INCIDENCE OF ILLEGAL SUBSTITUTION OF BEEF WITH INFERIOR-QUALITY ANIMAL SPECIES MEATS SOLD IN KERBALA

Hikmat Sahib Al-Nassir*, Raeed A.Kassim*, Bahaa H.Muhammed**

* College of Veterinary Medicine, University of Kerbala, Kerbala,|Iraq. **Hilla Surgical Hospital, Babel Governorate, Babel,Iraq**

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ABSTRACT

A surveillance study was conducted to determine the rates of mixing beef illegally with other selected animal species meats of inferior quality in Kerbala Governorate. Over an 8-month period (September 2012 to May 2013), a total of 120 samples of meat products viz., 40 local minced meats, 40 local beef burger and 40 imported beef burger were purchased from Kerbala city and analyzed by Agar Gel Immuno-Diffusion test (AGID) for detecting substitution of undesirable meat species. Meat extracts from camel, chicken and donkey were prepared. Rabbits were injected with meat extracts for preparation of hyper-immune sera, whereas, blood was collected to get the specific anti-sera. Positive result was recorded when precipitation line observed between the antibody and the corresponding meat extract. The incidence of adulteration was detected in 6 (5%) samples with camel meat and 23 (19.16%) samples with chicken meat, while none of the samples was found adulterated with donkey meat. Camel meat found in 4 (10%), 2 (5%) and 0 (0%) of local minced meat, local beef burger and imported beef burger respectively. However, chicken meat detected in 8 (20%), 10 (25%) and 5 (12.5%) of local minced meat, local beef burger and imported beef burger respectively.

It was concluded that substitution of meat products, in which inferior quality meat is mixed into beef, has been existed in Kerbala Governorate.

INTRODUCTION

Due to their high market value, meat and meat products are often targets for species substitution and adulteration (1). In recent years, interest in meat authenticity has increased as many consumers are concerned about the meat they eat. Therefore, there has been a need for a fast and routinely applicable meat species identification system. The first purpose of meat species identification is to detect adulteration or substitution and misrepresentation of meat for reason of health, religion and economics (2). Another
The purpose of meat species identification is to prevent indiscriminate slaughter of prohibited species for the religious reasons. However, meat species identification can also be adopted in veterinary forensic medicine.

Traditionally, species identification has been established through one of the three approaches: molecular biology-based methods, enzymatic immunological methods or chromatographic methods (3). Molecular biology-based methods use techniques such as Polymerase Chain Reaction (PCR) to identify species-specific nucleotide sequences or variations within the mitochondrial DNA for the basis of species recognition (4). The serological analysis method has been used for differentiating proteins. Following the parenteral introduction of the antigen, experimental animals react systemically by forming specific antibodies to the antigens. These antibodies which are present in the serum of donors (antisera) provoke, in the presence of homologous antigen, reactions which can be demonstrated directly or indirectly, according to the different serological techniques used (5). The biological method depends on the fact that certain antibodies develop in the blood of an animal which receives repeated injections of blood serum from another animal (6).

Meat species adulteration, substitution or mislabeling of meat products have been reported from different countries such as Canada, Australia, Britain and Egypt (7, 8, 9, and 10).

In a study conducted by (10), two hundred samples from beef meat products were subjected to analysis by Agar Gel Immuno-Diffusion (AGID) technique. Adulteration with chicken meat and pork was detected in minced meat, raw kofta, sausages and beef burger, while, donkey meat was detected in beef burger only. However in Egypt (11) examined samples of Kebab, grilled kofta and meat loaves and found that equine meat was present in all samples.

In South Africa, the results of a survey conducted by (5) revealed that 68% of samples contained species which were not declared on the product labeling, with the incidence being highest in sausages and burger. Soya was identified as undeclared plant protein in a large number of samples, while pork and chicken were the most commonly detected animal species. Unconventional species such as donkey, goat and water buffalo were also discovered in a number of products.

By conducting agar gel immune-diffusion, (12) detected chicken flesh at 1,3 and 5% levels in heated beef sausages.

Complement fixation test is another example of biological techniques. The principle of the test is same as in precipitation test but a compliment is used to reproduce the results for longer duration. However, it is a time taking method and ineffective in case of thoroughly cooked meat (13).

The main objective of this study was to find out the incidence rates of illegal substitution of raw or processed beef with other animal species meats of inferior quality sold in Kerbala.
MATERIALS AND METHODS

Meat samples:

A total of 120 beef meat samples of local minced meat, local beef burger, and imported beef burger (40 of each) were collected from Kerbala retail markets during the period from September 2012 to May 2013 and examined by AGID technique for detecting illegal substitution and adulteration.

Preparation of meat antigen:

Antigens from camel, chicken, and donkey meats were prepared and kept frozen until used. Preparation of antigen was adopted following the method of USDA-FSIS, (14). Meat was cut into small pieces and mixed with saline (NaCl 0.85%) at volume 1:3. Macerating 1-2 min was done and stands for 90 min., then filtration was applied. Samples were centrifuged and the supernatant was taken. Before immunization of rabbits the supernatant was filtered.

Preparation of Anti-sera:

Hyper-immune sera were prepared in rabbits by repeated subcutaneous injection of meat extracts. Nine female New-Zealand breed rabbits at 8-12 weeks old were clinically examined and allowed a minimum of 7 days of adaptation prior to the experiment. Rabbits were divided to 3 equal groups and were immunized for production of the target anti-sera. Blood was collected to get the specific anti-sera after 4 weeks of injection which was a period necessary to build up a primary immunological response (14).

Agar Gel Immuno-Diffusion Test (AGID):

Based upon the formation of specific immuno-precipitin lines resulting from the diffusion of meat extract (Antigens) and specific antisera (Antibodies) according to Ouchterlony (15).

A solution of 100 ml of 0.05M borate buffer (pH8) containing 0.9% NaCl and 3% polyethylene glycol was prepared. A total of 1.8g/100 ml of agarose was dissolved. The agarose solution was allowed to cool, and the gel was then poured on the gel plates. The plates were used within a day, or stored for a week or so at 4C. Circular holes (wells) of 2mm diameter with 1 cm distance between every two wells were cut in a row on the plate using a gel punch and a template.
RESULTS

The results shown in Table 1 indicated that out of 120 beef samples, adulteration was detected in 6 (5%) samples with camel meat and 23 (19.16%) samples with chicken meat, while none of the samples was found adulterated with donkey meat. Camel meat was detected in 4 (10%) samples of local minced meat and 2 (5%) sample of local beef burger, while imported beef burger was found free of adulteration with camel meat (Table 1). The same Table shows adulteration of samples with chicken meat in 8 (20%), 10(25%) and 5 (12.5%) of local minced, local beef burger and imported beef burger respectively. Table 2 displays 2 (5%) samples of local beef burger adulterated with camel meat, while none of the imported beef burger samples mixed with camel meat. The same Table also shows chicken meat detected in 10 (25%) samples of local beef burger and 5 (12.5%) samples of imported beef burger.

Table 1: Incidence rates of mixing beef with other species meats in tested meat sample.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sample</th>
<th>No.</th>
<th>Camel</th>
<th>No.</th>
<th>%</th>
<th>Chicken</th>
<th>No.</th>
<th>%</th>
<th>Donkey</th>
<th>No.</th>
<th>%</th>
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<td>Chicken</td>
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<td></td>
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<td></td>
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<tr>
<td>Local minced</td>
<td>40</td>
<td>4</td>
<td>10</td>
<td>8</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
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<tr>
<td>Local beef burger</td>
<td>40</td>
<td>2</td>
<td>5</td>
<td>10</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Imported beef burger</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>12.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Total</td>
<td>120</td>
<td>6</td>
<td>5</td>
<td>23</td>
<td>19.16%</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</table>

Table 2: Difference of mixing incidences between Local Beef Burger and Imported Beef Burger.

<table>
<thead>
<tr>
<th>Meat samples</th>
<th>No.</th>
<th>Camel</th>
<th>Chicken</th>
<th>Donkey</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>No</td>
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<td>Local beef burger</td>
<td>40</td>
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<td>Total</td>
<td>80</td>
<td>2</td>
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<td>15</td>
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DISCUSSION

In recent years, interest in meat authenticity has increased. Authentication methods can be categorized into the areas where fraud is most likely to occur: meat origin, meat substitution, meat processing treatment and non-meat ingredient addition. At the present time, methods aiming for investigating meat and meat product authenticity may be based either on the analysis of protein composition or on the analysis of nucleic acids (16).

Meat retail stores, food manufacturers or food processing factories may add different varieties of meats to species-specific meat so as to add bulk or make up the volume of the product. Low priced or low valued meat species may be adulterated with higher valued meat species. Meat products which are adulterated with less desirable species may threaten the public health and species identification is becoming an important practice (17).

The results of this study revealed that the adulteration rates for local minced meat were 10% for camel and 20% for chicken as presented in Table 1. The adulteration of this product with chicken meat was higher than those reported by (10) who found the adulteration rates for minced meat were 6% for each of chicken and pork.

The results of the present study indicated that the fraud in local beef burger was 5%, 25% with camel meat and chicken meat respectively (Table 1). However, the adulteration rates of the imported beef burger was also found high with chicken 12.5%, but none of the product samples was adulterated with camel meat (Table 1). It can be concluded that chicken was mostly incriminated for substituting beef in this location which is obviously explained by its lower market price.

The findings of the present study which are shown in Table 1 indicated that the highest total incidence rate of adulteration in the examined meat products was 19.16% with chicken meat and to a lesser extent 5% with camel meat. The high incidence rates of adulteration with chicken meat was also reported by (18), who found the adulteration rates of cattle meat with chicken meats were 34%, 32% and 32% for raw kofta, sausages and beef burger respectively.

The differences of adulteration incidences between local beef burger and imported beef burger with camel, chicken and donkey meats are illustrated in Table 2. Once again, the results revealed that chicken was incriminated as the main fraud in the imported beef burger (12.5%), while both chicken meat 25% and camel meat 5% adulterated the local product of beef burger. It might be useful to mention that camel meat is less desirable meat simply because it is firm in consistency and containing coarse fibers which are difficult to be cooked and digested (12).

Fortunately, the results of the current study as shown in Table 1 indicated that donkey meat was not detected in any of the 120 samples examined during the study and that can be explained as donkey meat is both socially and officially prohibited for religious reasons. However, in a study conducted in Egypt, (10) used an alternative method
based on conventional PCR (Polymerase Chain Reaction) analysis and found the adulteration rates with donkey meat were 18% for raw kofta, 8% for sausages, 7.7% for beef burger and 7% for minced meat.

The findings of this study indicated that adulteration of meat products and meat substitution are malpractices in meat industry, in which inferior or cheaper quality meat such as chicken meat and camel meat are mixed into superior quality and such fraudulent substitution has been existed in this location.

**REFERENCES**


