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Mislabelling of beef and poultry products sold in Malaysia

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ABSTRACT

Meat species specification is important for consumer protection and increases concern in food labelling regulations enforcement. Although regulations exist for processed meat products, information on the prevalence of meat products mislabelling and regulatory compliance in Malaysia is lacking. In this study, 143 prepacked (beef and poultry) meat products (sausages, cold cut meats, cooked whole muscle meats, breaded products, meatballs and ground meats) were purchased from several national and international supermarket chains in Malaysia. These samples were analysed for the presence of common meat species (buffalo, cattle, chicken, goat, sheep, duck and goose) and meats prohibited by Islamic laws ("Haram") (cat, dog, monkey, pig and rat) using species-specific primers. The results showed that 112 (78.3%) samples were mislabelled, attributed by the false declaration of species and/or presence of undeclared meat species. The mislabelled products consisted of 17/28, 3/4, 6/8, 19/25, 48/56, and 19/22 of sausage, cold cuts, cooked whole muscle meat, breaded product, ground meat, and meatball samples, respectively. Buffalo DNA was detected in 40 out of the 58 samples labelled as beef. The presence of undeclared chicken and buffalo DNA were detected in 33/58 and 62/84 of beef and chicken products, respectively. The five "Haram" meat sources, however, were not detected in all meat products tested. The presence of chicken or buffalo DNA in these products could be attributed to unintentional cross contamination from food processing equipment, especially meat grinder, and lack of proper cleaning or inadequate hygiene. In conclusion, this study shows that majority of the samples are not legally compliant, signifying that substitution and mislabelling of meat products are commonplace in Malaysia. Strict implementation of the Malaysia Food Regulations 1985 alongside with regular surveillance and monitoring programmes are compulsory to alleviate and deter mislabelling issues.

1. Introduction

Meat species authenticity is a significant field of food forensics which ensures food quality and safety to the consumers and conserves the laws related to meat and meat products. Authentication is the process by which a food is verified together with its label description (Ashurst & Dennis, 2013). Briefly, meat authenticity evaluation will resolve numerous issues, such as fraudulent substitution of meats of high commercial value with inferior quality and cheaper meats (Cawthorn, Steinman, & Hoffman, 2013), addition of meat species which are not declared on the product label (Bottaro, Marchetti, Mottola, Shehu, & Pinto, 2014; Okuma & Hellberg, 2015) and accidental mislabelling of meat products (D'Amato, Alechine, Cloete, Davison, & Corach, 2013). Mislabelling attributed to fraudulent substitution is most likely intentional and economically motivated for lucrative profits (Everstine, Spink, & Kennedy, 2013). In some cases, these substitutions impinge on both economy and public health (Galal-Khallaf, Ardura, Mohammed-Geba, Borrell, & Garcia-Vazquez, 2014; Premanandh, 2013). Even when mislabelling may not impact food safety and public health, there is the concern that consumers will be deceived into believing that they are getting the product they desire (D'Amato et al., 2013). Amaral, Santos, Melo, Oliveira, and Mafra (2014) reported that the much preferred Alheira sausages made from game meat were adulterated or substituted with less valuable cow meat. According to them, this constituted mislabelling.

The presence of undeclared meat species in most cases may be intentional, but in some instances the presence of undeclared meat species might be unintentional such as cross contamination from equipment during processing. Cross contamination of meat products from processing equipment's can occur in manufactur-

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ing plants where more than one species is processed due to the improper or insufficient cleaning of equipment. This may lead to an unintentional mislabelling issue (Bottaro et al., 2014). Intentional or unintentional, consequences of mislabelling incidents in meat products cause a loss of consumer's trust in the food supply chain, as well as the regulatory bodies. Detecting less desirable and objectionable species in meat products is of paramount importance for economic, health, religious and ethical reasons. For instance, according to Muslim dietary laws (Halaal) and Jewish dietary laws (Kashrut), consumption of pork is prohibited (Schröder, 2003), while for the Hindu, consumption of beef and beef products are also prohibited (Bonne & Verbeke, 2008). Proper labelling and correct declaration of meat species in commercial meat products are crucial to ensure fair trade, freedom of choice and compliance with legislation.

Meat safety and quality related issues, especially mislabelling and malpractices in the food chain, have attracted public scrutiny due to increased awareness among consumers in getting correct information and their demand for transparency. Hence, meat authenticity and traceability from production to consumption should receive the highest priority from the governments, both national and international regulatory agencies. Meat authentication can be performed by physical, chemical, histological, anatomical, biological and molecular analysis (Sentandreu & Sentandreu, 2014; Singh & Neelam, 2011). Molecular techniques such as PCR have become indispensable for meat authentication (Vlachos, Arvanitoyannis, & Tserkezou, 2013). Recently, DNA-based species identification techniques have received wider acceptance because of their reliability, superior stability and universality of DNA in all tissues and cells (Ali, Razzak, & Hamid, 2014). Furthermore, it tends to be more specific, sensitive and applicable even to heat processed products (Cheng, He, Huang, Huang, & Zhou, 2014; Ulca, Balta, Çağın, & Senyuva, 2013). Numerous publications are focussing on advances in detection techniques, publications on food fraud or mislabelling incidents, however, are limited. This study is the first to report a mislabelling incident in meat products sold in Malaysia.

The objectives of this study were to detect the presence of species substitution, cross contamination by other species in various commercial meat products and to determine the extent of mislabelling of these products. Common meat species (buffalo, cattle, goat, sheep, chicken, duck and goose) and "Haram" meat species prohibited under Islamic laws (pig, cat, dog, monkey and rat) were tested by utilising species-specific primers to investigate the incidence of mislabelling and substitution in commercial meat products sold in Malaysia.

2. Materials and methods

2.1. Samples

One hundred forty-three raw and processed prepacked meat consisted of 84 samples labelled as "chicken", 58 samples labelled as "beef" and one sample labelled as "duck", produced by different manufacturers (consisting of 44 different brands) were purchased from various national and international supermarket chains in Malaysia. The meat products were categorized into six categories, sausage (n = 28), cold cuts (4), cooked whole muscle meat (8), breaded products (25), ground meats (56) and meatballs (22). All meat products were transferred to the laboratory within 1 h in cooler containing ice (4 °C) and stored at −20 °C until analysis. The species declared on the packaging of each sample was recorded for subsequent results interpretation. Fresh cut beef from imported Brazil cattle, Pakistan water buffalo (hereafter referred to as buffalo), chicken, duck and pork were purchased (500 g each) and served as positive controls.

2.2. DNA extraction

Approximately 10 g of meat was taken from several different areas from each sample and the sample was minced using a knife. Dedicated chopping board and knife were used for each sample to avoid cross contamination. DNA was extracted from each sample by using GF-1 Tissue DNA Extraction Kit (Vivantis, Malaysia) according to manufacturer's instruction. The concentration and purity of extracted DNA were determined by measuring absorbance at 260, 280 and 230 nm using the Multiskan^{TM} GO Microplate Spectrophotometer (Thermo Scientific, USA). The SkanIt^{TM} Software version 3.2 (Thermo Scientific, USA) was used to perform calculations to measure the data, where the concentration of dsDNA and its purity were calculated from absorbance at 260 nm and ratio of absorbance at 260/280 nm and 260/230, respectively.

2.3. Multiplex PCR amplification and detection

All meat products were tested for the presence of seven common meat species, namely buffalo (*Bubalus bubalis*), cattle (*Bos taurus*), goat (*Capra hircus*), sheep (*Ovis aries*), chicken (*Gallus gallus*), duck (*Anas platyrhyncos*) and goose (*Anser albifrons*). In addition, detection for the presence of cat (*Felis catus*), dog (*Canis lupus familiaris*), monkey (*Macaca fascicularis*), pig (*Sus scrofa*) and rat (*Rattus rattus*) which are forbidden meat species in Islamic foods was conducted as all these products were certified halal by the Department of Islamic Development Malaysia (JAKIM). All multiplex PCR amplification reactions were performed with a TProfessional Standard Gradient 96 Thermocyler (Biometra, Germany) using species-specific primers. Twenty-five microliter PCR reaction mixture contained 50 ng of purified DNA in 1 × Taq buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 1 U Taq DNA polymerase (Promega, Madison, USA) and species-specific primers (Integrated DNA Technologies, Singapore) of respective concentrations. Each PCR run included a no-template control, positive control DNA and a negative control DNA. Three separate multiplex PCR amplifications were performed for identification of meat species.

The first multiplex PCR for the detection of buffalo, cattle, goat and sheep was performed as described by Zarringhabaie, Pirany, and Javanmard (2011), using 0.4 μM for each of the primers. PCR conditions were as follow: initial denaturation at 94 °C for 3 min, 35 cycles of denaturation at 94 °C for 45 s, annealing at 62 °C for 1 min, extension at 72 °C for 45 s, and a final extension at 72 °C for 10 min. The expected sizes of specific PCR amplicon for mitochondrial cytochrome b gene (*cytb*) of buffalo, goat, cattle and sheep were 124, 330, 472 and 585 bp, respectively. The second multiplex PCR for the detection of chicken, duck and goose was conducted as described by Hou et al. (2015), using 0.4 μM of chicken- and duck-specific primers and 0.8 μM of goose-specific primers. The mitochondrial 12S rRNA, *cytb* and D-loop genes of chicken, duck and goose, respectively, were targeted. PCR was conducted as follow: initial denaturation at 95 °C for 5 min, 35 cycles of denaturation at 95 °C for 30 s, annealing at 56 °C for 30 s, extension at 72 °C for 45 s, and a final extension at 72 °C for 5 min. The expected sizes of PCR amplicons were 131, 238, 387 bp for the chicken, duck and goose, respectively. Multiplex PCR for the detection of five forbidden meat species in Islam was performed as described by Ali et al. (2015), using 0.4 μM for each of the primers. Three mitochondrial genes were targeted in this multiplex PCR, NADH dehydrogenase subunit 5 (*ND5*) for detection of pig and monkey, ATPase subunit 6 (*ATPase 6*) for dog and rat, and *cytB* for cat. Thermocycler settings were as follow: initial denaturation at 94 °C for 3 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 59.5 °C for 30 s, extension at 72 °C for 30 s, and a final extension at 72 °C for 5 min. The expected sizes of PCR amplicons were 108, 129, 141, 163 and 172 bp for rat, monkey, pig, dog and cat,