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REVIEW ARTICLE

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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 were 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.

Key words: 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 (Frank and Hahn 2003, Mafra et al. 2008; Abbas et al. 2018). 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 (WHO 2015). Generally, meat products adulteration included substitution or addition of animal proteins of cheaper prices or plant proteins like soybean, forgery weights of actual constituents (Dooley et al. 2004). In Islam, foods containing pig, donkey and dog sources are Haram for Muslims to consume. Hence, it is an important task for food control laboratories to be able to carry out species differentiation of raw materials to be used for industrial food preparation and the detection of animal species in food products (Luo et al. 2008).Burger" is a popular meat product consumed by many people all over the world which is prepared from ground red meat, particularly beef as raw material. However, some other undeclared types of meat may also be substituted as adulterants. Kofta meatballs are commonly produced by emulsifying fine ground meat with starch of some sort, mixing salt and certain herbs, finally shaping into balls. (Purnomo, 1990). Luncheon and sausages are important industrial meat products that are considered as the most acceptable food products, widely consumed and used for fast meats. It is usually consist of finely chopped meat and fat with or without some added cereals, cured with salt and nitrite and heat processed (Ranken, 1984). 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 (Zahran and Hagag 2015). Furthermore, adulteration includes the introduction of meat or inclusion of cartilage or bones, which have been treated to resemble something palatable (Pointing and Teinaz 2004), in addition that United States determined that food adulteration usually refers to noncompliance with health or safety 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 fined anther method as DNA based methods that are more reliable ,more specific methods for detection of meat species adulteration, fast and inexpensive (Girish et al., 2005; Jia-qin et al., 2008; Yin et al., 2009). Real-time PCR is a highly 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. Highly specific RT-PCR does not require post PCR processing, as the results are obtained throughout the reaction (Zeitler et al., 2002; Huang and Pan, 2004 and 2005). 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 (Edris et al., 2012; Pascoal et al., 2005).Mitochondrial DNA (mtDNA) molecules coupled polymerase chain reaction represents a fast, sensitive and highly specific alternative to protein-based methods (Mafra et al., 2008). PCR has demonstrated to be a useful tool for the determination of minute amounts of different species, even in complex foodstuffs (Fajardo et al., 2008; Mafra et al., 2008). By using conventional multiplex PCR; many targets can simultaneously be amplified, which helps in detection of many species in a short period of

time (Bai et al., 2009; Ghovvati et al., 2009; Girish and Nagappa, 2009). Doosti et al., (2011) indicated that 7.58% of the total samples were containing Halal (lawful or permitted) meat and have another meat. These findings showed that molecular methods such as PCR and PCR-RFLP are potentially reliable techniques for detection of meat type in meat products 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 in various regions in Kalubia Governorate Egypt. All samples were transported to the laboratory under refrigeration, and were immediately processed or stored frozen at -20 °C for the next steps. Also, one sample of fresh raw pork,dog and donky meat was provided to be used as positive control.

Samples preparation

DNA extraction

DNA extraction from samples was performed using the QI Aamp 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, 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 provided in the kit.

Oligonucleotide Primer: Primers used were supplied from **Metabion (Germany)** are listed in table (1).

PCR amplification

Primers were utilized in a 25- µl reaction containing 12.5 µl of EmeraldAmp 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 performed in an applied biosystem 2720 thermal cycler.

Analysis of the PCR Products

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products was loaded in each gel slot. Gelpilot 100 bp DNA ladder (Qiagen, gnbh, Germany) and Generuler 100 bp ladder (Fermentas, Germany) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

RESULTS AND DISSCUSSION

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

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

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

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 Samples	Pork meat			**Equine meat**			**Dog meat**		
	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	-	-

Table (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.

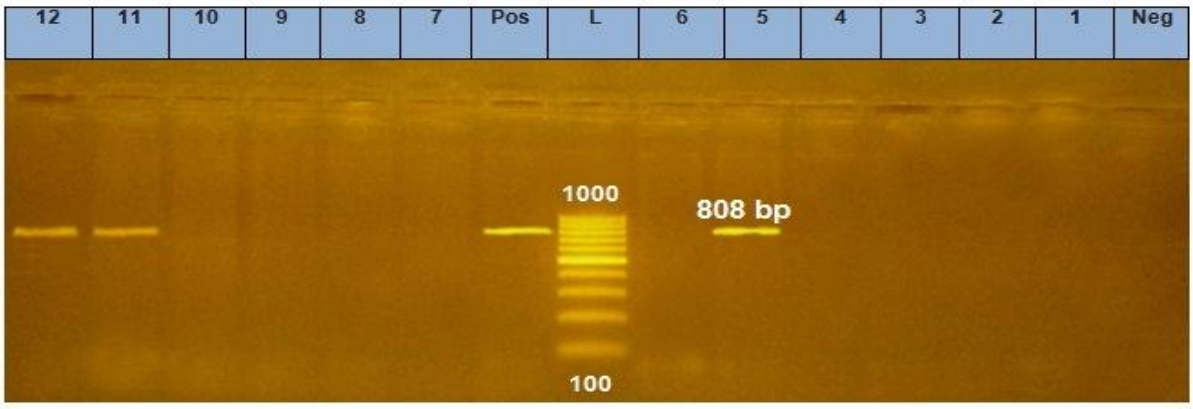

Figure 1. Agarose gel electrophoresis of PCR amplicon (343 bp) showing Dog adulteration in samples No. 5,11 and 12 at lanes 2, 3, 4,6,7,9 and 13. Lane M, 1kb plus DNA ladder.

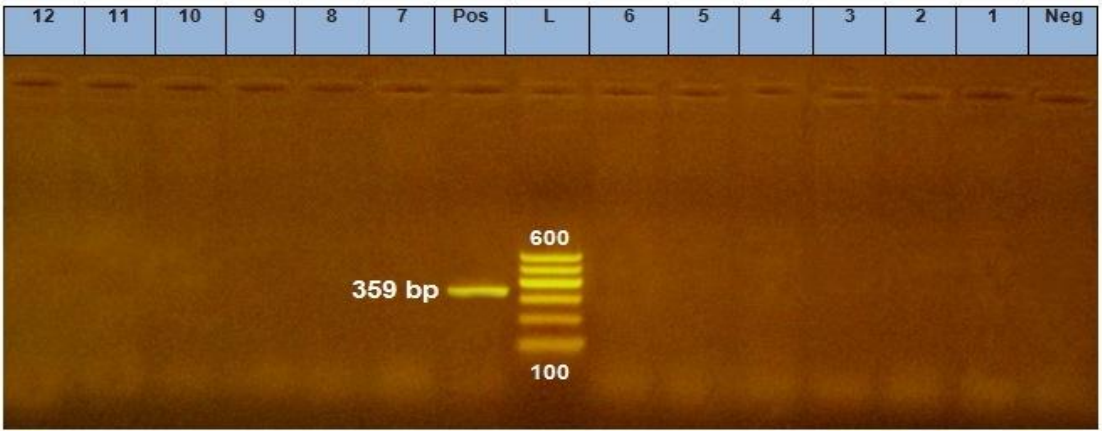

Figure 2. Agarose gel electrophoresis of PCR amplicon (343 bp) showing negative Equine adulteration in samples at lanes 2, 3, 4,6,7,9 and 13. Lane M, 1kb plus DNA ladder.

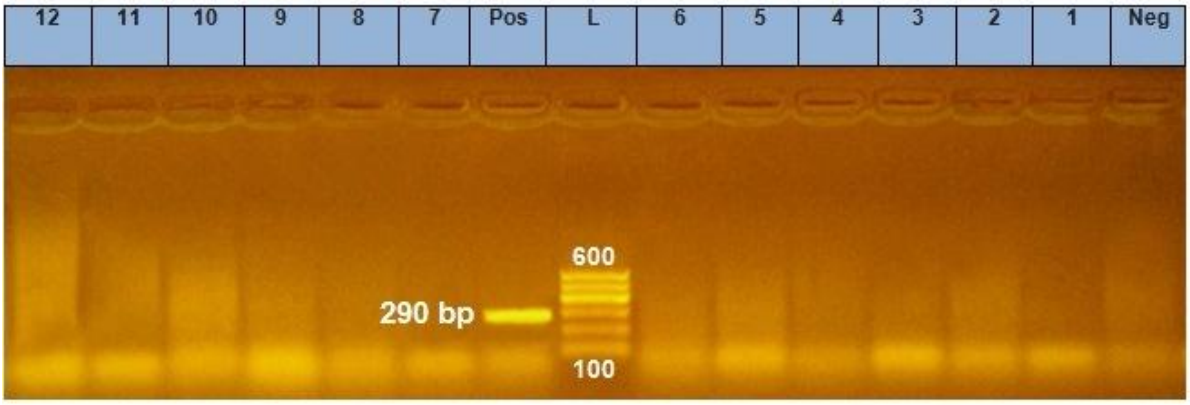

Figure 3. Agarose gel electrophoresis of PCR amplicon (343 bp) showing negative Pork adulteration in samples at lanes 2, 3, 4,6,7,9 and 13. Lane M, 1kb plus DNA ladder.

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 (Chemistry Center of Western Australia, 1999; MAFF, 1999, Odumeru, 2003; El-Sangary and Gabrail, 2006 and Abd El-Nasser et al., 2010), by adding different types of meats to species-specific meat product so as to add bulk or make up the volume of the product, fraudulent substitutions of lower valued or low priced meat species may substitute higher valued meat species. Meat and meat products are very susceptible to spoilage and also expensive as compared to other food types. Therefore, their composition and quality has always been interested. 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 and rapid performance with high sample numbers. Mitochondrial Cytochromeb gene and 12Srna can be used for specie. Specific PCR technique Wintero et al. 1990 compared immune diffusion, 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 (Meyer et al 1996 and Koh et al

1998). 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. (2014) found that eight luncheon meat (72.7%) and 6 (54.5%) of sausage samples were adulterated with addition of other meat types, the author added that only one sample each of luncheon and sausage was adulterated with addition of pig meat. Also Abbas et al. (2014) stated that 6 (8.82%) out of 68 fermented sausages were found to contain Haram (unlawful 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. (2010) 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 2015) was performed on Egyptian meat products labelled as 100% beef, using PCR-RFLP technique, revealed 12% adulteration. Donkey, sheep and goat meat were the main contaminating species in a partial agreement of the current results. In the same line Ahmed et al. (2011) detected higher adulteration rate with PCR than gel immune diffusion 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 performed in Suez Canal cities in Egypt (Mosaad 2017) revealed detection of sheep, chicken and equine species in 80%, 50% and 10% in that order of examined oriental sausage samples besides the absence of beef meat in 20% of samples. Beef luncheon specimens were found mixed with chicken in 70% and equine species in 10% of samples. Furthermore, beef burger meat products were mislabelled with chicken species in 100% of samples in addition to 30% adulteration with equine species. Results of that recent study were in line to the present finding though with higher percentages of adulteration. Moreover the international studies were parallel to the present findings as regards chicken and equine adulteration though with varying percentages in oriental sausage, beef luncheon and beef burger samples (Flores-Munguia et al. 2000; Ghovvati et al. 2009; Cawthorn et al. 2013). Also Sakalar and Abasiyanik (2011) 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 less desirable species may cause health risk and species identification is becoming a common and important practice (Ong et al., 2007 and Ali, 2008). Species identification of meat and meat products is important because of health, ethical, and economic reasons.Gada et al (2019) found that the total beef samples analysed showed 87.5% adulteration and mislabelling with one or more species. They were mostly mixed with chicken meat or their by-products (72.5%) followed by donkey (12.5%) and lastly human (2.5%) that was detected in manually prepared Kofta sample Khalid et al 2019 reported that PCR application on 96 beef meat and meat product samples gathered randomly 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 launcheon) uncovered 6 positive for donkey tissue (3 from sausage, 2 from minced meat and 1 from kofta) and 2 positive for horse tissue (from sausage). This basic PCR strategy effectively distinguished adulteration of raw and processed beef meat samples with horse and donkey tissue. This work also highlights on the severity of the meat adulteration problem 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 adultration 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 necessary to apply this technique by quality control laboratories for routine assessment of meat fraud in a rapid and reliable way.

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