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Animal species authentication in meat products using polymerase chain reaction technique

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ABSTRACT

Animal species authentication in meat products is crucial for ensuring food safety, quality and compliance with labeling regulations, it nvolves verifying the species origin of meat to prevent adulteration, fraud and ensure consumer trust. In this study Forty-five samples of meat products were collected, including 15 samples each from luncheon meat, sausage, and minced meat from different companies. DNA from each sample was extracted (using QIAamp DNA mini kit) directed to PCR using (according to Emerald Amp GT PCR master mix) and special Oligonucleotide primers for the cattle species (Beef cytochrome-b), the equine species (Equine mtDNA), the poultry species (Chicken cytochrome-b) and the pig species (Porcine 12S Rrna-tRNA Val). The results showed that all samples contained cattle species additionally 80% of the luncheon meat samples, 100% of the sausage samples, and 90% of the minced samples contained poultry species. PCR based method provide robust and reliable approach of authenticating animal species in meat products. Contributing significantly to food safety, quality assurance and regulatory compliance.

INTRODUCTION

Meat products, being the richest source of protein, provide the consumers with vital nutrients such vitamins, fatty acids, proteins, and trace minerals **(Uddin et al. 2021)**.There have long been concerns about the adulteration or substitution of meat for a variety of reasons, including wholesomeness, ethical and religious considerations, public health, and unhealthful competitiveness in the meat industry. Consequently, there should be a greater focus on the identification of animal species and foreign

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tissues in meat products, especially processed ones. Furthermore, In order to prevent fraudulent substitution of commercially valuable meat species with inferior, less expensive, or undesirable alternatives, the presence of undeclared species, the substitution of vegetable proteins for animal meat, and the accurate labeling of food, it is crucial to identify the authenticity of meat in meat products **(Ballin et al. 2009),** and to evaluate the composition of food and give consumers the information they need to ensure food safety **(Stamoulis et al. 2010).**

In numerous countries, regulations for food labeling mandate the declaration of the meat species used in processed meat products for consumers. This is necessary due to religious food ethics, medical reasons, and personal food preferences **(Doosti at al. 2014)**

Various analytical methodologies have been utilized in the identification of meat species, drawing from anatomical, histological, microscopic, organoleptic, chemical, electrophoretic, chromatographic, and immunological approaches. In this context, protein biomarkers specific to each species have been discovered through the application of electrophoretic and chromatographic methodologies **(Chou et al. 2007)**, or enzyme-linked immunosorbent assay (ELISA) **(Chen and Hsieh 2000)** and isoelectric focusing (IEF) **(Scarpeid et al. 1998)**. Moreover, the analysis by immunoassay, based on the use of antibodies raised against a specific protein, often presents cross-reaction with closely related species **(Meyer et al. 1994).**

However, due to their intrinsic constraints, the majority of these methodologies have been rendered obsolete by the more contemporary DNA-oriented molecular approaches. Over the past few decades, numerous techniques utilizing polymerase chain reaction have been suggested as efficient tools for ascertaining the source species in meat and its products, owing to their exceptional precision and sensitivity, along with prompt analysis duration and economic feasibility (**Sumathi, G. et al. 2015)**.

The authors exhibit high specificity, and sensitivity, and are distinguished by swift processing and economical pricing. Nevertheless, the occurrence of inhibitors in food, especially in meat items, may impede iriser binding and lower amplification effectiveness, resulting in a diminished level of sensitivity typically attainable through PCR when testing food samples **(Bottero 2003)**

Therefore, the proposed study aims to identification the animal species of meat (chicken, equine and pork) in meat products (minced meat, sausage, and luncheon), using PCR-based method.

2. MATERIALS and METHODS

2.1. Sample collection

A total of forty five meat products samples, including luncheon, sausage, and minced meat (15 samples of each) were collected from various retailers and markets in Beni-Suef Governorate. All samples were kept in a sterile plastic bag, and stored at -20 °C to prevent DNA degradation.

2.2. Extraction of DNA

It was performed according to QIAamp DNA mini kit (**Catalogue no.51304**) instructions. Briefly, 25 mg of the sample was incubated with 20 µl of proteinase K and 180 µl of ATL buffer at 56؛C overnight. After incubation, 200 µl of AL buffer was added to the lysate, incubated for 10 min at 72؛C, 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.

2.2. Cycling conditions of the primers during PCR

Temperature and time conditions of the primers during PCR are shown in Table (B) according to specific authors and Emerald Amp GT PCR master mix (Takara) kit.

2.3. DNA Molecular weight marker

The ladder was mixed gently by pipetting up and down. 10 µl of the required ladder were directly loaded.

2.4. Agarose gel electrophoreses

It was performed according to Sambrook **et al. (1989**) with some modification. Twenty µl of each PCR product samples, negative control and positive control were loaded to the gel. The power supply was 1-5 volts/cm of the tank length. The run was stopped after about 30 min and the gel were transferred to UV cabinet. The gel was photographed by a gel documentation system and the data was analyzed through computer software.

Table A. Oligonucleotide primers sequences and cycling conditions of the different primers during cPCR (Source: Midland Certified Reagent Company_ oilgos (USA)

Primer	Sequence5'-3'	Product size	Cycling conditions						Reference
			Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension	
Porcine 12S Rrna-tRNA Val	CTACATAA-GAATATCA CCCAC A C A T T - GTGGGATC TTCTAGGT	290 bp			52˚C 30 sesc.	72˚C 30 sec.			Tasara et al. 2005
Equine mtDNA	ccc tca aac att tca tca tga tga aa gct cct caa aag gat att tgg cct ca	359 bp			60˚C 40 sec.	72˚C 40 sec.			Maede. 2006
			94˚C 5 min.	94˚C 30 sec.			35	72˚C 10 min.	
Chicken cyto-chrome-b	GGGACACC CTCCCCCTT AATGACA GGAGGGCT GGAAGAAG GAGTG	266 bp			60˚C 30 sec.	72˚C 30 sec.			Doosti et al. 2014
Beef cyto-chrome-b	GCCATATA CTCTCCTTG GTGACA GTAGGCTT-GGGAATAG TACGA	271 bp			57˚C 30 sec.				

Table B. Preparation of PCR Master Mix according to Emerald Amp GT PCR master mix (Takara) Code No RR310 A kit

Component	Volume/reaction
Emerald Amp GT PCR mastermix (2x premix)	12.5 µl
PCR grade water	4.5 µl
Forward primer (20 pmol)	1 µl
Reverse primer (20 pmol)	1 µl
Template DNA	6 µl

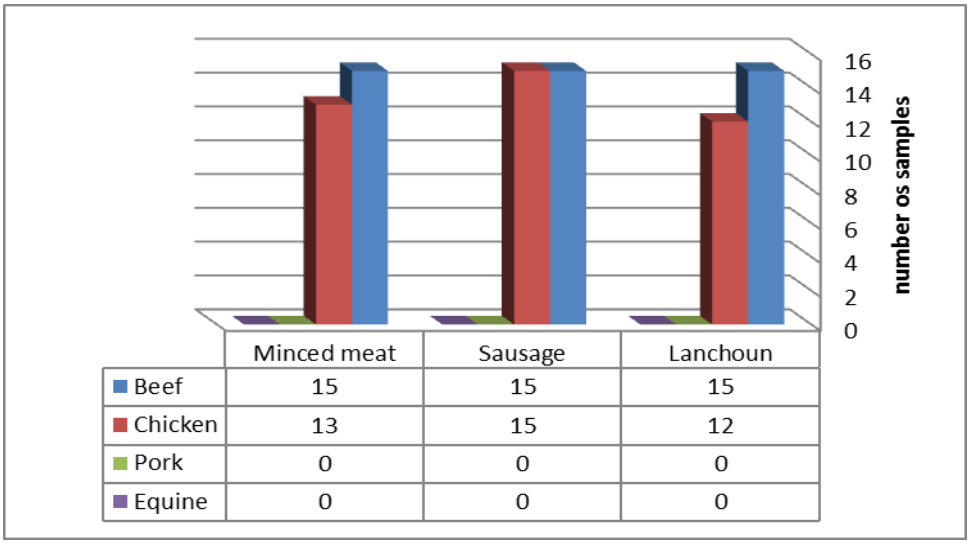
Results and discussions:

Application of PCR technique for detection of adulteration of such products was given, 12 samples of luncheon (80%) **(Figure 2)** and 13 samples of minced meat (86%) **(Figure 4)** were adulterated with chicken, while all samples (100%) of sausage were adulterated with chicken **(Figure 3)**. None of the examined product samples were adulterated with equine or pork meat **(Figure 2)**. All examined product samples were positive to cattle meat and the adulterated samples were intermixed with chicken meat. **(Figure 2, 3, 4)**

Table 1. Identified meat species in examined labeled beef products (n = 45).

Product	Number of positive samples to different meat species				% of mislabeling
	Pork	Equine	Chicken	Beef	
Luncheon (n=15)	0	0	12	15	80%
Sausage (n=15)	0	0	15	15	100%
Minced meat (n=15)	0	0	13	15	86%

Figure 1. Number of intermixed beef products samples with equine, pork or chicken meat



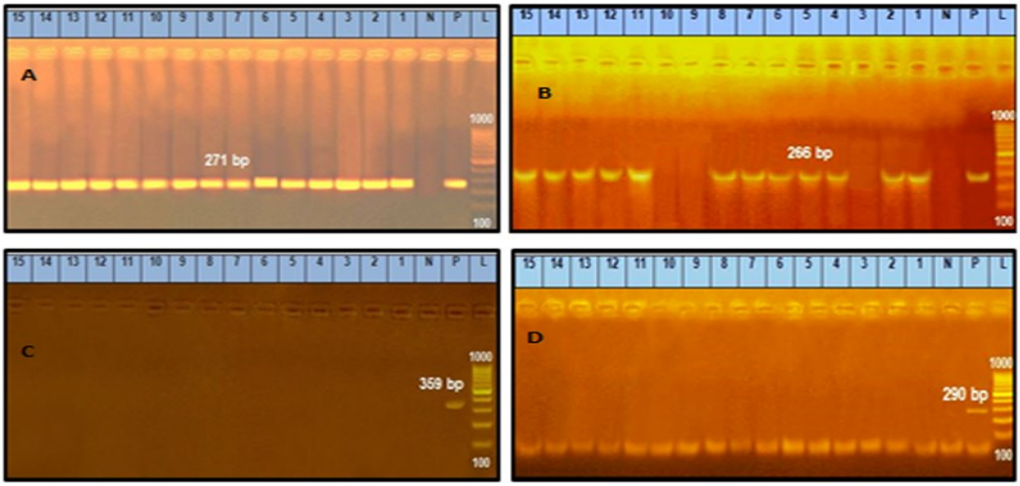


Figure 2 (A) Agarose gel electrophoresis of uniplex PCR of cyt b gene for cattle (271 bp) meat for demonstration of luncheon adulteration. Lane L: 100pb ladder. Lane P: control positive for of cyt b gene for cattle meat .Lane N: control negative.
(B) Agarose gel electrophoresis of uniplex PCR of cyt b gene for chicken (266 bp) meat for demonstration of luncheon adulteration. Lane L: 100pb ladder. Lane P: control positive for of cyt b gene for chicken meat. Lane N: control negative.
(C) Agarose gel electrophoresis of uniplex PCR of mtDNA gene for equine meat (359 bp) for demonstration of luncheon adulteration. Lane L: 100pb ladder. Lane P: control positive for of cyt b gene for equine meat. Lane N: control negative.
(D) Agarose gel electrophoresis of uniplex PCR of 12-sRNA gene for pork meat (290 bp) for demonstration of luncheon adulteration. Lane L: 100pb ladder .Lane P: control positive for of Rrna-tRNA gene for pork meat. Lane N: control negative.

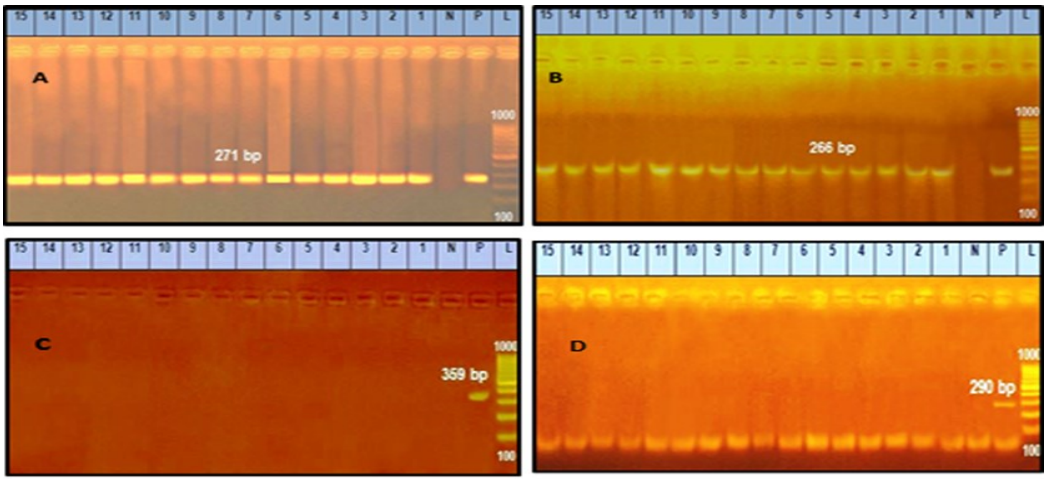


Figure 3 (A) Agarose gel electrophoresis of uniplex PCR of cyt b gene for cattle (271 bp) meat for demonstration of sausage adulteration. Lane L: 100pb ladder. Lane P: control positive for of cyt b gene for cattle meat. Lane N: control negative.
(B) Agarose gel electrophoresis of uniplex PCR of cyt b gene for chicken (266 bp) meat for demonstration of sausage adulteration. Lane L: 100pb ladder. Lane P: control positive for of cyt b gene for chicken meat. Lane N: control negative.
(C) Agarose gel electrophoresis of uniplex PCR of mtDNA gene for equine meat (359 bp) for demonstration of sausage adulteration. Lane L: 100pb ladder. .L ane P: control positive for of cyt b gene for equine meat. Lane N: control negative.
(D) Agarose gel electrophoresis of uniplex PCR of Rrna-tRNA gene for pork meat (290 bp) for demonstration of sausage adulteration. Lane L: 100pb ladder. .Lane P: control positive for of Rrna-tRNA gene for pork meat. Lane N: control negative.

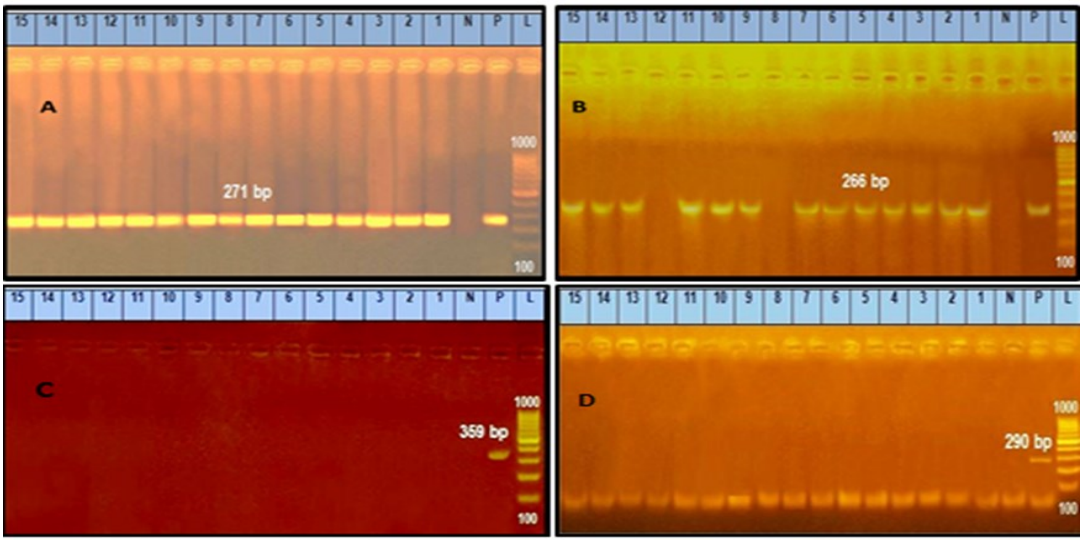


Figure 4 (A) Agarose gel electrophoresis of uniplex PCR of cyt b gene for cattle meat (271 bp) for demonstration of minced meat adulteration. Lane L: 100pb ladder. Lane P: control positive for of cyt b gene for cattle meat. Lane N: control negative.
(B) Agarose gel electrophoresis of uniplex PCR of cyt b gene for chicken (266 bp) meat for demonstration of minced meat adulteration. Lane L: 100pb ladder. Lane P: control positive for of cyt b gene for chicken meat. Lane N: control negative.
(C) Agarose gel electrophoresis of uniplex PCR of mtDNA gene for equine meat (359 bp) for demonstration of minced meat adulteration. Lane L: 100pb ladder. Lane P: control positive for of cyt b gene for equine meat. Lane N: control negative.
(D) Agarose gel electrophoresis of uniplex PCR of Rrna-tRNA gene for pork meat (290 bp) for demonstration of minced meat adulteration. Lane L: 100pb ladder. .Lane P: control positive for of Rrna-tRNA gene for pork meat. Lane N: control negative

According to **(Table 1, Figure 1)**, results revealed that 88.8% of the meat samples (40/45) were positive for chicken spp. The results of this study demonstrated a high rate of species fraudulence; as 80% of luncheon , 100% of sausage and 86% of minced meat had been adulterated with poultry tissues.

The obtained values of the current study were nearly similar to the results obtained by **Abuelnaga et al. (2021)** who examined 140 samples of meat products including sausage minced meat and beef luncheon (20 samples of each products) and results revealed that 20 (100%), 18 (90%), 18 (90%) of minced meat, beef luncheon and sausage samples were positive for addition of chicken tissue respectively**.** On the other hand, the results of the current study were greater than the findings of **Mehdizadeh et al. (2014)** and **Omran et al. (2019)**, which showed that undeclared chicken meat was present in 87.5% of all commercial meat items that were evaluated.

Poultry meat is less expensive in Egypt than beef meat products so it is common to mix meat products with poultry tissues such as skin, gizzard, cartilage and bone, which could lead to this kind of adulteration. This case is a clear example of the fraudulent substitution of a lower-value, less expensive meat species with a higher-value, more expensive meat species, indicating an economic fraud **(Abuelnaga et al. 2021).**

In the present study it was cleared that the adulteration rate of the meat samples by horse meat was (0%) which in accordance to **El-Shazly et al. (2016),** while it was lower than these results obtained by **El-Shewy (2007); Abd El-Nasser et al. (2010**); **Jaayid (2013); Zahran and Hagag (2015); Abd El- Razik et al. (2019); and Omran et al. (2019)**.

The findings of this study revealed that none of the meat samples tested positive for pig spp., aligning with the results of **El-Shazly et al. (2016)** and **Galal-Khallaf (2021).** How-

ever, these results were lower than the values reported by **Meyer et al. (1996), Partis et al. (2000), and Yosef et al. (2014).**

Additionally, **Abd El-Nasser et al. (2010)** discovered that 35.7% and 41.7% of examined minced meat and sausage samples were contaminated with pork meat.

Adulteration usually refers to noncompliance with health or safety standards **(FDA, 2000)**. The most significant issue with meat species adulteration has to do with religious beliefs because, in some faiths, like Islam, pork meat consumption is prohibited. Because it prevents unfair competition among producers and gives customers more options, food composition, and authenticity are becoming increasingly critical issues. Meat products must bear accurate labels that indicate their species content in accordance with EU labeling laws **(European Commission 2001)**. Numerous factors are taken into consideration when evaluating the quality of these items, including the dishonest substitution of lower-value meat for higher-value commercial meat **(Fajardo et al. 2008)**. While most protein-based techniques can identify the species of origin in raw meat, several authors have demonstrated that these techniques are noticeably less sensitive when evaluating foods that have undergone heat processing **(Rodriguez et al. 2004).** Polymerase chain reaction (PCR) represents a fast, sensitive, and precise alternative to protein-based methods **(Mafra et al. 2008)**. PCR has reported to be an accurate tool for the detection of minute amounts of different species, even in complex foodstuffs **(Fajardo et al. 2008; Mafra et al. 2008)**. Real-time PCR is the most used quantitative mtDNA-based method; however, in most laboratories, the implementation of this approach is still hindered by the high cost of the reagents and equipment. As an alternative, several multiplex PCR-based methods for quantitative analysis have been tried and approved. **(Mafra et al. 2007)**

CONCLUSION

PCR-based method is reliable and efficient method to ensure meat products integrity**,** prevent fraud and comply regulatory standards.

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