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Development of a PCR assay for the detection of dog meat adulteration in mutton

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In many developing countries the food analyst is confronted with the difficult task of providing proof of fraudulent substitution of more expensive meats with cheaper meats. Recently there was a disclosure about adulteration of beef products with equine meat which had become a global problem affecting some large global companies and international food brands. The Sri Lankan food market can also be affected due to the importation of processed beef products (e.g. McDonalds). Dog and cat meat in food and animal feed is prohibited according to the European meat hygiene law, for ethical and medical reasons. Molecular methods facilitate accurate and more reliable analysis of meat adulteration. Polymerase Chain Reaction (PCR) is a potent method to amplify specific DNA sequences, with high sensitivity. The aim of this study was to establish a qualitative PCR method to detect dog meat adulteration in mutton. Cytochrome b (Cyt b) gene is often used to compare multiple phylogenies and therefore the diversity of the Cyt b gene has been used to detect the source of meat. DNA was extracted from mutton and dog meat samples as well as mutton contaminated with 1%, 5% and 20% of dog meat. Another set of meat samples containing the same composition of dog meat and mutton was boiled for 45 minutes in water and DNA was extracted. Primers were designed to amplify a 809 bp region of dog Cyt b gene and a 157 bp region of goat Cyt b gene. After performing the PCR assay, the products were electrophoresed on a 2% agarose gel containing 0.5 µg/ml ethidium bromide and visualized under UV transe-illuminator. The results indicated that adulteration as low as 1% of dog meat could be detected using the PCR assay. A clear relationship was observed between the intensity of the PCR product and percentage of dog meat indicating that it could be used as a semi quantitative method as well. The results also show that this assay is valid for both raw and cooked (boiled) meat samples with about the same sensitivity. Furthermore, dog specific primers were checked with chicken, swine, beef, and goat cytochrome b gene sequence in NCBI using blast tool, and also a PCR was performed in order to verify the specificity. The dog specific primers did not amplify chicken, swine, beef, and goat DNA while goat specific primers did not amplify other DNA. Thus, the primers used in the assay ensure exclusion of false positive results. Non template controls were included in each assay to confirm the absence of contaminations. A qualitative PCR based method for detection of dog meat adulteration in mutton has been developed in this study which can also be used for both raw and cooked meat.

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