme reheating of the reaction mixture was done for 25 min at 65 °C. For the final RFLP analysis and separation of specific DNA fragments, 1 μL of digested PCR-products were loaded in the lab-on-a-chip-well of 1 K DNA kit and run by using Experion Automated Electrophoresis Station (Bio-Rad Laboratories, USA).

2.7. Commercial meat products analysis

Extracted DNAs of commercial chicken and beef frankfurters, nuggets and meatballs were exploited for the detection of cat meat using the SP-PCR (feline-specific) primer. We have considered the affirmative detection of deliberately cat meat spiked dummy commercial frankfurters, nuggets and meatballs as a positive control. The presence of 141-bp of endogenous control was analyzed to confirm the quality of DNA of the samples collected from the six different supermarket chains located across three different states of Malaysia.

3. Results and discussions

3.1. Feline species specificity analysis

For the in-silico analysis, nucleotide sequences of mitochondrial cytb gene of all species were taken from the NCBI database and aligned with the feline cytb specific SP-PCR primer binding sites.
demonstrated the feline specificity by complete matching with feline cytb gene and three to nineteen (14–86%) nucleotide mismatching with the other non-target species (Fig. 1b) providing the theoretical supports to our findings. Moreover, phylogenetic tree analysis (Fig. 1a) and the number of mismatches (Fig. 1b & 3a) reflected the divergence between the target and rest of the species DNA. The maximum and minimum genetic distance were observed between cat with cuttlefish (1.608) and cat with beef and buffalo (0.160), which reduced the risks of cross
positive amplification during the experimental study. In this experiment, the feline specificity was extended to check with the total 14 non-targets of animal, 5 fish and 5 common plant species which are frequently used for making different type of food products. In Fig. 1c (lane 1) and Fig. 2 (lane 1, 7 & 13), clearly showed that the primers are cat or feline specific, as the target amplicon (69 bp) was amplified only from the samples of feline meat or feline meat spiked product. Moreover, a pair of eukaryotic primer amplified the 141-bp target of the 18S rRNA gene from all the tested samples (Fig. 1c, lanes 1–25), which confirmed the existence of adequate DNA template.

Previously, six different PCR assays using cytb gene (331 bp) (Irine et al., 2013); cyt b gene (180 bp) (Tobe and Linacre, 2008); cyt b gene (672 bp) (Abdel-Rahman et al., 2009), (Abdulmawjood et al., 2003); 12S rRNA (108 bp) (Martin et al., 2007) and ND4 gene (274 bp) (Ilhak and Arslan, 2007) have been reported for cat meat detection. However, the majority of these assays have been regarded with longer amplicons size (≥180 bp) which would possibly be fragmented underneath different meat products processing treatments and lead PCR amplification failure. The recent studies esteemed the short-amplicon based PCR assays due to its higher stability over the longer-ones (≥150 bp) for species authentication under the complex background of food products (Rahman et al., 2016). Therefore, we have used this short-amplicon based (69-bp) SP-PCR assay with mitochondrial-cytb-gene target for F. catus detection in commercial meat products.

### 3.2. Feline meat spiked dummy meat products analysis

Lower priced meat are deliberately mixed in commercial meat products (Amin et al., 2016) and the food forgeries were reported in a number of meat products such as frankfurters, dried meats, burgers, ground meat, nuggets, meatballs and sausage (Kane and Hellberg, 2016). Recently, the ‘Food Safety Authority of Ireland’ discovered 37 % horsemeat in beef burgers and ground beef products and 85 % pork meat in salami (Kane and Hellberg, 2016). The latest scandal of cat meat in curry and selling of cat meat as rabbit meat (Amin et al., 2016) headed us to screen the commercial meat products adulteration with cat meat.

We observed the feline specific DNA from all lab-made (Table 1) feline meat spiked dummy beef and chicken frankfurters, nuggets and meatballs (Fig. 2 & Table 2). The results proved its sensitivity and efficiency to identify feline derivatives from any food processing products. Recently, Rahman et al. (2015) and Rashid et al. (2015) was identified 0.01 % (w/w) and 0.1 % (w/w) dog and monkey meat in dummy burgers and meatballs respectively, using a lab-on-a-chip-based detection method. Thus the LOD of 0.01 % in three different meat products (Fig. 2, lanes 4, 10 & 16) was an acceptable conclusion, as we used the short-length (69 bp) target in a highly sensitive microfluidic lab-on-a-chip based automated electrophoretic platform.

### 3.3. Lab-on-a-chip based SP-PCR-RFLP analysis

Lab-on-a-chip-based capillary electrophoretic assay made SP-PCR-RFLP patterns more specific, reliable and highly sensitive to distinguish and differentiate digested banding profiles in commercial meat products. It also proved its dominance over other capillary electrophoresis based assay such as DNA sequencing and probe hybridization methods for commercial sample analysis. Therefore, the amplified feline-specific PCR products (69 bp) was digested using AluI restriction enzyme since the theoretical study (Fig. 3a) revealed the availability of restriction sites yielding 43- and 26-bp length of two distinct fragments. Moreover, internal control of 141 bp of 18S rRNA gene produced two fragments (14- and 127 bp) with one AluI restriction cut-site during the in-silico analysis. In Fig. 3b, AluI digested feline meat contaminated (beef and chicken) frankfurters (lanes 2 & 4), (beef and chicken) nuggets (lanes 6 & 8) and (beef and chicken) meatballs (lanes 10 & 12) were separated by the Bio-Rad Experion Automated Electrophoresis Station. The 43-, 26- and 127-bp fragments were clearly visible both in the gel image (Fig. 3b) and the electropherograms (Fig. 3b) reflecting the target amplicon. However, 14-bp of eukaryotic control (141 bp) was not visible (Fig. 3b, lanes 2, 4, 6, 8, 10 & 12) because it integrated with the lower marker (15 bp) of the DNA ladder (1500 bp). The molecular size of the amplified products and restriction digestion of feline frankfurters, nuggets and meatballs using lab-on-chip are presented in Table 3. Among the previously developed feline specific assays, Abdulmawjood et al. (2003) described PCR-RFLP method with a longer fragment of 981 bp without any heat treating effect. Besides, this method was not verified in commercial meat products under the extensive autoclaving condition and might not be appropriate for processed meat products sample analysis due to larger-sized DNA target (981 bp). Therefore, the importance of this developed short-amplicon based SP-PCR-RFLP assay for commercial sample analysis using lab-on-a-chip is easily intelligible.

### 3.4. Screening of commercial meat products from supermarket chains

Frankfurters, nuggets and meatballs are the most common and popular meat products which are consumed all over the world including Malaysia, China, Indonesia and Europe (Rohman et al., 2011). Low-cost meat is usually added to higher valued meat products to reduce the production cost and financial benefit. Recently, horse meat was found in meatballs (Ali et al., 2015a) and chicken nuggets in Europe (Rahman et al., 2016). Therefore, for the screening of commercial beef and chicken frankfurters, nuggets and meatballs, 0.01 % (w/w) cat meat-spiked beef and chicken frankfurters (Fig. 4b, lanes 1 & 2), nuggets (Fig. 4b, lanes 3 & 4) and meatballs (Fig. 4b, lanes 5 & 6) were used as a positive control in the presence of 141 bp of eukaryotic 18S rRNA gene. In Fig. 4b (lanes 1–6), all the positive control (0.01 %)

Table 2

| Item     | No Feline meat spiked dummy commercial products | Existence of target | No of Total sample | Positive detection | Detection Probability (%) |
|----------|------------------------------------------------|--------------------|--------------------|--------------------|---------------------------|
| Frankfurters | 1 Raw beef frankfurters | + | 9 | 9/9 | 100% |
|           | 2 Autoclaved beef frankfurters | + | 9 | 9/9 | 100% |
|           | 3 Raw chicken frankfurters | + | 9 | 9/9 | 100% |
|           | 4 Autoclaved chicken frankfurters | + | 9 | 9/9 | 100% |
| Nuggets  | 1 Raw beef nuggets | + | 9 | 9/9 | 100% |
|           | 2 Autoclaved beef nuggets | + | 9 | 9/9 | 100% |
|           | 3 Raw chicken nuggets | + | 9 | 9/9 | 100% |
|           | 4 Autoclaved chicken nuggets | + | 9 | 9/9 | 100% |
| Meatballs | 1 Raw beef meatballs | + | 9 | 9/9 | 100% |
|           | 2 Boiled beef meatballs | + | 9 | 9/9 | 100% |
|           | 3 Raw chicken meatballs | + | 9 | 9/9 | 100% |
|           | 4 Boiled chicken meatballs | + | 9 | 9/9 | 100% |
| Total feline meat spiked sample | + | 108 | 108/108 | 100% |

“+” denotes presence of target feline DNA.
Fig. 3. (a) In-silico analysis with restriction cut site and (b) amplification of feline specific DNA target (69 bp) and internal control (141 bp) using SP-PCR-RFLP assay from deliberately cat meat spiked commercial meat products with the endogenous control. Clear 69 bp feline specific and 141 bp sized endogenous control before AluI digestion (lanes 1, 3, 5, 7, 9 & 11) and with 127 bp, 43bp and 26 bp size (lanes 2, 4, 6, 8, 10, and 12) using lab on a chip. In the gel view, SP-PCR products and endogenous control from (beef and chicken) frankfurters (lanes 1 & 3), (beef and chicken) nuggets (lanes 5 & 7) and (beef and chicken) meatballs (lanes 9 & 11) before AluI digestion; and (beef and chicken) frankfurters (lanes 2 & 4), (beef and chicken) nuggets (lanes 6 & 8) and (beef and chicken) meatballs (lanes 10 & 12) after AluI digestion. Electropherograms are demonstrated by respective labels.

Table 3
Molecular sizing of feline specific PCR amplicon and Lab-on-a-chip based restriction patterns from raw and treated feline meat spiked dummy commercial chicken and beef frankfurter, nuggets and meatballs samples.

|          | Frankfurters | Nuggets          | Meatball         |
|----------|--------------|------------------|------------------|
| PS (bp)  |              |                  |                  |
|          | frankfurters | nuggets          | meatball         |
|          | -beef        | -beef            | -beef            |
|          | autoclaved   | autoclaved       | autoclaved       |
|          | raw          | raw              | raw              |
|          |              |                  |                  |
|          |              |                  |                  |
| 141      | 140 ± 1      | 141 ± 1          | 147 ± 1          |
| 127      | 130 ± 0.6    | 133 ± 0.5        | 127 ± 0.9        |
| 14       | 72 ± 0.2     | 72 ± 0.4         | -                |
| 69       | 69 ± 0.8     | 65 ± 0.7         | -                |
| 43       | 49 ± 0.1     | 45 ± 0.7         | -                |
| 26       | 23 ± 0.3     | 24 ± 0.0         | -                |

PS-product size; bp-base pair.
was amplified with feline specific SP-PCR products from each of the spiked commercial samples of frankfurters, nuggets and meatballs. On the other hand, there was no feline specific SP-PCR amplicon from pure commercial products of beef and chicken frankfurters, nuggets and meatballs (Fig. 4b, lanes 7–24). Note that, homogeneous amplification of eukaryotic primers (141 bp) form all the commercial products (Fig. 4b, lanes 1–24) reflected the presence of ample DNA template and the assay performance was validated with the absence of cat meat derivatives in commercial meat products (Fig. 4b lanes 7–24 & Table 4). Thus, total 378 samples of two types (chicken and beef) with three commercial meat products (frankfurter, nuggets and meatball) of total six different brands purchased from total six supermarket located at Kedah, Penang and Kuala Lumpur of Malaysia (Fig. 4) were negative for feline meat detection using feline SP-PCR based on lab-on-a-chip. The result is acceptable as the Government of Malaysia is devoted to set up Malaysia as a Halal-hub and strongly observing for the assessment of Halal Status of foods in different food products.

4. Conclusion

Note that, a highly sensitive SP-PCR-RFLP assay using electrophoresis based lab-on-a-chip is documented for the repetitive analysis of cat meat detection in popular commercial meat products. A set of feline specific primers were analyzed using cat and other twenty-four (24) species of animal, plant and fish DNA. Thus the feline specific target was amplified only from the feline DNA template, which ratified the specificity and self-standing capacity of the applied biomarkers. The LOD of 0.01 % (w/w) feline meats was found in all commercial dummy meat products (frankfurters, nuggets and meatballs) using SP-PCR assay. The successful digestion of the SP-PCR products using Alu restriction enzyme yield feline specific restriction patterns (43- and 26-bp) from all the deliberately cat meat spiked dummy commercial products. Thus the endpoint detection and separation of restriction digested SP-PCR-RFLP products using lab-on-a-chip confirmed the feline species authenticity and declined any dubious results. Henceforward, the amazing stability and established sensitivity of this assay initiates...
its application for the screening of larger quantity of samples of three major commercial meat products (frankfurters, nuggets and meatballs) of total six brands from different supermarket chains across Malaysia (three-states) for feline species detection. The screening of total of 378 samples of commercial meat products from six different supermarket chain of Malaysia, were negative for feline species detection. Thus the assay showed it acceptability to the regulatory bodies and quality control laboratories for commercial meat products screening. It also showed the reflection of the reliability of the halal status of Malaysian commercial meat products.

Authors statement

All the authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Compliance with ethics requirements

All experiments were conducted with the Ethical clearance ref. no: NANOCAT/25/04/3013/MMR (R), University of Malaya.

Declaration of Competing Interest

The authors do not have any conflict of interest to publish this manuscript.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jfca.2020.103565.

Table 4
Analysis results of screening commercial meat products of different brands and supermarket chains across Malaysia.

| Item | No of Commercial meat product | Existence of target | No of Total sample | Positive detection | Detection Probability (%) |
|------|--------------------------------|---------------------|---------------------|---------------------|---------------------------|
| Meat Products | 1 Beef frankfurters (B = 1, S = 1, L = 3, r = 3) | — | 27 | 0/27 | 100% |
| | 2 Chicken frankfurters (B = 3, S = 3, L = 3, r = 3) | — | 81 | 0/81 | 100% |
| | 3 Beef nuggets (B = 1, S = 1, L = 3, r = 3) | — | 27 | 0/27 | 100% |
| | 4 Chicken nuggets (B = 3, S = 3, L = 3, r = 3) | — | 81 | 0/81 | 100% |
| | 5 Beef meatballs (B = 3, S = 3, L = 3, r = 3) | — | 81 | 0/81 | 100% |
| | 6 Chicken meatballs (B = 3, S = 3, L = 3, r = 3) | — | 81 | 0/81 | 100% |
| Brands | 1 BA (P = 6, S = 1, L = 3, r = 3) | — | 162 | 0/162 | 100% |
| | 2 BB (P = 1, S = 1, L = 3, r = 3) | — | 27 | 0/27 | 100% |
| | 3 BC (P = 1, S = 1, L = 3, r = 3) | — | 27 | 0/27 | 100% |
| | 4 BD (P = 3, S = 1, L = 3, r = 3) | — | 81 | 0/81 | 100% |
| | 5 BE (P = 2, S = 1, L = 3, r = 3) | — | 54 | 0/54 | 100% |
| | 6 BF (P = 1, S = 1, L = 3, r = 3) | — | 27 | 0/27 | 100% |
| Supermarkets | 1 SA (P = 6, S = 1, L = 3, r = 3) | — | 162 | 0/162 | 100% |
| | 2 SR (P = 1, S = 1, L = 3, r = 3) | — | 27 | 0/27 | 100% |
| | 3 SC (P = 3, S = 1, L = 3, r = 3) | — | 81 | 0/81 | 100% |
| | 4 SD (P = 1, S = 1, L = 3, r = 3) | — | 27 | 0/27 | 100% |
| | 5 SE (P = 2, S = 1, L = 3, r = 3) | — | 54 | 0/54 | 100% |
| | 6 SF (P = 1, S = 1, L = 3, r = 3) | — | 27 | 0/27 | 100% |
| Total commercial product’s sample | — | — | 378 | — | 378/378 | 100% |

“B” brand, “S” Supermarket, “L” Location, “r” for replicate and “—” denotes absence of target DNA.

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