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Detection of Meat Products Adulteration by Polymerase Chain Reaction (PCR) Assay in Kalubia Governorate, Egypt

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Abstract

Environmental forensic, food quality and safety regulatory agencies are interested in meat products adulteration due to they are very rich source of proteins, containing all the essential amino acids and other nutrients for human. The detection of meat products adulteration is necessary for legal, economic, religious, and public health reasons. Lately, there are increasing concerns regarding for adulteration of meat products with that of other species as pork, dog, and donkey species. So, the aim of this study is using Polymerase Chain Reaction (PCR) assay in detection of adulteration of (Burger, Kofta, Lunshon and Native sausage samples) by pork, equine and dog meat species, samples survey was collected from Kalubia governorate markets. We found that some raw kofta and some Native sausage samples were adulterated by dog meat by 33.3% and 66.7% respectively. While other samples were free from adulteration.

Keywords: Polymerase Chain Reaction (PCR) assay; Meat products adulteration by pork; Equine and dog meat

Introduction

Food adulteration is a worldwide important for legal, economic, religious, public health or medical reasons and inconsistent with federal or state standards [1-3]. Processed meat refers to meat that has been turned out through salting, fermentation, drying, smoking or other processes to improve flavor or preservation, as luncheon, kofta, burger, and native sausages [4]. Generally, meat products adulteration included substitution or addition of animal proteins of inexpensive costs or plant proteins as soybean, forgery weights of definite ingredients [5]. In Islam, foods containing pig, donkey and dog sources are Haram for Muslims to consume. Therefore, food control laboratories have an essential mission in carrying out species differentiation of raw materials to be applied for industrial food production and the recognition of animal species in food products [6]. Burger is a common meat product made of ground red meat, especially bovine beef as raw material, which are eaten by many people around the world. However, adulterants may also replace some other unreported types of meat.

Kofta meatballs are usually formed by emulsifying fine ground meat with starch of some kind, mixing salt and particular herbs, finally molding into balls [7]. Luncheon and sausages are important industrial meat products that are considered as the most acceptable food products, widely consumed and used for fast meats. Commonly it is consisting of fine-cleaved meat and lipids with or without some additional cereals, cooked with salt and nitrite and heat treated [8]. Intended mixing of meat products with other animals' meat other than declared have been reported in several countries including Egypt, such as the mixing of the donkey and dog meat instead of pure beef meat [9]. Furthermore, adulteration involves the addition of meat or inclusion of cartilage or bones, which have been treated to be like something pleasant [10], in addition that United States determined that food adulteration usually refers to noncompliance with health or security standards (Food and Drug Administration "FDA", 2000). Different analytical techniques that based on protein analysis have been applied for meat products fraud identifications are not specific enough, so we must fine anther method as DNA based methods that are more reliable, more specific methods for detection of meat species adulteration, fast and inexpensive [11-13]. Real-time PCR is a highly

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Table 1: Primers sequences, target genes, amplicon sizes and cycling conditions.

Target gene	Primers sequences	Amplified segment (bp)	Primary	Amplification (35 cycles)			Final extension	Reference
			Denaturation	Secondary denaturation	Annealing	Extension		
Porcine 12S Rrna-tRNA Val	CTACATAAGAATATCACCCAC	290	94˚C	94˚C	52˚C	72˚C	72˚C	Tasara et al. 2005
	ACATTGTGGGATCTTCTAGGT		5 min	30 sec	30 sec	30 sec	7 min	
Equine mtDNA	CCC TCA AAC ATT TCA TCA TGA TGA AA	359	94˚C	94˚C	60˚C	72˚C	72˚C	Maede, 2006
	GCT CCT CAA AGG GAT ATT TGG CCT CA		5 min	30 sec	40 sec	40 sec	10 min	
Dog cytB	GGAGTATGCTTGATTCTACAG	808	94˚C	94˚C	52˚C	72˚C	72˚C	Abdel-Rahman et al. 2009
	AGAAGTGGAATGAATGCC		5 min	30 sec	40 sec	40 sec	10 min	

sensitive, preferred method for quantitative DNA analysis. Unlike conventional PCR, which measures products at the end of the reaction, RT-PCR quantifies DNA by fluorescent emissions released throughout the reaction during each amplification cycle. The most useful RT-PCR assays are those that use fluorogenic molecules specific for the target amplicon and will only emit a fluorescent signal as a result of directly or indirectly binding to the target. The most specific RT-PCR does not entail post PCR processing, as the results are achieved throughout the reaction [14-16]. The advantage of DNA-based analysis includes the ubiquity, abundance and stability of DNA in all cell reported to be useful targets for species identification of foods. However, methods based on DNA amplification are still preferred, as they are less affected by industrial processing [17,18]. Mitochondrial DNA (mtDNA) molecules coupled polymerase chain reaction represents a rapid, sensitive and an extremely specific substitute to protein-based methods [2]. PCR has established to be a valuable tool for the determination of diminutive amounts of different species, even in complicated foodstuffs [2,19]. Many targets can instantaneously be amplified by the usage of traditional multiplex PCR, which aids in detection of many species in a short period of time [20,21]. Doosti et al. [22] revealed that 7.58% of the whole samples were including Halal (lawful or allowable) meat with another meat. These results showed that molecular methods as PCR and PCR-RFLP are potentially trustworthy techniques for recognition of meat kind in meat foodstuffs for Halal authentication. So current study aimed to identify species adulteration, substitution of meat products by using Polymerase Chain Reaction (PCR) technique, using species-specific primers as it is highly sensitive and potentially reliable technique.

Material and Methods

Samples collection

A total of 48 different commercial beef meat products, 5 gm of (Beef luncheon, Raw kofta, Egyptian sausage and Beef burger) 12 of each were randomly collected from markets various regions in Kalubia Governorate, Egypt. All samples were transported to the laboratory under refrigeration, and were immediately processed or stored frozen at -20ºC for the next steps. Also, one sample of fresh raw pork, dog and donkey meat was provided to be used as positive control.

Samples preparation

DNA extraction: DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 25 mg of the sample was incubated with 20 µl of proteinase K and 180 µl of ATL buffer at 56ºC overnight. After incubation, 200 µl of AL buffer was added to the lysate, incubated for 10 min at 72ºC, and then 200 µl of 100% ethanol was added to the lysate. The lysate was then transferred to silica column, centrifugated. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer supplied in the kit.

Oligonucleotide primer: Primers used were provided from Metabion (Germany) are scheduled in Table 1.

PCR amplification: Primers were applied in a 25- µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentrations, 4.5 µl of water, and 6 µl of DNA template. The reaction was done in an applied biosystem 2720 thermal cycler.

Analysis of the PCR products: The products of PCR were split by electrophoresis on 1.5% agarose gel (AppliChem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5 V/cm. For gel analysis, 15 µl of the products was filled in each gel slot. Gel pilot 100 bp DNA ladder (Qiagen, gnbh, Germany) and Gene ruler 100 bp ladder (Fermentas, Germany) were used to establish the fragment sizes. The gel was snapped by a gel documentation system (Alpha Innotech, Biometra) and the data was evaluated through computer software.

Results and Dissection

Amplification with species-specific oligonucleotide primers revealed a 290, 359 and 808 bp from pork, equine and dog genomic DNA, respectively (Figures 1-3).

Table 1 and 2 showed that raw kofta and Egyptian sausage samples were adulterated by dog meat by 33.3% and 66.7% respectively while other species were not detected in all samples. It is very important for food hygienic purposes and lawful authentication detection of meat species adulteration, substitution of meat products which was reported from different countries such as Canada, Australia, United Kingdom and Egypt [23-27], by adding various kinds of meats to species-specific meat product to add bulk or make up the size of the product, fraudulent replacements of lower valued or low priced meat species may replace higher valued meat species. Meat and meat products are very susceptible to decomposition and also expensive as compared to other food types. Therefore, their structure and quality has always been concerned. Among the techniques used for species identification, PCR is a DNA based technique allowing the detection of very low amounts of nucleic acid probes and the determination of their sequence via the amplification of DNA or RNA individual strains. This method has some advantages such as high sensitivity

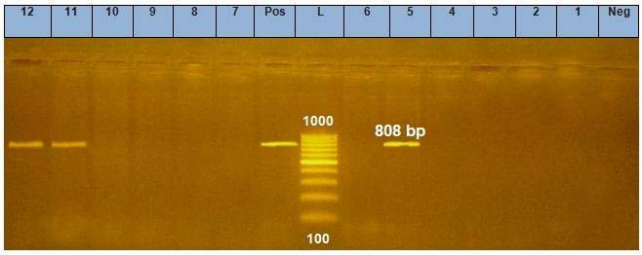

Figure 1: Agarose gel electrophoresis of PCR amplicon (343 bp) showing Dog adulteration in samples No. 5, 11 and 12 at lanes 2, 3, 4, 6, 7, 9 and 13. Lane M, 1kb plus DNA ladder.

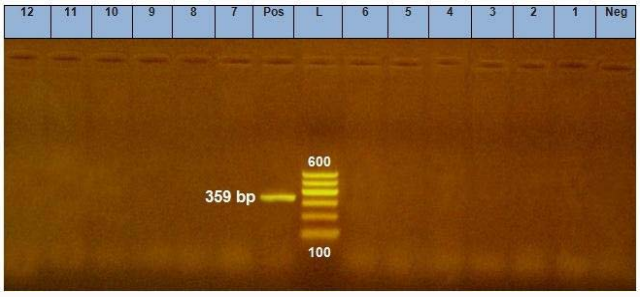

Figure 2: Agarose gel electrophoresis of PCR amplicon (343 bp) showing negative Equine adulteration in samples at lanes 2, 3, 4, 6, 7, 9 and 13. Lane M, 1kb plus DNA ladder.

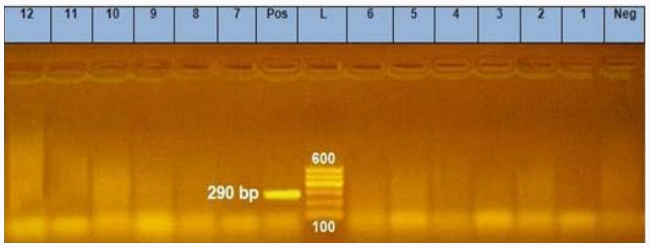

Figure 3: Agarose gel electrophoresis of PCR amplicon (343 bp) showing negative Pork adulteration in samples at lanes 2, 3, 4, 6, 7, 9 and 13. Lane M, 1kb plus DNA ladder.

and rapid performance with high sample numbers. Mitochondrial Cytochromeb gene and 12S rna can be used for species.

Specific PCR technique Wintero et al. [28] compared immunodiffusion, immune electrophoresis, isoelectric focusing, and DNA hybridization for determining species of meat. They concluded that DNA hybridization was more reliable and sensitive than other methods, though it was complicated and time-consuming. Similarly, the high cost and complexity associated with this technique have been reported by other researchers [29,30]. We found that kofta and native sausage were adulterated by dog meat by 33.3% and 66.7% respectively which in accordance with Yosef et al. [31] established that eight luncheon meat (72.7%) and 6 (54.5%) of sausage samples were adulterated with adding of other meat varieties, the author added that only one sample of luncheon and sausage was mixed with addition of pig meat. Also, Abbas et al. [32] stated that 6 (8.82%) out of 68 fermented sausages were found to include Haram (illegal or prohibited) meat. They added that molecular methods as PCR are potentially reliable techniques mitochondrial DNA segment (cytochrome-b gene) for detection of meat type in meat products for Halal authentication. In this regards, Abd El-Naseer et al. found that 57% and 66.7% of examined minced meat and sausage samples were adulterated with addition of other meat, out of which 35.7% and 41.7% were adulterated with pork meat, while 7% and 8% were adulterated with donkey meat, respectively. Zahran and Hagag were performed on Egyptian meat products identified as 100% beef, using PCR-RFLP technique, showed 12% adulteration. Donkey, sheep, and goat meat were the major contaminating varieties in a partial agreement of the current results. In the same line Ahmed et al. [33] noticed a higher adulteration ratio with PCR than gel immunodiffusion method in the beef burger with chicken at 69%, in raw kofta with pork at 45.5% and donkey at 18% in a similar Upper Egyptian locality. Species-specific PCR in another study done in Suez Canal cities in Egypt (Mosaad 2017) showed recognition of sheep, chicken and equine species in 80%, 50% and 10% in that order of analyzed oriental sausage samples besides the deficiency of beef meat in 20% of samples. Beef luncheon samples were found combined with chicken in 70% and equine species in 10% of specimens. Furthermore, beef burger meat products were mislabeled with chicken species in 100% of samples in addition to 30% adulteration with equine species. Results of that current study were in line to the present finding though with higher percentages of adulteration. Moreover, the global researches were parallel to the current findings regarding chicken and equine adulteration although with variable ratios in oriental sausage, beef luncheon and beef burger samples [21,34,35]. Also, Sakalar and Abasiyanik [36] declared that 40% of the commercially labeled meat products were adulterated with different meat species which were not referred in their labels. These meat products which contain fewer suitable species may cause health risk and species recognition is becoming a frequent and valuable practice [37,38]. Species identification of meat and meat products is important because of health, ethical, and economic reasons. Gada et al. found that the total beef samples examined showed 87.5% adulteration and mislabeling with one or more species. They were frequently mixed with chicken meat or their by-products (72.5%) tracked by donkey (12.5%) and finally human (2.5%) that was noticed in a manually prepared Kofta sample Khalid et al. reported that PCR application on 96 beef meat and meat product samples assembled haphazardly from street vendors and prominent retail markets (24 of burger, 16 of minced meat, 24 of kofta, 16 of sausage, 7 of raw meat and 9 of luncheon) exposed 6 positive for donkey tissue (3 from

Table 2: Incidence of adulteration of beef luncheon, Raw kofta, Sausage and Beef burger samples by using PCR sample number (48 samples 12 of each product).

Species	Pork meat			Equine meat			Dog meat		
Samples	Sample No	positive	%	Sample No	positive	%	Sample No	positive	%
Beef luncheon	12	-	-	12	-	-	12	-	-
Raw kofta	12	-	-	12	-	-	12	4	33.3
Native sausage	12	-	-	12	-	-	12	8	66.7
Beef burger	12	-	-	12	-	-	12	-	-

sausage, 2 from minced meat and 1 from kofta) and 2 positive for horse tissue (from sausage). This elementary PCR strategy efficiently recognized adulteration of raw and treated beef meat specimens with horse and donkey tissue. This work also focuses on the seriousness of the meat adulteration challenge in Egypt.

Conclusion

We concluded that these results might be useful for effective control of adulterated consumer meat products and violations of labeling requirements for meat products. PCR species determination can also be used to monitor meat adulteration for any meat products.

The Governments should apply more restricted laws through various religious, political, educational and scientific bodies to create an awareness program for its citizens with regard to the religious and aspects of the food being consumed. It is essential to employ this technique by quality control laboratories for regular evaluation of meat fraud in a quick and reliable manner.

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