



MODIFIED, INNOVATIVE METHOD FOR DETECTION OF ADULTERATION OF MEAT PRODUCTS WITH MEAT OF OTHER SPECIES

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Summary

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The aim of this study was to detect illegal adulteration of beef meat products with meat from other species. Samples (n=120) of industrial and handmade beef products were randomly collected from retail outlets in Assiut city, Egypt: raw beef burger, oriental beef sausage, beef kofta, and beef luncheon (30 samples each). Samples were analysed using agar gel immunodiffusion (AGID) and modified AGID (MAGID) assays. Results showed that 17.5% of examined products were adulterated with chicken meat. MAGID detected that 14.1% of samples were adulterated with donkey meat, whereas all AGID results were negative. Human tissue was detected in 8.3% (AGID) and 10% (MAGID) of examined samples. Histological examination was then used to detect foreign tissue, and all categories of products were found to be adulterated, and some of them – contaminated with human blood cells. Polymerase chain reaction analysis confirmed that MAGID was more accurate and sensitive than AGID, especially for false negative AGID results. Consumers are advised not to consume too much of the studied meat products to avoid exposure to adulterated or contaminated products that might constitute a health hazard.

Key words: beef meat, chicken meat, donkey meat, modified agar gel immune diffusion

INTRODUCTION

Consumers are increasingly concerned about the authenticity of the meat they eat. There has been a dramatic rise in the popularity of various meat products with a

long shelf-life and relatively low cost, allied with significant changes in lifestyles (Jiang *et al.*, 2020).

Meat and meat products are often subjected to counterfeiting, mislabelling, and similar fraudulent activities. The substitution of ingredients with meat from other animal species is one of the many forms of food adulteration (Pierina & Maria, 2021). Occasionally, high-value meat is adulterated with cheaper meats to add bulk or increase the volume of the product for economic reasons. Meat products adulterated with meat from less desirable species may threaten public health; therefore, species identification is becoming an important practice (Goyal *et al.*, 2022).

There are several techniques for meat species identification ranging from simple techniques based on morphology to sophisticated molecular techniques.

Immunological techniques are mainly based on the principles of antigen–antibody reactions. The specificity and simplicity of immunoassays make them suitable for regulatory purposes. Overnight on-site screening tests for species identification based on Ouchterlony's (1948) agar gel immunodiffusion (AGID) assay have been further developed by Cutrufelli *et al.* (1991, 1992). The AGID assay is relatively simple to perform but it requires more antibodies and a relatively long incubation time and has only moderate sensitivity (Hsieh *et al.*, 1996). We modified this test to increase sensitivity and accuracy, thereby reducing false negative results.

Polymerase chain reaction (PCR) methods are also used for meat species identification. PCR can exploit mitochondrial DNA (mtDNA) to increase test sensitivity further on the basis of the high copy number (approximately 2,500 copies) of mtDNA in cells against just a few copies of genomic DNA per cell. Therefore, mtDNA can be more efficient at detecting species-specific DNA (Teletchea

et al., 2005; Musto, 2011; Omran *et al.*, 2019).

Histological techniques can also be used to identify unauthorised tissues in processed meat products. They are relatively simple and inexpensive and can provide an economic tool for evaluating meat adulteration and ensuring hygiene and meat quality (Sohrabi *et al.*, 2020).

The aim of this study was to analyse beef products available in retail markets in Assiut, Egypt, for adulteration with chicken and donkey meats and human tissue using standard AGID, a novel modified AGID (MAGID) method and histological techniques, confirmed using PCR.

MATERIALS AND METHODS

Collection of samples

A total of 120 different industrial and handmade beef product samples were randomly collected from restaurants, butchers, hypermarkets, and local shops in various localities in Assiut city, Egypt (raw beef burger, raw beef oriental sausage, raw beef kofta, and beef luncheon; 30 samples from each product). In each group except for beef luncheon, 20 samples were industrial frozen samples and 10 – handmade chilled samples. All samples were wrapped.

Preparation of meat sample extracts

A sample (25 g) of meat product was mixed with 50 mL physiological saline and then subjected to stomaching for 1–2 min using a laboratory blender stomacher 400, rested for 1 h, and then filtered through filter paper (Hsieh *et al.*, 1995).

Preparation of antisera

Hyperimmune sera were prepared in rabbits via repeated subcutaneous injection of meat antigens. Female New Zealand white rabbits aged 8–12 weeks were clinically examined and confirmed as healthy before the experiment. The rabbits were divided into four equal groups according to the number of antigens used (at minimum two rabbits for each group and another group as control). Rabbits were immunised for production of the target antisera.

Preparation of meat antigens for immunisation

Antigens from beef and chicken and donkey meats and human placenta (as human tissue) were prepared according to methods of the USDA-FIS (2005) and kept frozen at -20°C until used.

Gel plate preparation

The method used followed that of Beard (1970), with some modifications. The Ouchterlony AGID assay was the best one of routine serological tests. In the present experiment, the agar gel was prepared as follows: one gram of agarose and 0.8 g of NaCl were dissolved in 100 mL of deionised distilled water in a sterile glass flask. The mixture was then boiled in a microwave oven for 3 min until it became clear. Approximately 20 mL of liquefied agarose was dispensed into 100×15 mm Petri dishes to a thickness of 2.8–3.0 mm. The plates were left to cool on a levelling table in a dust-free environment at room temperature ($30\pm 2^{\circ}\text{C}$) with the lids off to permit escape of water vapour. The lids were left off for at least 15 min but not longer than 30 min to avoid change in electrolyte concentration of the agar due to evaporation, which may have an adverse effect on the formation of precipitin lines. Once the gel had solidified, a tem-

plate cutter was used to cut wells (a 7-well pattern, with a center well surrounded by six evenly spaced wells). The wells were 5.3 mm in diameter, with the edges of the peripheral and central wells separated by 2.4 mm. Five patterns were cut into each gel plate. The agarose plugs were removed manually. Control wells were resealed using drops of agarose. The plates were used on the day they were prepared.

Agar gel immunodiffusion test (AGID)

The AGID method is based on the formation of specific immune-precipitin lines resulting from the diffusion of meat extract (antigens) and specific antisera (antibodies). The test was done as per Ouchterlony (1968): 1) Using a micropipette, approximately 50 μL of sample extract (antigen) was transferred to each peripheral well on the gel plate and the same volume of antibody (antiserum of target species) was placed into the central well (the wells being filled as near level as possible without overflowing); 2) Each plate was then covered and allowed to rest for a few minutes before moving to avoid the risk of spillage; 3) The plates were then incubated in a humid chamber at room temperature and examined daily for at least 48 h against light and dark backgrounds for precipitin lines.

Precipitin lines may be detected after approximately 48 h (Dennis, 2010). The interpretation of the results was done as follows: 1) The Petri dish lid was removed, and the plates were read over an intense narrow beam of light against a dark background (a microscope illuminator works well and allows for varying light intensity and position); 2) A specific positive result was recorded when a precipitin line formed on the center line between the central (antiserum) and a peripheral (anti-

gen) well, visible as a light haze; 3) The type of reaction varied with the concentration of antibody in the sample being tested. The positive control serum line was used as the basis for reading the test, and if the line was not distinct, the test was regarded as not valid and was repeated. In this case, the MAGID method was applied.

Modified agar gel immunodiffusion test (MAGID)

To improve the sensitivity and accuracy of meat species identification, especially where false negative results were obtained from low levels of antibody or antigen concentration, MAGID was applied.

Using the previously examined agar gel plates, after recording the results, the same quantity (as above) of antigen and antiserum was added to the relevant wells after 24 h. Each plate was then treated as above (rested and incubated) and examined for precipitin lines over 48 h. This modified method improved and increased the intensity of precipitin line formation.

Scoring of AGID and MAGID results

After 24–48 h, the intensity of the precipitin line formed between the antigen and antiserum wells was scored as follows: weak positive (+), moderate positive (++), strong positive (+++), very strong positive (++++), negative (–), and negative with unspecific lines (–LI).

Polymerase chain reaction tests

The samples were also subjected to PCR analysis illustrated by Omran *et al.* (2019) to confirm the sensitivity and accuracy of the AGID and MAGID results.

Histological examination

Some of the positive samples of raw beef products (burger, sausage, kofta, and

luncheon) previously examined using AGID, MAGID, and confirmed by PCR were selected for histological examination.

The tissues were fixed in 10% neutral buffered formalin and embedded in paraffin and routinely processed for light microscopy. Sections (6 µm) were cut from the paraffin-embedded blocks and stained using haematoxylin and eosin for histological study. The slides were observed under a microscope (N-180 M, NOVEL, Beijing, China) equipped with an electronic eyepiece (MD130, OME-TOP system, New Taipei City, Taiwan) to detect unauthorized tissues. Images were processed using Photoshop CS software (Adobe, San Jose, CA, USA) according to the method of Mokhtar *et al.* (2018).

RESULTS

Table 1 shows results of AGID and MAGID tests performed to detect adulteration of raw beef burger samples with chicken meat, donkey meat, or human tissue.

The AGID results indicated that the examined industrial frozen raw beef burger samples contained beef and all were free from donkey and human tissue but 5 (25%) of the 20 samples were adulterated with chicken meat. The MAGID results also indicated that all of these samples contained beef and no human tissue, with 5 (25%) adulterated with chicken meat; however, MAGID also indicated that 1 sample (5%) was adulterated with donkey meat.

AGID indicated that all of the raw handmade chilled beef burger samples contained beef and were free from chicken and donkey meats, but human tissue was detected in 4 (40%) out of the 10 samples. MAGID results for the same samples indi-

Table 1. Results of agar gel immunodiffusion (AGID) and modified agar gel immunodiffusion tests (MAGID) to detect adulteration in raw beef products (beef burger, oriental sausage, kofta, and luncheon) with chicken meat, donkey meat, and human tissue. Data are given as percentage (number of positive samples/total examined samples)

	Beef			Chicken			Donkey			Human tissue		
	AGID	MAGID		AGID	MAGID		AGID	MAGID		AGID	MAGID	
Raw beef burger	Industrial frozen	100% (20/20)		25% (5/20)	25% (5/20)		0% (0/20)	5% (1/20)		0% (0/20)	0% (0/20)	
	raw beef burger											
	Handmade chilled	100% (10/10)		0% (0/10)	0% (0/10)		0% (0/10)	40% (4/10)		40% (4/10)	40% (4/10)	
	raw beef burger											
Raw beef oriental sausage	Total	100% (30/30)		16.6% (5/30)	16.6% (5/30)		0% (0/30)	16.6% (5/30)		13.3% (4/30)	13.3% (4/30)	
	Industrial frozen	100% (20/20)		20% (4/20)	20% (4/20)		0% (0/20)	5% (1/20)		0% (0/20)	0% (0/20)	
	raw beef oriental											
	sausage											
Raw beef kofta	Handmade chilled	100% (10/10)		0% (0/10)	0% (0/10)		0% (0/10)	40% (4/10)		20% (2/10)	30% (3/10)	
	raw beef oriental											
	sausage											
	Total	100% (30/30)		13.3% (4/30)	13.3% (4/30)		0% (0/30)	16.6% (5/30)		6.6% (2/30)	10% (3/30)	
Beef luncheon	Industrial frozen	100% (20/20)		35% (7/20)	35% (7/20)		0% (0/20)	30% (6/20)		0% (0/20)	0% (0/20)	
	raw beef kofta											
	Handmade chilled	100% (10/10)		0% (0/10)	0% (0/10)		0% (0/10)	10% (1/10)		40% (4/10)	50% (5/10)	
	raw beef kofta											
Total % of most adulterating species	Total	100% (30/30)		23.3% (7/30)	23.3% (7/30)		0% (0/30)	23.3% (7/30)		13.3% (4/30)	16.6% (5/30)	
	Industrial frozen	100% (20/20)		35% (7/20)	35% (7/20)		0% (0/20)	30% (6/20)		0% (0/20)	0% (0/20)	
	raw beef kofta											
	sausage											
Total % of most adulterating species	Total	96.6% (29/30)		16.6% (5/30)	16.6% (5/30)		0% (0/30)	0% (0/30)		0% (0/30)	0% (0/30)	
	Industrial frozen	100% (20/20)		35% (7/20)	35% (7/20)		0% (0/20)	30% (6/20)		0% (0/20)	0% (0/20)	
	raw beef kofta											
	sausage											
Total % of most adulterating species	Total	21/120		17.5	17.5		0	14.1		8.3	10	
	Industrial frozen	100% (20/20)		35% (7/20)	35% (7/20)		0% (0/20)	30% (6/20)		0% (0/20)	0% (0/20)	
	raw beef kofta											
	sausage											

cated that all contained beef and were free from chicken meat and that 4 (4%) were adulterated with donkey meat and contained human tissue.

The total findings of the examined raw beef burger samples using AGID and MAGID showed that 30 (100%), 5 (16.6%), and 4 (13.3%) of the 30 samples contained beef, chicken, and human tissue, respectively. Using AGID, no samples were shown to be adulterated with donkey meat, but using MAGID, 5 (16.6%) samples were shown to be adulterated with donkey meat.

The results of AGID and MAGID tests performed to detect adulteration of raw beef oriental sausage samples with chicken, donkey, and human tissue showed that all examined samples of industrial frozen raw oriental sausage contained beef and did not contain human tissue. However, 4 (20%) of the 20 samples were adulterated with chicken meat. Donkey meat was not detected using AGID, whereas it was detected in 1 sample (5%) examined using MAGID.

All of the raw handmade chilled beef oriental sausage samples contained beef, and none contained chicken or donkey meat, but human tissue was detected in 2 (20%) out of the 10 samples examined using AGID. MAGID results showed that all the examined samples contained beef and none of them were adulterated with chicken meat. However, 4 (40%) and 3 (30%) of the samples contained donkey meat and human tissue, respectively.

Total results for the raw beef oriental sausage samples showed that beef and chicken meat were detected in 4 (13.3%) out of the 30 samples examined using AGID; donkey meat was not detected, and that 2 (6.6%) contained human tissue. MAGID results also showed that 100% of the examined samples contained beef and

4 (13.3%) were adulterated with chicken meat. However, MAGID indicated that 5 (16.6%) and 3 (10%) out of the 30 examined samples were adulterated with donkey meat and human tissue, respectively.

All industrial frozen raw beef kofta samples examined using AGID and MAGID contained beef and did not contain any human tissue, but 7 (35%) of the 20 samples were adulterated with chicken meat. None of the samples examined using AGID showed adulteration with donkey meat, but MAGID indicated that there were 6 samples (30%) adulterated with donkey meat.

All handmade chilled raw beef kofta samples contained beef and none contained chicken or donkey meat by AGID, but human tissue was detected in 4 (40%) of the 10 samples. MAGID also showed that 100% of samples contained beef and that none was adulterated with chicken meat. However, 1 (10%) and 5 (50%) of the samples contained donkey meat and human tissue, respectively.

The total results for raw beef kofta showed that all the samples examined using AGID contained beef and that chicken meat was detected in 7 (23.3%) out of the 30 examined samples. Donkey meat was not detected in any of the samples, but 4 (13.3%) contained human tissue. MAGID also showed that all samples contained beef and that 7 (23.3%) were adulterated with chicken meat. Furthermore, donkey meat and human tissue were detected in 7 (23.3%) and 5 (16.6%) out of the 30 examined samples, respectively.

For the examined beef luncheon samples, AGID and MAGID produced the same results. Beef and chicken meat were detected in 29 (96.6%) and 5 (16.6%), respectively, out of the 30 examined samples. Donkey meat and human tissue were not found in any of the samples.

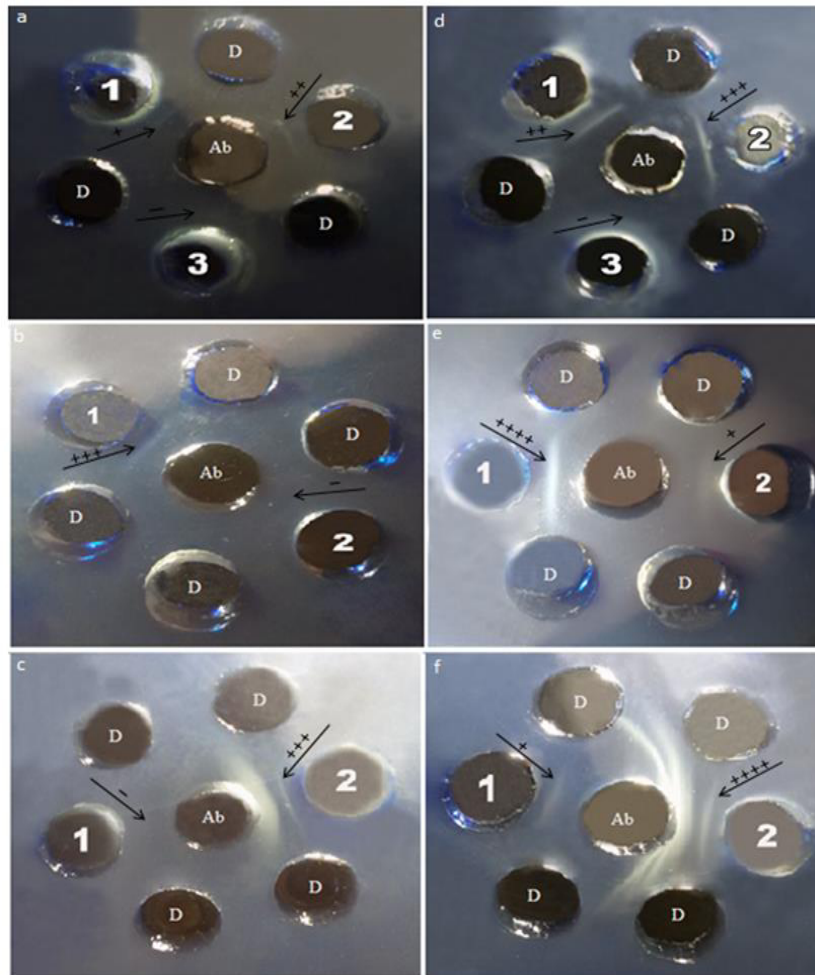


Fig. 1. Comparison between the intensity of precipitin lines formed in the agar gel immunodiffusion (a–c) and modified agar gel immunodiffusion (MAGID) tests (d–f) for detection of adulteration in beef product samples. Note how the positivity scores on the arrows are higher with MAGID. Ab: antibody of meat species; 1, 2 and 3: antigen of meat product samples; D: distilled water.

Fig. 1 shows examples of AGID and MAGID results, illustrating how MAGID improved upon AGID as follows: false negative (–) became weak positive (+), weak positive (+) became moderate positive (++), moderate positive (++) became strong positive (+++), and strong positive (+++) became very strong positive (++++).

The AGID test not only permits identification of meat that has been used to adulterate a product but also provides an indication of the amount of adulterant added by examining the intensity of the immunological reaction (the precipitin line formation) between the antigen and antiserum. For example, the chicken precipitin line is more intense than that for

beef (Fig. 2), indicating that the amount of chicken meat in the product might have been higher than that of beef. Also, the AGID test may be performed on semi-cooked or semi-heated products, such as luncheon, in which incomplete denaturation of the protein content allows the reaction with antiserum prepared from raw antigen.

PCR tests were conducted to confirm the sensitivity and accuracy of the AGID and MAGID tests performed in this study (Table 2). For the raw beef burger samples, MAGID proved to be more efficient. In some of the examined samples, especially in the handmade chilled samples, chicken meat was detected using PCR but not using AGID or MAGID. Also, MAGID detected donkey meat, and this was confirmed using PCR. However, although human tissue was detected using AGID and MAGID, it was not detected using PCR.

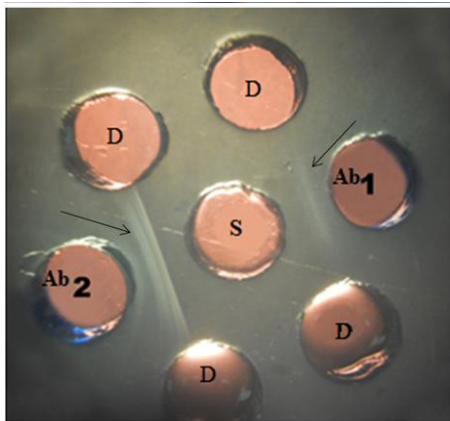


Fig. 2. Example result of the agar gel immunodiffusion test to detect adulteration of a beef product sample with chicken meat. Precipitin lines are shown with arrows. With antisera of beef (1) and chicken (2), according to intensity of precipitin lines (→) the amount of chicken was more than that of beef. Ab: antibody, S: antigen of meat product, D: distilled water.

In some of the raw beef kofta samples, chicken meat was not detected using AGID and MAGID (especially in all handmade chilled samples) but was detected using PCR. Donkey meat was detected using MAGID but was confirmed in only two samples using PCR. Human tissue was detected using AGID and MAGID but was detected in only one sample using PCR.

In beef luncheon, chicken meat was detected in four samples using AGID and MAGID but was detected in five samples using PCR. Donkey meat and human tissue were not detected using AGID, MAGID, or PCR.

Comparing PCR results with those of AGID and MAGID, it was noticed that chicken meat was detected using PCR more often than using AGID or MAGID, particularly in the handmade chilled meat product samples. Donkey meat was not detected using AGID but was detected more frequently using MAGID than using PCR. AGID and MAGID detected human tissue in 9 and 11 handmade chilled samples, respectively, whereas PCR did not detect human tissue in any of the examined meat product samples except in one handmade chilled kofta sample.

Histology results showed that the industrial frozen raw beef burger samples were adulterated with collagenous fibres, lung tissue, and various plant materials (Fig. 3), along with cartilage, spongy bone, and endochondral ossified epiphyseal plates. The raw handmade chilled beef burger samples were mixed with smooth muscle, collagenous fibres, tendon, spongy bone, blood vessels, the small intestine of a large ruminant, cardiac muscle, brain tissue, internal organ (uterus), plant material, and food additives.

The industrial frozen raw oriental beef sausage samples were adulterated with

Table 2. Application of conventional PCR technique for confirmation of adulteration of beef meat products with chicken, donkey species and human tissue

	Type	Chicken			Donkey			Human tissue		
		AGID	MAGID	PCR	AGID	MAGID	PCR	AGID	MAGID	PCR
Raw beef burger	Industrial frozen raw beef burger	(3/5) 60%	(3/5) 60%	(4/5) 80%	(0/5) 0%	(1/5) 20%	(1/5) 20%	(0/5) 0%	(0/5) 0%	(0/5) 0%
	Handmade chilled raw beef burger	0% (0/5)	(0/5) 0%	(5/5) 100%	(0/5) 0%	(4/5) 80%	(1/5) 20%	(4/5) 80%	(4/5) 80%	(0/5) 0%
Raw beef oriental sausage	Industrial frozen raw beef oriental sausage	(1/5) 20%	(1/5) 20%	(4/5) 80%	(0/5) 0%	(1/5) 20%	(1/5) 20%	(0/5) 0%	(0/5) 0%	(0/5) 0%
	Handmade chilled raw beef oriental sausage	(1/5) 20%	(1/5) 20%	(3/5) 60%	(0/5) 0%	(1/5) 20%	(0/5) 0%	(2/5) 40%	(3/5) 60%	(0/5) 0%
Raw beef kofta	Industrial frozen raw beef kofta	(2/5) 40%	(2/5) 40%	(4/5) 80%	(0/5) 0%	(4/5) 80%	(2/5) 40%	(0/5) 0%	(0/5) 0%	(0/5) 0%
	Handmade chilled raw beef kofta	(0/5) 0%	(0/5) 0%	(4/5) 80%	(0/5) 0%	(1/5) 20%	(0/5) 0%	(3/5) 60%	(4/5) 80%	(1/5) 20%
Beef luncheon	Total	(4/10) 40%	(4/10) 40%	(5/10) 50%	(0/10) 0%	(0/10) 0%	(0/10) 0%	(0/10) 0%	(0/10) 0%	(0/10) 0%

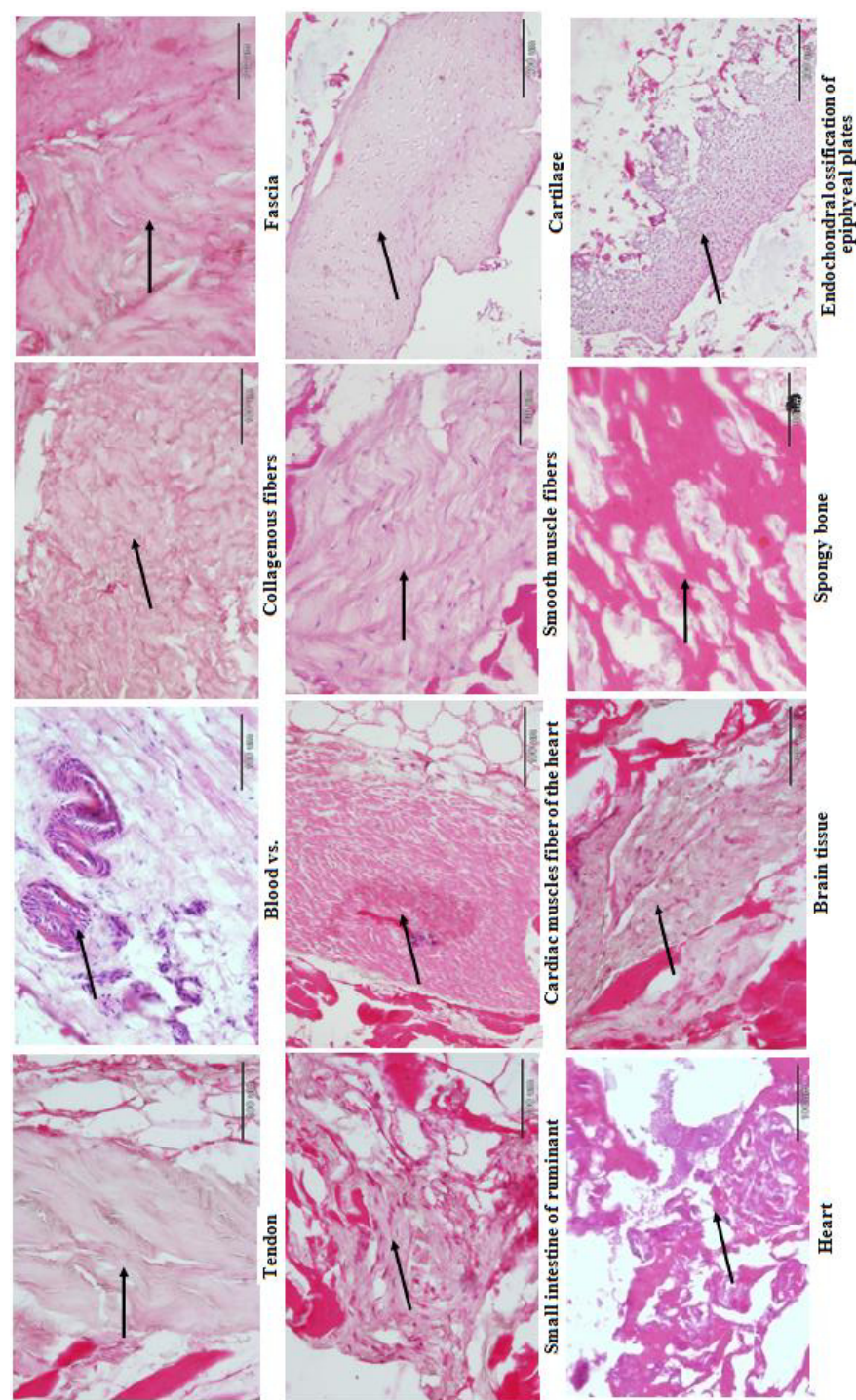


Fig. 3. Histology results showing adulteration with unauthorized tissue of beef product samples (beef burger, oriental sausage, kofta, and luncheon) stained by haematoxylin and eosin stain.

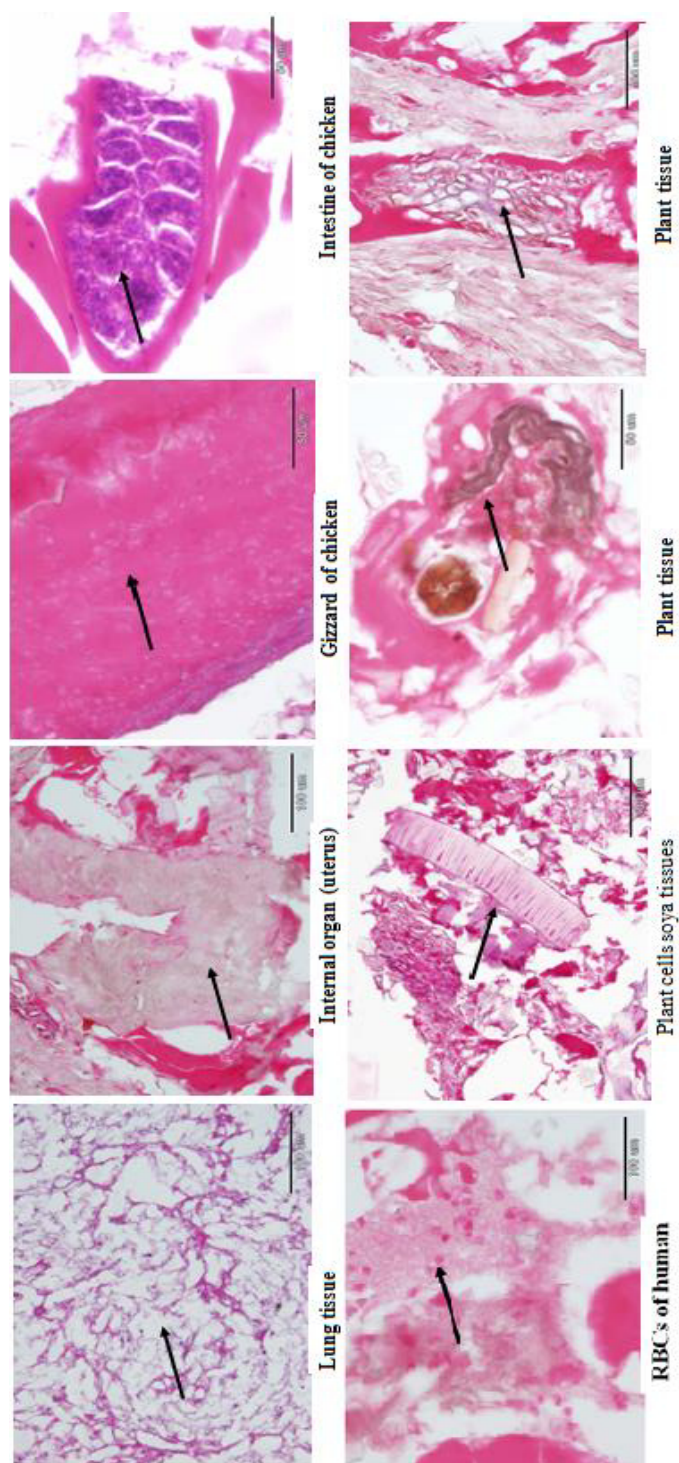


Fig. 3 (cont'd). Histology results showing adulteration with unauthorized tissue of beef product samples (beef burger, oriental sausage, kofta, and luncheon) stained by haematoxylin and eosin stain.

smooth muscle, tendon, spongy bone, blood vessels, heart, plant cells, and chicken intestine. Results indicated that this product had been adulterated to increase its bulk using fat, smooth muscle, and plant material.

The industrial frozen raw beef kofta samples contained smooth muscle, tendon, lung tissue, artery, heart, chicken gizzard, chicken intestine, and plant material, whereas the handmade chilled raw beef kofta samples contained smooth muscle, fascia, lung tissue, plant material, and human red blood cells.

The beef luncheon samples showed the presence of blood vessels, brain tissue, chicken intestine, and plant material, though the majority of the beef luncheon samples were adulterated only with plant material.

DISCUSSION

Identification of meat species is an important task in food quality control. Intentional adulteration of meat products with other-than-declared meat species can bring the manufacturer considerable economic profit. However, adulteration is hazardous for consumers, as it may lead to dangerous allergic reactions. Meat species can be identified using serological (Reddy *et al.*, 2000), histological (Tremlova, 2000), and molecular biological methods (Omran *et al.*, 2019).

Most samples of industrial frozen raw beef burger showed adulteration (with chicken meat), whereas most samples of handmade chilled beef burger contained donkey meat and human tissue (as confirmed using MAGID). For these products, the AGID results of this study agreed with those of Abd El-Aziz (2009) and Al-Nassir *et al.* (2014), who found that 100% of the burger samples they examined con-

tained beef with 25% being adulterated with chicken. Al-Nassir *et al.* (2014) found that none of the local beef burger samples they tested were adulterated with donkey meat. In this study, adulteration rates were lower than those in the study by Abd El-Aziz (2009), who found adulteration with chicken at a rate of 32%. Flores-Munguia *et al.* (2000) and Abd El-Aziz (2009) detected undeclared equine meat in 9 of 23 burger meat samples and donkey meat in 2% of the samples, respectively. In this study, the results for raw beef oriental sausage agreed with those of Abd El-Aziz (2009), who found that 100% of the examined raw beef oriental sausage samples contained beef and that the samples were not adulterated with donkey meat. The rate of adulteration in this study was lower than that of Flores-Munguia *et al.* (2000), who recorded undeclared equine meat in 5 of 17 samples, and Abd El-Aziz (2009), who found an adulteration rate of 32% with chicken meat.

In the industrial frozen beef oriental sausage, adulteration with chicken meat was the most frequent, whereas donkey meat and human tissue were detected at a high rate in the handmade chilled beef oriental sausage samples (MAGID).

In the frozen raw beef kofta, adulteration with chicken and donkey meats was the most frequent, whereas in the handmade chilled kofta, presence of human tissue was the most frequent (MAGID). These results agreed with those of Abd El-Aziz (2009) who found that 100% of raw beef kofta samples contained beef and none were adulterated with donkey meat, although they also noted a lower rate of adulteration with chicken meat (34%).

In this study, AGID showed overall adulteration rates of 17.5% and 8.3% for chicken meat and human tissue, respectively, whereas MAGID showed overall

adulteration rates of 17.5%, 14.1%, and 10% for chicken meat, donkey meat, and human tissue, respectively.

The availability and sensitivity levels of each anti-species antiserum to specific adulterants may make detection of low levels of adulterating species in ground meat products difficult (Mageau, 1991). In addition, interference by certain meat product ingredients, such as salt, spices, and soy proteins (Hsieh *et al.*, 1996), further limits the use of the AGID technique. The precipitation reaction was considered positive if a pronounced positive reaction zone occurred on the center line between the central and peripheral wells (between antiserum and antigen). This effect was obtained via equilibration of antiserum and antigen to concentrations of 1:99 and 1:9, respectively, when precipitation zones were sufficiently sharp and did not diffuse (Siklenka *et al.* 2004).

The shortcoming of the AGID test is that in samples with low levels of antibody or antigen the results are difficult to interpret as positive; thus, a false negative result may be reported. False positive results occasionally arise because of non-specific interactions leading to a relatively diffuse precipitate that may sometimes be recognised by its abnormal appearance and its solubility in water (Hitchcock & Crimes, 1985). For these reasons, this study worked on a modification of AGID to improve sensitivity and to minimise incorrect results, especially false negative results from low levels of antibody or antigen. Thus, for MAGID, after recording results of the AGID test, equal quantities of antigen and antiserum were added to the same wells on the previously examined AGID agar gel plates. This modification can improve and increase the intensity of the precipitin lines formed between the antigen and serum wells.

Identification of meat species in heat-processed products is hindered by progressive denaturation of the protein markers, leading to loss of solubility and antigenicity (Hitchcock & Crimes, 1985). False positive results may also occur because of cross-reactions (Reddy, 2000) or accidental cross contamination during processing, through improper handling or the use of shared equipment (Owusu-Apenten, 2002).

Microscopic/histological examination is one of the oldest methods used to analyse foodstuffs for composition and to detect contamination or intentional adulteration. The object is usually a qualitative examination, i.e. detection of the presence of individual tissues and evaluation of their acceptability or suitability for the given product.

The present study focused on unauthorised tissues as inedible components added to beef burger, sausage, kofta, and luncheon, including lung tissue, heart, bone, cartilage, fascia, intestine, large amounts of fat or plant material, tendon, and other illegal substances illustrated in our results. In accordance with our findings, different studies were performed for the detection of unauthorised tissues in the beef products. Histological examinations performed in the USA on different brands of hamburger showed the presence of blood vessels, plant material, cartilage, and bone (Prayson *et al.*, 2008). A similar histological evaluation of hamburgers showed the presence of unauthorised tissues including blood vessels, cartilage, and plant material (Sepehri, 2008). A comprehensive study by Sadeghi *et al.* (2011) identified different percentages of unauthorised tissues, including heart muscle and tendon, in sausage samples. Smooth muscle and soya tissues were detected histologically in meat products by

Rokni *et al.* (1999). A wide range of unauthorised tissues have been detected in sausage samples, including gizzard, soya, lung tissue, spongy bone, and cartilage (Latorre *et al.*, 2015; Mokhtar *et al.*, 2018).

In this study, histological examination of the meat products indicated that they did not meet Egyptian standards 1972–2005, 1688–2005, 1973–2005, and 1114–2005 (ES, 2005a,b,c,d). These standards require that the meat used for industrial frozen beef burger, sausage, kofta, and luncheon preparations must be free from cartilage, bone, tendon, blood vessels, clotted blood, nerve tissue, and connective tissue. Also, the mixture of minced meat used for industrial frozen beef burger, sausage, and luncheon preparations must be free from tissues of the reproductive system, stomach, intestine, esophagus, and urinary bladder. Our histological evaluation of these meat products markedly showed that the formulations used in their preparation did not respect the Egyptian food standards or hygiene food regulations and that overall the products were not of good quality.

It was found that in some handmade chilled samples, chicken meat was not detected using AGID or MAGID but was detected using PCR. When these samples were examined histologically, the results did not show any chicken tissue. The possible explanation is that these beef products were contaminated with chicken tissue that could not be detected histologically.

The histology results for some of the beef luncheon showed a complete absence of beef tissue and the presence of only plant tissues, even though the samples gave positive reactions with AGID, MAGID, and PCR. The possible explanation is that dry meat matter was used in

the manufacturing of the luncheon instead of fresh beef. This would constitute mislabelling and represent a case of meat product fraud. Within the last decades, a significant increase in food fraud incidence has been observed, including false labelling and undeclared use of food additives or fillers to replace skeletal muscles in the product to achieve economical profit (Everstine *et al.*, 2013; Ordunaa *et al.*, 2015). Such cases have increased consumer awareness, and food fraud has gained much attention. It is the producer's responsibility to ensure the quality of meat products.

Human tissue was not detected in any of the examined beef samples except one (handmade beef chilled kofta) using PCR. The histological examination showed the presence of human red blood cells, indicating possible contamination with blood (from bleeding due to injuries) during the meat processing operation, which can be an important source of infection and diseases.

The potential sources of undeclared chicken meat in the evaluated beef samples are varied, including mechanically recovered meat (currently most often produced from chicken carcasses), accidental cross contamination (because of improper handling), and the use of shared equipment; sometimes spice contamination can occur during processing (Surowiec *et al.*, 2011; Keyvan *et al.*, 2017). In meat plants processing both poultry and ruminant meat products, cross contamination of the meat species may occur during operations. It may be inevitable that one species of meat is mixed with another during meat processing operations, such as cutting and grinding, via knives, choppers, and cutting boards (Zarringhabaie *et al.*, 2011). The use of waste products that have lower nutritional value than pure beef is problem-

atic, as these may also be contaminated with food-borne pathogens, and a too low cooking temperature in final products poses a potential health risk for consumers (Ayaz *et al.*, 2006; Doosti *et al.*, 2014).

Another serious concern from regulatory, health, and ethical standpoints was the detection of undeclared donkey meat in some of the examined beef product samples. The presence of equine meat, regardless of its amount, is unacceptable to consumers as many would find it disgusting and repulsive to eat (Ali *et al.*, 2014). Because equines are not commercially processed for human consumption and not being considered as a conventional species in the domestic meat supply chain in Egypt, such meat might have been processed under unsanitary conditions, posing potential risks to human health. The use of donkey meat in a supposedly beef product can be categorised as adulteration, the intentional mixing of lower-cost (inferior and cheaper) meat species into a higher-cost product (Bourguiba-Hachemi & Fathallah, 2016), and could be a case of intentional substitution for economic gain (Cawthorn *et al.*, 2013).

Human tissue was detected in some of the examined handmade chilled beef products. In this study, human placenta was chosen as the antigen added to the studied products to identify human tissue and to detect human placenta. Recent research has indicated benefits of placenta consumption by women in the postpartum period, providing increased energy, improved lactation, decreased risk of baby blues and postpartum depression, increased iron stores, decreased postpartum vaginal bleeding, and quicker uterine involution (Selander *et al.*, 2013).

Criminal or illegal marketing or mixing with prohibited meat sources, such as

human tissues, or biological waste, such as placenta, may be practiced without detectable features, especially if the product is prepared manually in local shops (Hayes 2016). Human placenta might be released from hospitals in some countries for pharmaceutical companies to extract hormones or for use in cosmetics, but sporadically enforced policies and absence of standard nationwide regulations to ensure medicinal use or correct disposal as biological waste may lead to its use as a processed meat adulterant in the form of mince, with all its macroscopic features lost, especially if prepared manually in local shops (Cremers & Low, 2014).

The adulteration or contamination of meat products with different meat species or human tissue is unacceptable as it represents a potential health risk for consumers and is a source of zoonotic diseases. Fraudulent substitutions of expensive meat with cheaper meat or addition of undeclared species in meat products may cause concerns for consumer protection. Religious strictures, perceived or real health concerns, and cultural likes and dislikes are the main drivers of the need for species identification for consumer protection (Ahmed *et al.*, 2011). The usual reasons given for meat adulteration are economic. The high price of meat and negligence or carelessness of consumer safety encourages small- and medium-sized enterprises and traditional market sellers to substitute beef with other kinds of meat in their products (Roostita *et al.*, 2014).

CONCLUSIONS

In this study, most of the examined meat products were adulterated with one or more species. All the categories of meat products, including industrial raw frozen

and handmade chilled products, were adulterated, and some handmade chilled meat products were contaminated with human red blood cells. Chicken meat was the most frequently detected undeclared meat in the examined products. The presence of donkey meat in some of the examined meat products has become a widespread problem in retail markets. The occurrence of one sample positive for human tissue, in handmade chilled beef kofta, was not anticipated. Each immunological, molecular, biological and histological method used in the study has its own advantages and limitations. MAGID is more successful and sensitive than AGID, especially in resolving false negative results of AGID. Consumers are advised not to consume the studied meat products excessively to avoid exposure to adulterated products that may pose a health hazard.

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