



## IDENTIFICATION OF MEAT SPECIES IN SOME MEAT PRODUCTS IN ASSIUT CITY

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### **ABSTRACT:**

Meat adulteration constitutes an important problem in Egypt. Adulteration of meat may occur by substitution of low priced or even banned meat species for that high priced one. In this study, agar gel immunodiffusion test (AGID) and polymerase chain reaction (PCR) techniques were applied for detection of meat adulteration. Meat extract from beef, chicken, pork and donkey were prepared. Hyperimmune sera were prepared in rabbits by subcutaneous injection of meat extracts and blood was collected to get the specific antisera. Positive results indicated by appearance of clear precipitation line between the antibody and the corresponding antigen with assurance that no cross reaction occurred between species. Two hundred samples from beef meat products (50 minced meats, 50 raw kofta, 50 sausages and 50 beef burger) were subjected to analysis by AGID technique. The incidence of adulteration of minced meat with each of chicken and pork were 6%. The rate of adulteration was 34% and 26% in raw kofta, 32% and 14% in sausage and 32% and 2% in beef burger, respectively. Donkey meat was detected only in beef burger at rate of 2%.

For application of PCR technique specific primers for chicken, pork and donkey meat species were prepared; their molecular weights were 420, 343, and 350 bp, respectively. Deoxyribonucleic acid (DNA) was extracted from tested samples for detection of the previous species in these tested samples. Out of suspected and negative adulterated samples examined by AGID technique, fifty samples were reanalyzed by PCR technique. By using PCR technique the adulteration rates with chicken were 57%, 63.7%, 66.7% and 69% in minced meat, raw kofta, sausages and beef burger, respectively. The adulteration rates with pork were 35.7%, 45.5%, 41.7% and 23% in minced meat, raw kofta, sausages and beef burger, respectively. The adulteration rates with donkey meat were 7%, 18%, 8% and 7.7% in minced meat, raw kofta, sausages and beef burger, respectively.

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### **INTRODUCTION:**

**Meat species adulteration means that meat products contain undeclared meat species; as a result, the meat ingredients are not consistent with the label. One possible reason of high**

**adulteration rate occurred in processed meat products is accidental contamination resulting from improper handling or processing. For instance, if the grinder is not cleaned before other meat is put through, ground meat will**

contain small amounts of the previous ground meat. Another reason is deliberate adulteration of processed meat products with inexpensive meat for economic gain, because it is more difficult to detect adulterant in cooked or ground meat than in fresh or intact meat. After grinding, heating, and/or curing processes which may cause the change of meat texture, color, appearance, or even flavor, the origin of meat is easily concealed in a meat mixture (Chemistry Center of Western Australia, 1999; MAFF, 1999; Odumeru, 2003 and Ayaz *et al.*, 2006).

The most important concern for consumers, scientists and governments may be the species-related disease which can be transmitted to human. A non-bacterial disease known scrapie is raising concern among people, because the occurrence and spread of bovine spongiform encephalopathy (BSE) is thought to be established by feeding cattle with scrapie-infected sheep tissues (Wilesmith *et al.*, 1988). It has been reported that both human new variant Creutzfeldt-Jakob disease (nvCJD) and BSE belong to the family of fatal TSE diseases, and they share the same infection mechanism, namely the abnormal prion protein (PrP<sup>sc</sup>), which aggregates in cytoplasmic vesicles in the brains of infected individuals and animals and is highly resistant to heat (Irani and Johnson, 2003). Several studies have reported that PrP<sup>sc</sup> failed to completely inactivate after treatment at 121°C for 60 min or after even more severe heat treatments (Brown *et al.*, 1990; Taylor *et al.*, 1994 and Appel *et al.*, 2001).

Reddy *et al.* (2000) raised antisera in rabbits using native and heated testicular antigens from cattle, sheep, goat, or buffalo. To overcome the problem of absorption to make the antisera monospecific, antisera were raised also in phylogenetically related species. Cutrufelli *et al.* (1993) and Canadian Council on Animal Care (2002) use preferably young adult female Specific Pathogen Free rabbits (about 3.5–4 kg). Allow it a minimum of 7 days of acclimatization after its arrival. Injection sites must be sufficiently distant to prevent coalescence of the local inflammatory response. Wait for 3–4 weeks period is necessary to build up a primary immunological response.

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 Huang and Pan, 2005).

## **MATERIALS AND METHODS:**

### **Collected samples:**

**Two hundreds beef meat products samples of minced meat, raw kofta, sausages and beef burger (50 of each) were collected from Assiut City retail markets during the year 2008, and analyzed for detection of meat adulteration.**

### **Preparation of Meat antigen:**

Antigens from beef, chicken, pork and donkey meats were prepared and kept frozen at -20 until used. Twelve female New Zealand white breed rabbits at 10–12 weeks old were subjected to examination for health signs, free from any abnormalities, vaccinated with bacterial and viral vaccines before the experiment. Rabbits were divided into 4 groups according to the number of antigens used. Three rabbits were used for each group and 3 rabbits as control. Rabbits were immunized for production of the target antisera.

### **Meat Extraction and Antigen Preparation:**

Preparation of antigens was adopted after the method of USDA-FSIS, (2005). Meat was cut into small pieces and mixed with saline (NaCl 0.85%) at volume 1:3. Stomaching 1-2 min. was done and stands for 90 minutes. Filtration through whatman paper filters was applied. Samples were centrifuged and the supernatant was taken. Before immunization of rabbits the supernatant was filtered through bacteriological filter.

### **Species identification methods:**

#### **1-Agar Gel Immunodiffusion Test (AGID):**

Based on Ouchterlony method of Siklenka *et al.*, (2004), the Agar-gel immunodiffusion is notable for its qualitative ability to demonstrate similarities and resolve differences in related proteins based upon the formation of specific immunoprecipitin lines resulting from the diffusion of specific antigens and antibodies from wells or troughs cut into an agar matrix after they have reached their optimum proportions. As such, this procedure is ideally suited for meat species protein identification and the end point was the formation of specific immunoprecipitin lines resulting from the diffusion of meat extract and specific antiserum.

#### **2-Polymerase chain reaction method (PCR):**

Fifty samples (14 samples of minced meat, 11 of raw kofta, 12 of sausages and 13 of beef burger) were chosen from the suspected and negative adulterated samples examined by AGID to be reexamined by PCR.

**RESULTS:**

**Table 1: Incidence of adulteration of minced meat, raw kofta, sausage and beef burger samples examined by Agar Gel Immunodiffusion test (AGID)**

Species	Minced meat		Raw kofta		Sausage		Beef burger		Total	
	No	%	No	%	No	%	No	%	No	%
Beef	50	100	50	100	50	100	50	100	200	100
Chicken meat	3	6	17	34	16	32	16	32	52	26
Pork	3	6	13	26	7	14	1	2	24	12
Donkey meat	-	-	-	-	-	-	1	2	1	0.5

**Table 2: Incidence of positive, suspected and negative adulteration of minced meat samples examined by AGID**

Species	Positive		Suspected		Negative		Total
	No	%	No	%	No	%	
Beef	50	100	-	-	-	-	50
Chicken meat	3	6	2	4	45	90	50
Pork	3	6	19	38	28	56	50
Donkey meat	-	-	1	2	49	98	50

**Table 3: Incidence of positive, suspected and negative adulteration of raw kofta samples examined by AGID**

Species	Positive		Suspected		Negative		Total
	No	%	No	%	No	%	
Beef	50	100	-	-	-	-	50
Chicken meat	17	34	2	4	31	62	50
Pork	13	26	12	24	25	50	50
Donkey meat	-	-	9	18	41	82	50

**Table 4: Incidence of positive, suspected and negative adulteration of sausage samples examined by AGID**

Species	Positive		Suspected		Negative		Total
	No	%	No	%	No	%	
Beef	50	100	-	-	-	-	50
Chicken meat	16	32	3	6	31	62	50
Pork	7	14	7	14	36	72	50
Donkey meat	-	-	8	16	42	84	50

**Table 5: Incidence of positive, suspected and negative adulteration of beef burger samples examined by AGID**

Species	Positive		Suspected		Negative		Total
	No	%	No	%	No	%	
Beef	50	100	-	-	-	-	50
Chicken meat	16	32	3	6	27	54	50
Pork	1	2	26	52	23	46	50
Donkey meat	1	2	3	6	46	92	50

**Table 6: Incidence of adulteration of minced meat, raw kofta, sausages and beef burger samples examined by PCR**

Species	Minced meat (14 samples)		Raw kofta (11 samples)		Sausage (12 samples)		Beef burger (13 samples)		Total (50 samples)	
	No	%	No	%	No	%	No	%	No	%
Chicken meat	8	57	7	63.6	8	66.7	9	69	34	68
Pork	5	35.7	5	45.5	5	41.7	3	23	18	36
Donkey meat	1	7	2	18	1	8	1	7.7	5	10

**Table 7: Comparative results of positive adulterated samples in minced meat examined by AGID and PCR**

Species	AGID (50 samples)		PCR (14 samples)	
	No	%	No	%
Chicken meat	3	6	8	57.1
Pork	3	6	5	35.7
Donkey meat	-	-	1	7.2

**Table 8: Comparative results of positive adulterated samples of raw kofta examined by AGID and PCR**

Species	AGID (50 samples)		PCR (14 samples)	
	No	%	No	%
Chicken meat	17	34	7	50
Pork	13	26	5	35.7
Donkey meat	-	-	2	14.3

**Table 9: Comparative results of positive adulterated samples of sausages examined by AGID and PCR**

Species	AGID (50 samples)		PCR (14 samples)	
	No	%	No	%
Chicken meat	16	32	8	57.1
Pork	7	14	5	35.7
Donkey meat	-	-	1	7.2

**Table 10: Comparative results of positive adulterated samples of beef burger with AGID and PCR**

Species	AGID (50 samples)		PCR (13 samples)	
	No	%	No	%
Chicken meat	16	32	9	69.2
Pork	1	2	3	23.1
Donkey meat	1	2	1	7.7

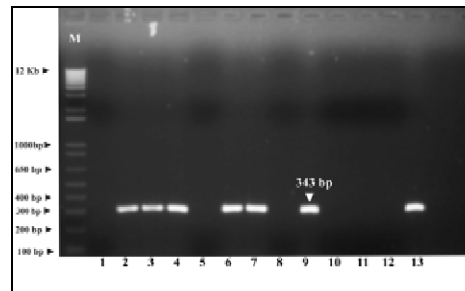
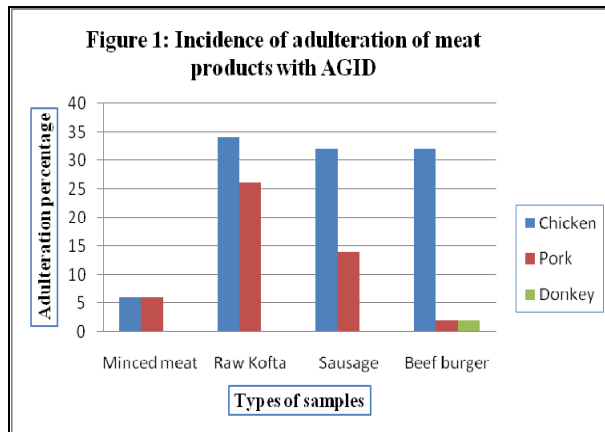


Figure 2: Agarose gel electrophoresis of PCR amplicon (343 bp) showing pork adulteration in samples No. from 1 to 13 at lanes 2, 3, 4, 6, 7, 9 and 13. Lane M, 1kb plus DNA ladder

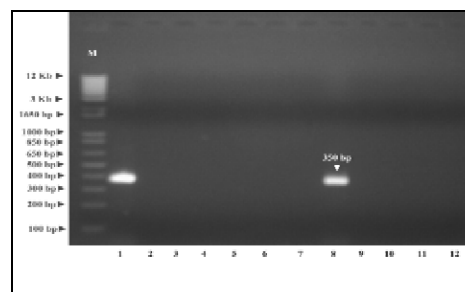
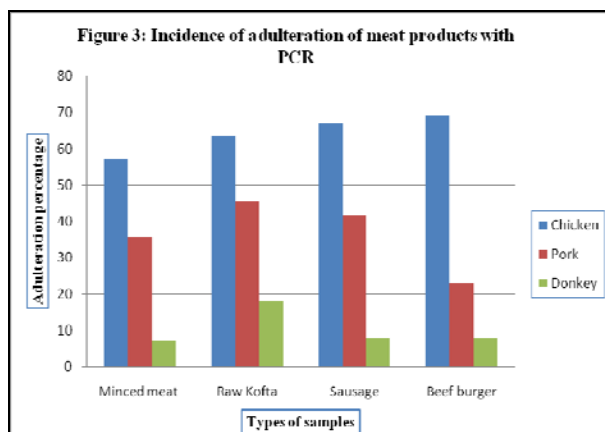


Figure 4: Agarose gel electrophoresis of PCR amplicon (350bp) showing donkey adulteration in samples No. from 1 to 12 at lanes 1 and 8. Lane M, 1kb plus DNA ladder

## DISCUSSION:

Meat species adulteration is a worldwide problem, which violates food labeling laws, constitutes economic fraud, and raises ethical, religious and food safety concern. Meat species adulteration, substitution or mislabeling of meat products has been 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). Food manufacturers or food processing factories may

add different types of meats to species-specific meat product so as to add bulk or make up the volume of the product. Low priced or lower valued meat species may substitute higher valued meat species. 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). Mixing of different species followed by grinding and/or heat-processing aids to the difficulties of discrimination of meat origin and limits the detectability of many analytical techniques.

Fraudulent substitutions of expensive meat with cheaper one or addition of undeclared species in meat products may cause concerns for consumer protection and other economic reasons. El-Shewy (2007) examined samples of kabab, grilled kofta and meat loaves and he found that equine meat was present in all samples.

This study aimed to evaluate the use of AGID and PCR for species specification of meat products. The AGID test was used also by Cordal de Bobbi *et al.*, (1985) for qualitative identification of fresh ground meat samples of beef, sheep, pork, horse and rabbit by the double agar gel diffusion test and by immune-electrophoresis. It has been proved that AGID is sensitive and specific without cross-reactivity with the other tested meat species. There was no apparent cross-reaction with any of the different tested proteins. Only the tested protein gives Ag-Ab reaction with the prepared antiserum.

AGID test was used in this study and found to be simple, inexpensive and rapid technique for species identification of meat and meat products in comparison to other techniques. AGID was applied on 50 samples of each of the following beef meat products; minced meat, raw kofta, sausage and beef burger for detecting the adulteration with chicken, pork and donkey meat as presented in Tables 1-5. The results of AGID test revealed that the adulteration rates for minced meat were 6% for each of chicken and pork as shown in Tables 1 and 2. The adulteration rates for raw kofta were 34% with

chicken and 26% with pork as summarized in Tables 1 and 3 and Figure 1. The adulteration rates in sausage were 32% for chicken and 14% for pork. In beef burger the adulteration rates were 32% for chicken and 2% for each of pork and donkey as presented in Tables 1 and 5.

Out of fifty samples from each product suspected and negative adulterated meat products samples examined by AGID technique, an alternative method based on conventional PCR analysis to confirm the results of the adulteration which recorded by AGID technique. The results of PCR showed that the adulteration rates for minced meat, raw kofta, sausages and beef burger with chicken were 57%, 63.6%, 66.7% and 69% respectively as presented in Table 6 and illustrated in Figure 3. The incidence of adulteration rates for minced meat, raw kofta, sausage and beef burger with pork were 35.7%, 45.5%, 41.7% and 23% respectively. While, the adulteration rates with donkey meat were 7% for minced meat, 18% for raw kofta, 8% sausages and 7.7% beef burger. Using of target DNA was successfully identified for each species tested as illustrated in Figures 2 and 4, and amplification was not affected by additives or processing. Also the presence of DNA from the other species did not affect the detection of target DNA's, similar observation was concluded by Kesmen *et al.* (2007). PCR analysis of species-specific mitochondrial DNA sequences is the most common method currently used for identification of meat species in food (Ahmed and Abdel-Rahman, 2007).



PCR products were examined for its specificity to meat species by identification of the corresponding species. The products showed species-specific DNA fragments of 420, 343 and 350 bp from chicken, pork and donkey meats respectively.

It was noticed that the sensitivity and accuracy of PCR in detection of species of meat and its adulteration greatly overcome potency of AGID test as present in Tables 7, 8, 9, and 10 as PCR depends on the detection of the specific DNA molecules which is a relatively stable allowing analysis of processed and heat treated food products (Beneke and Hagen, 1998). Failure of AGID to detect species adulteration may be attributed to addition of spices, salts and other ingredient (Hsieh *et al.*, 1996). Species identification in heat processed products is hindered by progressive denaturation of the protein markers, leading to loss of solubility and antigenicity (Hitchcock and Crimes, 1985). A well recognized drawback for PCR methods is that they are susceptible to contamination and thus delicate facilities and extreme caution is needed. On the contrast imunodiffusion techniques are suitable to be used as a field test.

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## التعرف على أنواع اللحوم الموجودة في بعض منتجات اللحوم في مدينة أسيوط محمود عبد الناصر على\*، حسين يوسف أحمد\*\*، دعاء محمد عبد العزيز\*\*

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أصبح الغش التجاري بكافة أنواعه ظاهرة عالمية واسعة الانتشار تستحق الاهتمام، ويعد غش اللحوم واحداً من أهم مشاكل الغش والتدليس التجاري التي يتعرض لها المجتمع المصري في الآونة الأخيرة، وذلك لعمل منتجات رخيصة الثمن والذي أصبح مشكلة هامة في مصر خاصة مع وجود الدخل المنخفض وارتفاع أسعار اللحوم بشكل ملحوظ. يعاني سوق اللحوم من ضعف الرقابة وانتشار الغش والتلاعب حيث يمكن أن يحدث غش اللحوم باستبدال اللحوم غالية الثمن بلحوم رخيصة الثمن أو ممنوعة. وكذلك يمكن خلط اللحوم الحمراء بلحوم رديئة أو غير صالحة للاستهلاك الآدمي، وذلك بخلطها مع بعضها البعض أو بفرمها وبيعها جاهزة لعمل بعض أنواع المأكولات خاصة في الأسواق الشعبية التي تكثر فيها اللحوم رخيصة الثمن. وهذا النوع من الغش يسبب الكثير من الأمراض مثل العدوى بالأمراض البكتيرية أو الفيروسية.

تم في هذه الدراسة جمع عدد 200 عينة من منتجات اللحم البقري من مدينة أسيوط خلال عام 2008م، وقد احتوت هذه العينات على 50 عينة من كل من اللحم البقري المفروم والكفتة النيئة والسجق البقري والبيف برجر. وقد تم إجراء كل من اختبار الترسيب خلال الطبقة الجلاتينية واختبار البلمرة للتعرف على غش اللحوم بيروتينات حيوانية من أنواع أخرى.

لتطبيق اختبار الترسيب في الطبقة الجلاتينية تم تحضير محلول ملحي لخالصة اللحوم لكل من لحوم البقر، لحوم الفراه، لحوم الخنازير ولحم الحمير. وتم تحضير الأمصال المضادة لهذه البروتينات بحقن الأرناب تحت الجلد بخالصة اللحوم المختلفة السابق ذكرها. ثم تم نزع الأرناب وجمع الدم لفصل الأمصال المضادة. تم اختبار الأمصال والمضادة للتأكد من خصوصيتها وفعاليتها مع التركيزات المختلفة. ظهرت النتيجة الايجابية في هذا الاختبار بوجود خط الترسيب الواضح بين المصل المضاد والمصل المقابل مع التأكد من عدم وجود تداخل في التفاعل بين الأنواع المختلفة.

تم تحليل جميع العينات موضع البحث بإجراء اختبار الترسيب خلال الطبقة الجلاتينية عليها. كان معدل الغش في اللحم البقري المفروم بكل من الفراه والخنزير 6% لكل منهما، وفي الكفتة النيئة 34%، 26%، في السجق 32%، 14% وفي البيف برجر 32%، 2% على الترتيب. أما لحم الحمير فقد وجد فقط في البيف برجر بنسبة 2%.

لتطبيق اختبار البلمرة تم تحضير بادئات مخصصة لكل من لحوم الفراه، الخنزير ولحم الحمير وكان الوزن الجزيئي لهم هو 420، 343، 350 زوج قاعدي على الترتيب. تم استخلاص الحمض النووي من العينات للتعرف على وجود الأنواع السابق ذكرها في هذه العينات أم لا.

اختيرت خمسون عينة من العينات التي كانت نتائجها سلبية أو مشكوك فيها مع تطبيق اختبار الترسيب خلال الطبقة الجلاتينية لكي يعاد تحليلها باختبار البلمرة. ويتطبيق اختبار البلمرة كان معدل الغش بلحوم الفراه بنسبة 57%، 63.7%، 66.7%، 69% في اللحم المفروم، الكفتة النيئة، السجق والبيف برجر على الترتيب. كان معدل الغش بلحوم الخنازير 35.7%، 45.5%، 41.7%، 23% في اللحم المفروم، الكفتة النيئة، السجق والبيف برجر على الترتيب. كان معدل الغش بلحوم الحمير 7%، 18%، 8%، 7.7% في اللحم المفروم، الكفتة النيئة، السجق والبيف برجر على الترتيب.

يخلص البحث إلى التوعية بعدم شراء اللحوم المفرومة أو المصنعة حيث أنه يسهل خلطها مع نوعيات رديئة من اللحوم، وكذلك عدم شراء اللحوم من مصادر غير موثوقة والابتعاد عن اللحوم مجهولة الهوية. كما يؤكد على أنه أصبح لزاماً تحرك المسؤولين وجمعيات حماية المستهلك لضبط حالات الغش والاستعانة بخبراء الطب البيطري للتكليف القانوني لهذه الحالات والتطبيق الحازم لقانون حماية المستهلك.

