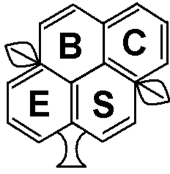

DETECTION OF MEAT PRODUCTS ADULTERATION WITH OTHER MEAT S' SPICIES

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Journal

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ABSTRACT

Species identification of animal tissues in meat products is an important issue to protect the consumers from illegal and / or undesirable adulterations for economic, religious and health reasons. So, the purpose of this investigation is to detect about undesirable meat species in commercial meat products by applications of polymerase chain reaction (PCR) in food analysis which have been developed based on DNA technology because of their rapid, simplify, specific and sensitivity.

In this study, PCR techniques were developed for detection of adulteration and identification of Ruminants, Pork, Poultry, Equines, Fish, Cat and Dogs s' meat species in examined meat products which were collected from different regions of Cairo and Giza governorates using specific primers. Both positive control (target DNA) of every one and the Marker (Mw) of all them are used, DNA fragments size of Ruminants, Pork, Poultry, Equines, Fish, Cat and Dogs s' meat species are 104, 290, 183, 359, 224, 672 and 808 bp.

DNA of Ruminants was detected in all samples with percentage 100%. On contrast, the DNA of Pork, Cat and Dogs were not detected in all previous samples. But, DNA of Poultry was detected in 77 samples with percentage 82% (approximately most of samples). Whereas, DNA of equines was detected in 54 samples with percentage 57% and then DNA of fish was detected in 45 samples with percentage 48% (approximately half of samples number).

INTRODUCTION

Bimolecular techniques have been extensively investigated as they offer undutiful advantages, such as having a high degree of specificity and being applicable even to heat processed products. Although DNA like proteins undergoes thermal denaturation. It has been observed that DNA can be still detected by short fragment amplification (**Meyer and Candrian 1996).**

Polymerase Chain Reaction (PCR) has been applied for the detection of different animal DNA fragments. **(Krcmar and Rencova 2001** and **Wang *et al.,* 2000)**. **Lahiff *et al.,* (2001)** developed a PCR to recognize ovine, porcine and poultry DNA in feedstuffs. **Myers *et al.,* (2003)** identified different species in feedstuffs using universal primers coupled with restriction end nucleases.

Bottero *et al.,* (2003) developed a method which involved the ability of primers to amplify wider target sequences. This PCR based assay demonstrated to be highly sensitive and useful in routine feedstuff analysis for the detection of all vertebrates.

Ahmed *et al.,* (2007) detect the different animal's meat, (buffalo, cattle, pig and sheep) used for species-specific analysis and RFLP for PCR products of mt-DNA cytochrome *b* gene to provide us with a simpler, quicker and cheaper alternative for sequencing to direct identification of meat animal's species.

A method utilizing PCR-restriction fragment length polymorphism (RFLP) in the mitochondrial genes was developed for beef, pork, buffalo, quail, chicken, goat, rabbit species identification and Halal authentication. PCR products of 359-bp were successfully obtained from the *cyt b* gene of these six meats. Enzymes were identified as potential restriction endonucleases to differentiate the meats **(Murugaiah *et al* 2009).** Also, **Farrokhi, and Joozani, (2011)** added that the identification of pork DNA in meat extracts is very important for Halal authentication and Muslim consumers demand protection from falsely labeled meat products. A pig-specific SYBR green I real-time PCR assay has been developed, using specific primers for pig mitochondrial DNA. Successful amplification has been obtained by DNA extracted from control meat samples

Spychaj *et al.,* (2009) demonstrated that PCR techniques, in combination with species-specific primers, PCR- -RFLP, PCR-SSCP and real-time PCR, allow identification of meat species occurring

independently or in mixtures with other meat species as well as meat subjected to thermal treatment or other technological processes in the course of industrial production. The results demonstrated that none of the samples were contaminated with porcine residuals, but 40% of sausages samples and 30% of cold cut samples were contaminated with poultry residuals. Also the ground meat samples were not contaminated with poultry residuals **Ghovvati *et al.,* (2009).**

Arslan, *et al.,* (2006) observed the effects of various cooking methods including boiling, roasting, pressure cooking, and pan frying on species determination of beef by PCR. The results indicated that with the exception of pan frying for 80 min, beef was determined in all meat samples including the broth and sauce of the roasted meat. The polymerase chain reaction (PCR) was applied to identify six meats (cattle, pig, chicken, sheep, goat and horse) as raw materials for products. Cattle, pig, chicken, sheep and goat fragments were amplified from cooked meat heated at 100 or 120°C for 30 min, but horse DNA fragments could not be detected from the 120°C sample. Detection limits of the DNA samples were 0.25 ng for all meats (**Matsunaga *et al.,* 1999**).

Lhak *et al.,* (2007) indicated that meat species (horse, dog, cat, bovine, sheep, porcine, and goat meat) was determined by the polymerase chain reaction (PCR) technique, using species-specific primers were accurately determined in all combinations by PCR. It is concluded that PCR can be useful for fast, easy, and reliable control of adulterated consumer meat products.

Karabasanavar, **(2011)** demonstrated that a highly species-specific polymerase chain reaction (PCR) assay was developed for the authentic identification of goat. Suitability of the developed goat species-specific PCR assay was confirmed for in raw, cooked (60, 80 and 100C for 30 min) and micro-oven-processed meat samples ($n = 20$ each). A sensitivity of 0.1% was established for detection of adulteration and limit of detection of goat DNA was 0.1 pg. This investigation presents a novel PCR assay with its newly designed primers that could be used for the authentic identification of goat species.

Unajak, *et al.,* (2011) apply the sensitive and specific method multiplex nested-PCR to identify commercial meat species. Different lengths of specific nested-PCR products were detected to be 350, 570, 750 and 1000 bp for chicken, pig, cow, and crocodile, respectively.

The system allowed detection with as little as 5 nanogram of DNA from either meat or blood sample. Detection sensitivity of individual species was improved, enabling the detection of DNA with as little as 1 pictogram. It was shown that the multiplex-PCR assay enhanced the sensitivity of routine species identification and allowed the use of blood as an alternative DNA source for detection.

Now the development of PCR technique makes easy to identify the meat species even from the cooked and spoiled meat in which protein is easily destroyed. Real time PCR is the revolution in this field in which we can identify and monitor the product during its amplification. Although no single technique is sufficient for differentiation of all types of meat species and meat products **(Singh, and Neelam, 2011).**

The aim of the present study was to:

1- Estimate the ability of species specific DNA primers (a) to detect the presence of beef, pork, chicken, fish, equine, cat and dog's DNA in meat products.
2- Estimate the effect of different processing techniques on the DNA extraction of beef sample.

MATERIALS AND METHODS

Samples:

94 meat product samples were collected from different societal level local markets, restaurants and fast food cars in twenty six regions across Cairo and Giza governorates during year 2010.

DNA extraction from the examined samples:

DNA extraction was performed using Prepman ultra sample preparation reagent. Applied Biosystem, USA, according to the used manual attached to the Kit as follows:

Twenty mg of each sample were transferred to 2ml microcentrifuge tube to which 400 µl of Prepman ultra sample preparation reagent was added. The tube then was incubated in a dry bath for 10min at 65C with frequent mixing. After reaching room temperature, the tube was centrifuged at 16000 rpm for 3min then 50 µl from the supernatant was transferred to a new labeled microcentrifuge tube.

Conventional PCR assay:

Primer sequences (table 3) were used to amplify the target genes of ruminants, pork, poultry, equines, fish, cat and dog DNA.

PCR amplification was performed in a final volume of 50 ml containing 75 mM Tris-HCL (Ph 8.8), 1 unit of Platinum Taq DNA Polymerase (Invitrogen, USA). 0.1mg/ml BSA (Roche Diagnostics GmbH, Mannhein, Germany), 0.2Mm each of dATP, dCTP, dGTP, dTIP (Pharmacia, Uppsala, Sweden), 2 Mm Mg Cl_2, 25 pmol of each primer and 250ng of DNA templates. Amplification was performed in a Thermal Circler Biometric (Applied Biosystems, CA) with the following cycling condition: 1- Thermal profile for Ruminants, Poultry, fish and pork after an initial heat denaturation step at 95 C° for 30 sec, 60 C° for 30 sec and 72 C° for 1 min. 2- Thermal profile for cat, dog and equines after an initial heat denaturizing step at 94 C° for 4 min, 35 cycles were programmed as follow: 95 C° for 1 min, 52 - 58C° for 1min, 72C° for 1 min and final extension at 72 C° for 10 min.

Following amplification, 10 µl of 50% sucrose solution were added to the PCR mixtures resulting in a total volume of 60 µl from which 25 µl were pipette into wells in 1.8% melting agarose (Fisher Scientific, USA). The PCR reaction samples were separated by horizontal gel electrophoresis (Hybaid, UK) and digital images were obtained using gel documentation system, USA **(Guan and Levin, 2002).**

Design of oligonucleatides of different animal species used in this study (Primers set b) according to **Dalmasso et al., (2004)** is shown in table (1).

Table (1) Oligonucleatides of different animal species (Primers set b):

Species	Gene	Primer sequence	Amplicon bp
Ruminants (beef, Buffalo and mutton)	16s rRNA	5- GAA AGG ACA AGA GAA ATA AGG -3 5- TAG GCC CTT TTC TAG GGC A -3	104
Pork	12s rRNA – tRNA	5- CTA CAT AAG AAT ATC CAC CAC A -3 5- ACA TTG TGG GAT CTT CTA GGT -3	290
Fish	12s rRNA	5- TAA GAG GGC CGG TAA AAC TC -3 5- GTG GGG TAT CTA ATC CCA G -3	224
Poultry	12s rRNA	5- TGA GAA CTA CGA GCA CAA AC 30 5- GGG CTA TTG AGC TCA CTG TT 30	183
Cat	SSR	5- CTC ATT CAT CGA TCT ACC AC -3 5- GTG AGT GTT AAA ACT AGT ACT AGA AGA -3	672
Dog	SSR	5- GGA GTA TGC TTG ATT CTA CAG -3 5- AGA AGT GGA ATG AAT GCC -3	808
Equines (horse and donkey)	Cytochrome-b	5- CCA TCC AAC ATC TCA GCA TGA TGA AA -3 5- GCC CCT CAG AAT GAT ATT TGT CCT CA -3	359

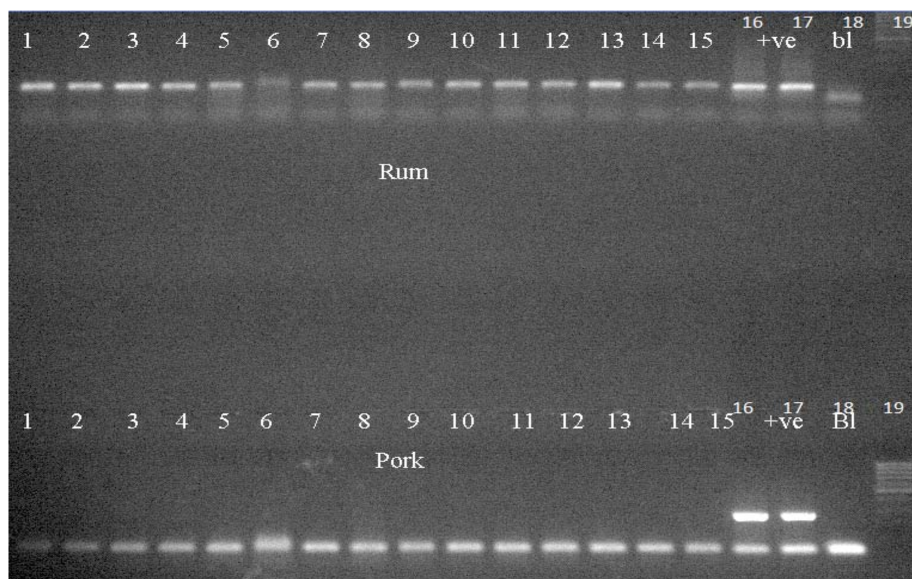
bp= base pair

RESULTS AND DISCUSSION

At present, a critical point concerning adulteration of meat and its products is represented by the reliability of the control tests. The low resolution efficiency of the microscopic method, which allows the detection of zoological classes but not of species, highlights the need for alternative analytical approaches

Technologies based on DNA analysis seem to fulfill this need. The present paper describes the application of PCR to detect ruminant, poultry, fish, pork, equine, dog and cat's species in meat products as shown in the following pictures from (1) to (6).

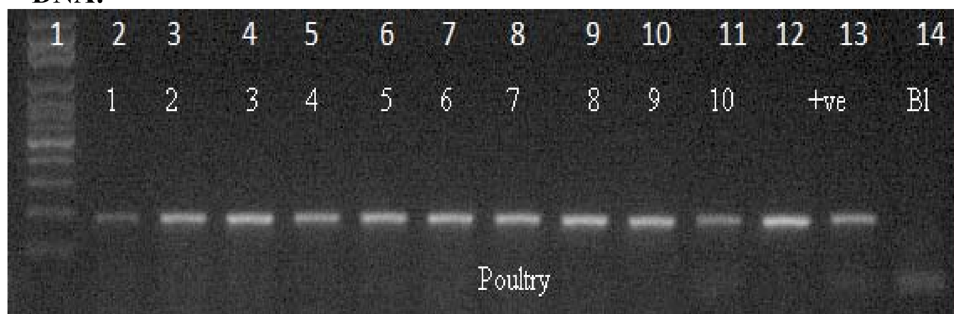
Picture (1) result of the examined samples for the detection of Ruminants DNA:



In the upper part, lane 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15 show Positive results (104 bp) of 15 representative ruminant samples, Lane 16 and 17 shows result of positive control sample (104 bp), Lane 18 shows result of a blank sample and Lane 19 shows the separation manner of 100 bp Marker.

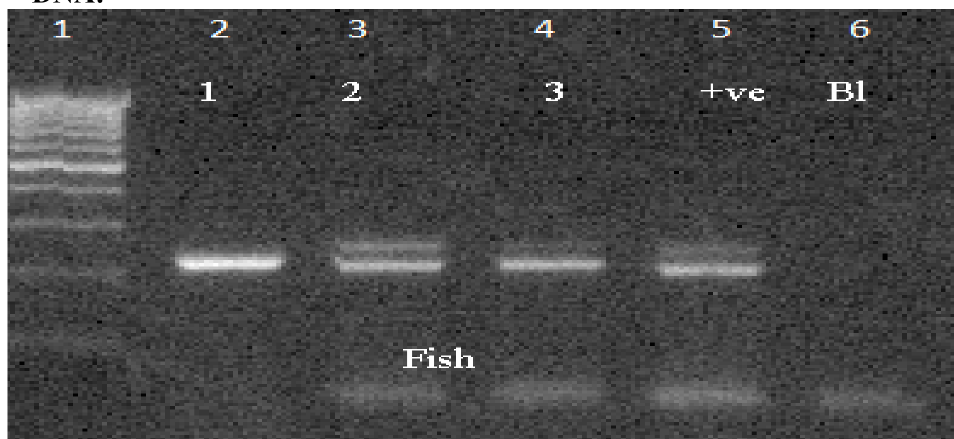
In the lower part, lane 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15 show negative results of 15 representative pork samples, Lane 16 and 17 shows result of positive control sample (290 bp), Lane 18 shows result of a blank sample and Lane 19 shows the separation manner of 100 bp Marker.

Picture (2) result of the examined samples for the detection of Poultry DNA:



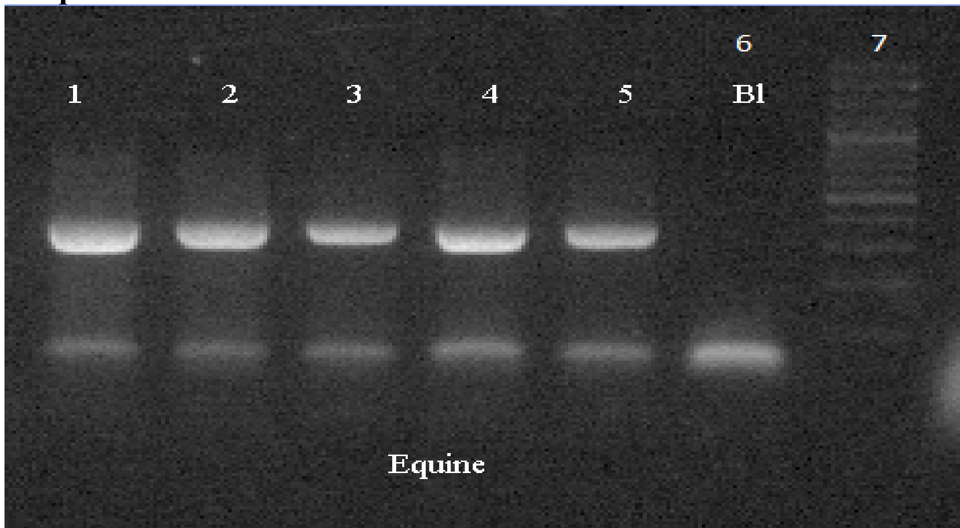
Lane 1 shows the separation manner of 100 bp Marker, lane 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 show positive results (183 bp) of 10 representative samples, lane 12 and 13 shows result of positive control sample (183 bp) and Lane 14 shows result a blank sample.

Picture (3) Result of the examined samples for the detection of Fish DNA:



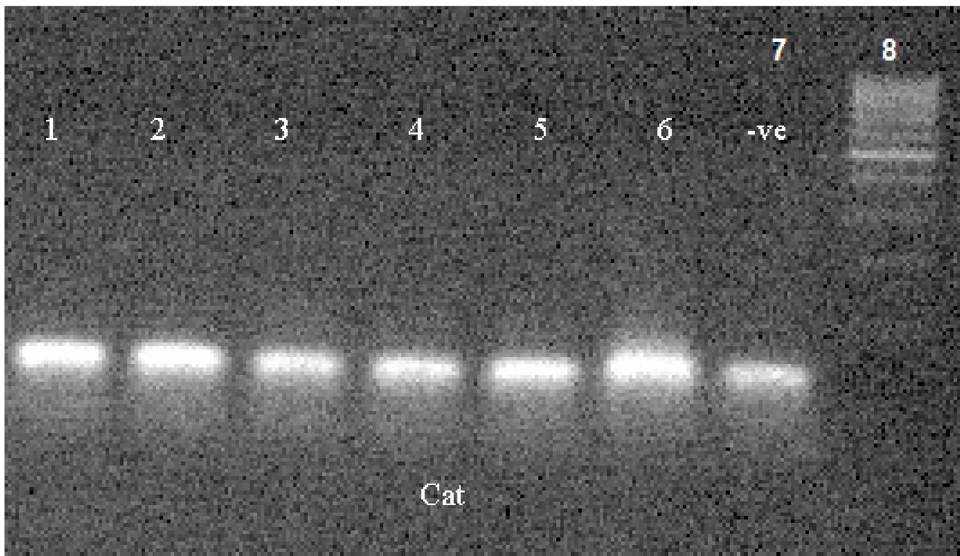
Lane 1 shows the separation manner of 100 bp Marker, Lane 2, 3 and 4 show positive results of 3 representative samples (224 bp), Lane 5 shows result of positive control sample (224 bp) and Lane 6 shows result of a blank sample.

Picture (4) Result of the examined samples for the detection of Equine DNA:



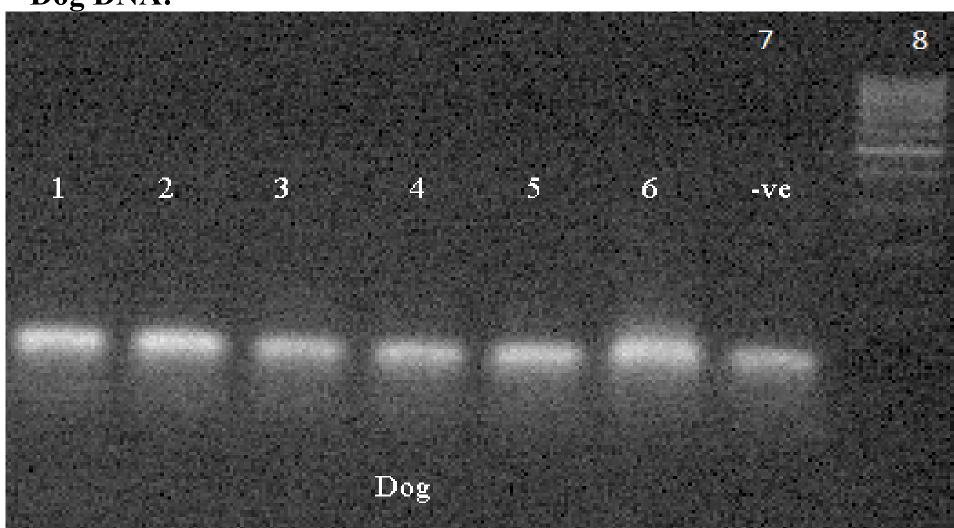
Lane 1, 2, 3, 4 and 5 shows Positive results of 5 representative samples (359 bp), Lane 6 shows result of a blank sample (359 bp) and Lane 7 shows the separation manner of 100 bp Marker.

Picture (5) Result of the examined samples for the detection of cat DNA:



Lane 1, 2, 3, 4, 5 and 6 show negative results of 6 representative samples (672 bp), Lane 7 shows result of negative control sample and Lane 8 shows the separation manner of 100 bp Marker.

Picture (6)) Result of the examined samples for the detection of Dog DNA:



Lane 1, 2, 3, 4, 5 and 6 show negative results of 6 representative samples (808 bp), Lane 7 shows result of negative control sample and Lane 8 shows the separation manner of 100 bp Mar

The collected meat product samples divided into to forty six and forty eight samples were purchased from twelve and sixteen regions in Cairo and Giza governorates respectively. The previous samples are distributed and illustrated in tables (2) and (3).

From data shown in table (2) it is indicated that all the samples contain ruminant's meat whereas, thirty nine adulated with poultry (85%), twenty samples adulated with equines (43%) and thirteen samples adulated with fish (28%). On contrast, all samples free from pork, dogs and cat's meat species from Cairo governorate. While, table (3) observed thirty eight samples adulated with poultry (79%),thirty four samples adulated with equines (71%) and thirty two samples adulated with fish (67%) from Giza governorate. Also, all samples contain ruminant's meat but free from pork, dogs and cat's meat species

Table (2) Tested meat product samples collected from different restaurants and fast food cars in Cairo governorate:

Samples name and their numbers		Source	Detected species by PC	Comment	Samples name and their numbers		Source	Detected species by PCR	Comment
Ground beef Kofta Shawarma	1 1 1	Helwan	1Ruminant + Poultry + fish. 2 Ruminant + Poultry.	Adulated with poultry and fish.	Hawawshi Sausage	2 2	Ramsis	2 Ruminant + Poultry. 2 Ruminant +Poultry + Equine	Adulated with poultry and equine
Ground beef Hawawshi	2 2	Ataba	2Ruminant + Poultry + Fish. 2Ruminant + Fish + Equine.	Adulated with poultry, fish and equine.	Kofta	7	El-Azher	4 Ruminant +Poultry + Equine 3 Ruminant +Poultry.	Adulated with poultry and equine
Kofta Hawawshi	2 1	Miser El-Adema	2Ruminant+Poultry. 1Ruminant+Poultry+Equine.	Adulated with poultry and equine	Sausage	5	El Husein	2 Ruminant +Poultry. 3 Ruminant + Poultry +Equine	Adulated with poultry and equine
Hawawshi	2	El-Mokatam	2 Ruminant +Poultry +Equine+ Fish.	Adulated with poultry, fish and equine	Sausage Kofta	2 4	El-Saida Zanab	2Ruminant+Equine. 1 Ruminant + Poultry +Equine 3Ruminant+Poultry.	Adulated with poultry and equine
Hawawshi Kofta	2 2	Madinet –Nasr	4 Ruminant +Poultry + Fish.	Adulated with poultry and fish.	Kofta Sausage	1 1	Bab - El-Sheria	1 Ruminant + Poultry +Fish. 1 Ruminant + Poultry + Equine + Fish.	Adulated with poultry, fish and equine
Hawawshi	2	Masaken El-Sheraton.	2 Ruminant +Poultry.	Adulated with poultry.	Hawawshi Kofta	2 2	Abo El-Reish	2Ruminant+Poultry+Equine. 2Ruminant+Poultry.	Adulated with poultry and equine

Table (3) Tested meat product samples collected from different restaurants and fast food cars in Giza governorate:

Samples names and their numbers		Source	Detected species by PCR	Comment	Samples names and their numbers		Source	Detected species by PCR	Comment
Kofta Shawarma	2 2	6-October	2 Ruminants. 2 Ruminants + Poultry.	Adulated with poultry	Hawawshi Sausage	2 1	Om El Masreen	3 Ruminants + Poultry + Equines + fish.	Adulated with poultry, fish and equine
Kofta Sausage	2 2	Embaba	4 Ruminants +Poultry +Equines +Fish	Adulated with poultry, fish and equine	Hawawshi Kofta	2 2	El Mahata street	4 Ruminants +Poultry +Equines +Fish	Adulated with poultry, fish and equine
Ground beef Hawawshi Sausage.	1 2 1	El-Dokki	1 Ruminants + Poultry + Equines + Fish. 1Ruminants +Equines +Fish. 2Ruminants +Fish.	Adulated with poultry, fish and equine	Sausage Kofta	2 1	Fasal	2 Ruminants +Poultry +Equines +Fish 1 Ruminants +Poultry	Adulated with poultry, fish and equine
Hawawshi Kofta	2 2	Bin-El-Sarauate	1 Ruminants +Equines +Fish. 1 Ruminants + Equines. 2Ruminants+poultry+Equine.	Adulated with poultry, fish and equine.	Hawawshi Shawarma	1 2	Al-Haram-	1 Ruminants +Poultry +Equines +Fish. 2 Ruminants +Poultry	Adulated with poultry, fish and equine
Hawawshi Sausage	2 2	El-Monieb	1 Ruminants + Fish. 1 Ruminants +Equines +Fish. 2 Ruminants +Poultry.	Adulated with poultry, fish and equine.	Hawawshi Sausage	2 1	Bolak El-Dakror	3 Ruminants +Poultry +Equines + Fish.	Adulated with poultry, fish and equine
Kofta	2	Giza square.	1 Ruminants +Poultry +Equines + Fish. 1 Ruminants +Equine +Fish.	Adulated with poultry, fish and equine	Hawawshi Kofta	2 2	El-Barageel	2 Ruminants +Poultry +Equines +Fish 2Ruminants+Poultry+Equines.	Adulated with poultry, fish and equine
Hawawshi Sausage	2 2	Jazerat El-Dahab	2 Ruminants +Poultry + Equine + Fish. 2Ruminants+Poultry+Equine.	Adulated with poultry, fish and equine	Hawawshi	2	Saft El-Laban	2 Ruminants +Poultry +Equines + Fish.	Adulated with poultry, fish and equine

It is resulted that the adulteration percentage in the Giza governorate is greater than for Cairo governorate especially with equines and fish's meat species. On contrast, the adulteration with poultry in Cairo governorate is greater than for Giza government.

The data obtained from this study agreed with that obtained by **Kiyoshi *et al.,* 2002** who used the same primer sequences in set (a) and ends with the same results ensuring its specificity and sensitivity. Also, **Higgins et al., 1992 and Dalmasso *et al.,* 2004** used the same primer sequence set (b) used in this study and came to the same conclusion (the specificity and the same level of sensitivity).

The negative effect of the heat treatment on DNA of beef sample agreed with that obtained from **Dalmasso *et al.,* 2004** who erased the advantage of relying on DNA based method over protein based methods as the protein can be destructed by heat treatments while DNA could resist destruction and can be detected even in short chains.

The PCR described in this paper proved to be very specific and sensitive, with a very low detection limit (0.05%) when DNA mixtures were tested. The same assay, applied on experimental mixtures of examined meat in vegetable, showed the same detection limit of the microscopic official method **(EU.C.1998).**

In conclusion, the PCR approaches proposed in this study can be considered as reliable and accurate methods for the control of food and /or feedstuffs. The test could be useful in the control of different products, and to verify the origin of the raw materials. Also, adulteration of any food or feed ingredients with any extraneous protein sources could be detected by this method of analysis.

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الكشف عن غش منتجات اللحوم بأصناف لحوم غير شرعية

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**معمل سلامة الغذاء– المركز الإقليمى للأغذية والأعلاف – مركز البحوث الزراعية - جيزة- مصر.

أنتشر الفساد فى الأونة الأخيرة خاصة بعد إنتشار مرضى أنفلونزا الطيور و الخنازير، والإمربإعدامها مما أدى إلى لجوء بعض معدومى الضمائر لغش منتجات اللحوم بها إلى جانب إضافة أصناف لحوم أخرى غير شرعية مثل لحم الحميروالقطط والكلاب والسمك لرخص ثمنه.

تم تجمبع عينات منتجات اللحوم من محلات و مطاعم وعربات الأغذية سريعة التجهيز فى مناطق مختلفة المستوى الإجتماعى على مستوى محافظتى القاهرة والجيزة ثم تجهيزها والكشف عن الحامض النووى (DNA) لهذه الأصناف من اللحوم كل على حدا مقارنة بلحم المقترات المفروض تواجدها (البقرى أوالجاموس أوالخراف) بواسطة جهاز ال PCR .

أوضحت النتائج أن كل العينات إحتوت على لحم المقترات وخالية من بعض اللحوم غير الشرعية مثل لحم الخنزيروالقطط والكلاب فى حين أحتوت ٧٧ و ٥٤ و ٤٥ عينة من إجمالى عدد العينات بنسب تتراوح ٨٢ و٥٧ و ٤٨ % على لحم الطيوروالحمير والسمك على التوالى.