

Identification of Adulteration with Camel Meat Using Polymerase Chain Reaction Assay

Alaa El-deein El- Morshedy , El-Saied A. El-daly, Adel I. El-Atabany, and Ahmed E. Tharwat

Food Control Department (Meat Hygiene) .Faculty of Veterinary Medicine, Zagazig University
ahmed_tharwat1983@yahoo.com

Abstract: Meat species adulteration is a common problem in the retail market. This study investigated the validity of polymerase chain reaction (PCR) to detect the adulteration of camel meat even in low level and heat treated meat emulsion of camel meat. The primer pair was designed based on mitochondrial D-loop gene for detection of adulteration of camel meat in admixed meat and meat products by polymerase chain reaction (PCR) assay. Amplification of 208-bp DNA fragments was observed from camel, without any cross-reaction with cattle, sheep, goat and chicken. The amplification was further confirmed by endonuclease enzyme Taq I restriction enzymes. No adverse effect of processing was found on PCR amplification of camel meat DNA extracted from processed meat and meat products, even from meat emulsion autoclaved at 121 °C, for 15–20 min. The detection limit for camel meat was found to be 0.05% in the admixed meat and meat products; however, very faint and inconsistent results were obtained in autoclaved meat emulsion at 0.05% level. The developed PCR assay was found to be specific for camel and could be a useful tool for detection of meat adulteration.

[Alaa El-deein El- Morshedy , El-Saied A. Eldaly, Adel I. El-Atabany, and Ahmed E. Tharwat **Identification of Adulteration with Camel Meat Using Polymerase Chain Reaction Assay.** Journal of American Science 2011; 7(12): 339-343]. (ISSN: 1545-1003). <http://www.americanscience.org>.

Keywords: Meat species. Adulteration. Detection. Processed meat products. Species-specific PCR assay

1. Introduction

Consumers require clear and accurate information to make informed choices about their diet and the foods they buy. The information given to consumers is essential for them choosing one food product over another. Consumer choice might also reflect lifestyle or religious concerns (e.g. vegetarianism, preference for organic products, absence of pork for Jewish and Muslims), or health concerns (e.g. absence of peanuts, lactose or gluten for individuals with particular allergies). Therefore, the description and/or labeling of food must be honest and accurate; particularly if food has been processed removing the ability to distinguish one ingredient from another. The information that must be given is enshrouded in law in most developed countries, so that food supplied must exactly what the labeling says it is. That is, the food must be authentic and not misdescribed (**Mark and Sandy, 2004**).

The invariable adulteration/substitution of camel meat with other meat species is common in minced meat products in some countries in Middle East particularly in Egypt due to low cost and easy availability of camel meat. However, detection of camel meat is difficult due to a lack of rapid and sound technique to differentiate them from other meat species especially in low percentage. The task is complicated if the meat is in processed form or heat treated. In the past, food authentication routinely involved the detection of species-specific proteins, when attempting to discern the origins of material for

human consumption (**Hitchcock and Crimes, 1985**). Such tests employed a variety of immunological and electrophoresis methods, but they were not without their problems. As a result of heating, the processing of foodstuffs can cause denaturation of the proteins under study and, in addition, protein expression is usually tissue dependent (**Hofmann, 1987**). Increasingly; however attention has now turned to DNA as a source of information. As DNA is more thermo stable than many proteins, analysis using nucleic acids are less liable to be disrupted by processing of foodstuffs. Furthermore, DNA is present in the majority of the cells of an organism, potentially enabling identical information to be obtained from many appropriate samples from the same source, regardless of the tissue of origin. Additionally, through the acquisition of sequence data, DNA can potentially provide more information than protein, due to the degeneracy of the genetic code and the presence of many non coding regions (**Ebbehoj and Thmosen, 1991, Hunt and Parker, 1997**). Furthermore, driven by the clinical arena, nucleic acid-based technologies are developing rapidly and the informed adoption of suitable methods by the food industry has the potential to greatly simplify methods of authentication. Therefore, the present study was aimed to develop and evaluate the designed camel-specific primer pair using PCR assay for its detection in meat products under various processing conditions.

2. Materials and Methods

DNA Extraction

The DNA was extracted from meat samples of camel, cattle (ox), sheep, goat and chicken collected from local slaughterhouses using Tissue Kit (ferments, USA) as per the instructions given by manufacturer. The same kit was also used for the extraction of DNA from heat-treated meat and meat emulsion. The DNA samples of camel breeds (Camelus dromedarius "one-humped camel") was used in this experiment.

Design of Oligonucleotide Primer Pair

Species-specific primer for the detection of camel DNA was designed based on mitochondrial D-loop gene (National Center for Biotechnology Information (NCBI) accession number AF475263). Then, alignments and comparisons of available gene sequences (NCBI GenBank database) of camel and other species were made before designing the primer pair. The primer design software DNASTAR (DNASTAR Inc., USA) was used for analysis of sequences and the designing of primer pair. The primer pair designed was synthesized from Metabion International, Germany. The details of primer pair used in the present investigation are

Given below:

L183 (forward: 5-AGC CTT CTC TTC AGT CGC ACA C-3)

H372 (reverse: 5-GCC CAT GAA AGC TGT TGC T-3)

PCR Amplification of DNA Fragment

The reaction mixture was prepared in a 500- μ l PCR tube (Axygen, USA) in a total volume of 50 μ l containing 5 μ l of 10 \times PCR buffer, 15 μ M MgCl₂, 200 μ M each of dNTP, 1–2 units of Taq DNA polymerase (Qiagen, USA), 20 pmol each of forward and reverse primer, 1 μ l of DNA template (20–30 ng), and remaining nuclease-free water (Fermentas, USA). The PCR conditions programmed on master cycler gradient thermocycler (Eppendorf, Germany) were as follows: initial denaturation at 94°C for 2 min followed by 30–35 cycles of denaturation at 94°C for 0.5 min, annealing at 60°C for 0.5 min, and extension at 72°C for 1 min. Then, final extension was done at 72°C for 5 min. The PCR product was kept at –20°C for further use.

Analysis of PCR-Amplified DNA Fragments

The submarine horizontal agarose gel electrophoresis was used for analysis of PCR products using 2% agarose in gel. For that, 0.4 g of agarose (Ambion, USA) was put in 20 ml of 1 \times TBE solution (Fermentas, USA.) and heated to completely dissolve the agarose. Then, 1 μ l (5%) of ethidium bromide solution was added as gel visualizing agent and mixed thoroughly. The electrophoresis was done

for 40 min at 80 V. The PCR product was finally analyzed using UV transilluminator. The ready to use 50-bp ladder (Fermentas, USA) was used in the present work as a molecular marker.

Digestion of PCR-Amplified DNA Fragments with Restriction Enzyme

Restriction digestion assay was performed in a final volume of 20 μ l by mixing 5 units of endonuclease enzyme Taq I (Promega) with 10 μ l PCR products. The mixture was incubated for 1 h at 37°C. Then, the digested DNA fragments were run on 2% agarose gel for 40 min at 80 v.

Evaluation of Specificity and Sensitivity

The specificity of this camel-specific PCR assay was cross tested with DNA of other non-targeted meat species used in the study. The sensitivity of the assay was tested in heat-treated admixed minced meat and emulsion-based meat products containing 5%, 1%, 0.5%, 0.1% and 0.05% camel meat. The non-targeted meat species had equal proportion in minced meat and meat emulsion. The specificity and sensitivity of this assay were tested in various manufacturing and processing conditions. The non-targeted meat species in this study were cattle, sheep, goat and chicken.

Preparation of meat products and heat treatments

Meat emulsion-based meat products such as kabab, patty and meat block were prepared as outline by **Mane et al. (2009)**. these products were given heat treatment at different temperatures to evaluate applicability of optimized PCR assay in meat and meat products.

3. Results and Discussion

Consumers concerns over food safety issues such as traceability of animal origin and authenticity of meat products are driving the requirement for reliable authentication methods. Of these methods, DNA-based methods offer the greatest potential because they are stable and not tissue dependent. In the present study, species-specific PCR assay was developed and employed for the detection of camel from other commonly used meat species using the primer pair designed based on mitochondrial D-loop gene. Further more, the application of DNA methods based on mtDNA facilitates the PCR amplification in case where the availability of DNA template after its extraction from cells is sufficient for detection, as mtDNA is several fold more abundant than nuclear genome and each cell carries multiple numbers of mitochondrion, depending on tissues and species. mtDNA evolves much faster than nuclear DNA and presents more sequence diversity, thus facilitating the

identification of closely related species

The developed primer pair successfully amplified the expected 208 –bp DNA fragments from DNA of camel meat breed mentioned in the "Materials and Methods" (Fig.1). The optimized PCR assay was further confirmed for its size using endonuclease enzyme Taq I, which cuts the 208 –bp DNA into 151bp and 57bp (fig. 2). This approach was earlier employed by many other workers for further confirmation of PCR products amplified from meat and meat products (**Chen *et al.*, 2005**)

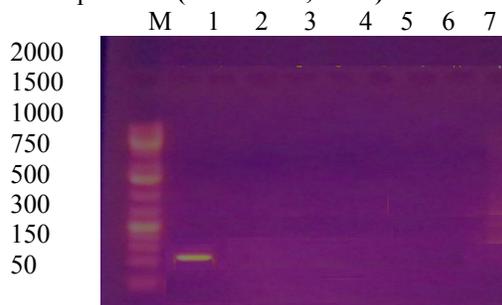
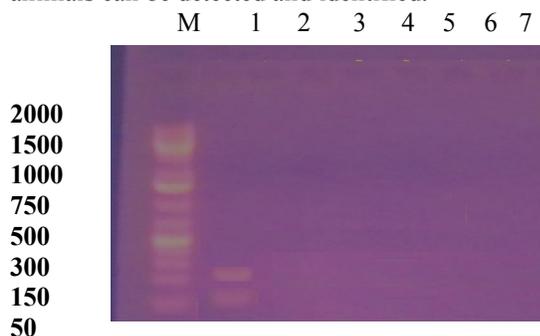


Fig (1) optimization of PCR for amplification of DNA extracted from muscle tissue of camel meat. Lane M 50bp ladder, lane 1 camel meat.

The optimized PCR assay with camel DNA was subsequently employed with DNA extracted from muscle tissues of cattle (ox), sheep, goat and chicken. After repeated testing, no cross-reaction was observed with DNA of cattle (ox), sheep, goat and chicken extracted from muscle tissues (Fig. 3).the use of PCR greatly improved and facilitated the detection of animal ingredients in food or feedstuff due to its simplicity, species specificity and high sensitivity .with species-specific primers and analysis of restriction fragment length polymorphism, material from cattle, sheep, goat, chicken and other animals can be detected and identified.



Fig(2) PCR amplified DNA fragment of camel mitochondrial D-loop gene digested with endonuclease enzyme Taq I. lane M 50 bp ladder, lane 1 endonuclease enzyme Taq I

The optimized camel-specific PCR assay was then evaluated for its efficiency to amplify the DNA extracted from heat –treated meat and meat emulsion to amplify the DNA it was found that these treatments and the ingredients used for emulsion preparation have no adverse effect on PCR amplification (Fig. 4).

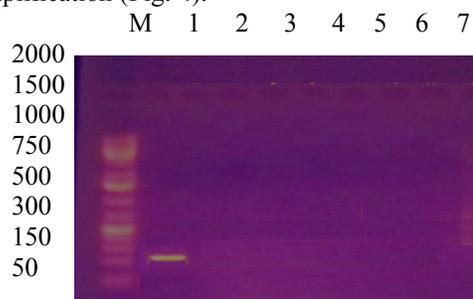


Fig (3) PCR amplification pattern of DNA fragment of mitochondrial D-loop gene in different meat species .lane M 50-bp ladder, lane 1 camel ,lane 2 cattle, lane 3 sheep, lane 4 goat, lane 5 chicken and lane 6 negative control.

In addition, no adverse effect on PCR amplification of DNA extracted from autoclaved meat emulsion at 121 c for 15-20 min .as mentioned in the earlier discussion, this may be due to heat stability and large number of copies of mitochondrial DNA in meat tissue, contributing to the survival of a sufficient number of DNA copies, even when these were subjected to extreme processing conditions of autoclaving (**Parties *et al.*, 2000**).

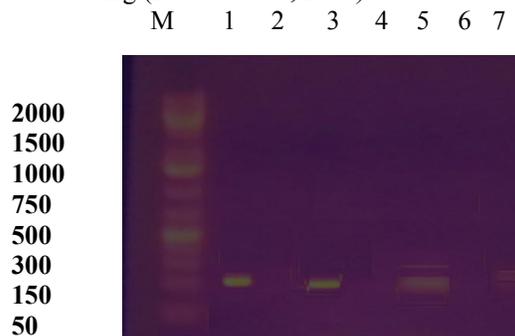


Fig (4) PCR amplification pattern of DNA fragment of camel mitochondrial D-loop gene in raw , cooked and autoclaved meat emulsion .lane M 50-bp ladder, lane 1 raw meat emulsion, lane 2 cooked meat emulsion ,lane 3 autoclaved meat emulsion, lane 4 raw meat emulsion , lane 5 cooked meat emulsion and lane 6 autoclaved meat emulsion., lane 7 negative control. Lanes 1-3 contain 100% camel meat and lanes 4-6 contain 10% camel meat mixed with cattle, sheep, goat and chicken.

Further, optimized camel-specific PCR assay was employed to test its sensitivity in the admixed meat emulsion containing camel meat, cattle (ox), sheep, goat and chicken. The developed PCR assay was found able to detect up to 0.05% level of meat adulteration, however, very faint amplification was observed at 0.05% level of adulteration (Fig. 5), but no effect was observed on PCR amplification of camel DNA in admixed meat products up to 0.05% level of adulteration (Fig. 6).this might be due to less sever condition of processing generally used for meat product preparation (Arsalan *et al.*, 2006).

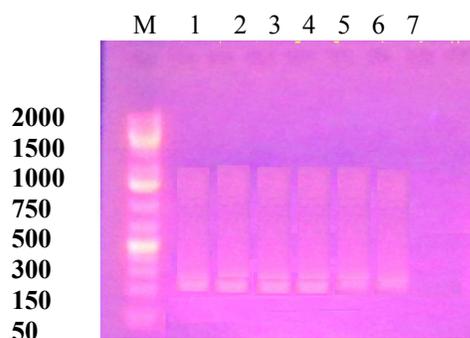


Fig (5) PCR amplification pattern of DNA fragment of camel mitochondrial D-loop gene in raw , cooked and autoclaved meat emulsion .lane M 50-bp ladder, lane 1 raw meat emulsion, lane 2 cooked meat emulsion ,lane 3 autoclaved meat emulsion, lane 4 raw meat emulsion , lane 5 cooked meat emulsion and lane 6 autoclaved meat emulsion., lane 7 negative control. Lanes 1-3 contain 5 % camel meat and lanes 4-6 contain 1 % camel meat mixed with cattle, sheep, goat and chicken.

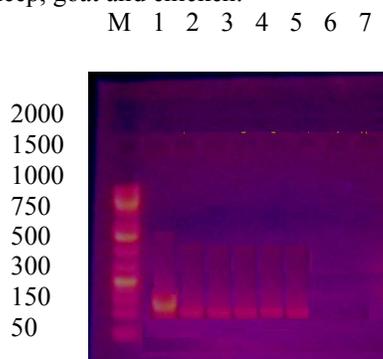


Fig (6) PCR amplification pattern of DNA fragment of camel mitochondrial D-loop gene in raw , cooked and autoclaved meat emulsion .lane M 50-bp ladder, lane 1 and 4 kabab, lane 2 and 5 patty ,lane 3 and 6 block, lane 7 negative control. Lane 1-3 contain 5% camel meat and lanes 4-6 contain 1% camel meat mixed with cattle, sheep, goat and chicken meat.

Conclusion

We have investigated the suitability of the methods for detection and identification of species in mix meat and heat treated products .the developed camel-specific PCR assay in the present study was found to be rapid, sensitive, authentic ,and cost –effective method for identification of camel in meat and meat products manufactured under different processing conditions. This assay was also efficient and effective in admixed meat and meat products and able to detect 0.05% level of camel meat in meat products containing multiple non-targeted meat species. Also no adverse effect of heat processing and ingredients used for emulsion preparation was observed on PCR amplification of desired DNA fragments. It is expected that this technique a useful laboratory tool for future species identification, especially for meat traceability.

Corresponding author

Ahmed E. Tharwat
Food Control Department (Meat Hygiene).
Faculty of Veterinary Medicine,
Zagazig University
ahmed_tharwat1983@yahoo.com

References

- Hitchcock, C. and Crimes,A.(1985):Methods for meat species identification: a Review in Meat Sci., 15:215-224.
- Hofmann, K. (1987): Fundamental problems in identifying the animal species of muscle meat using electrophoretic methods.In fleishwirts. 67:820-826.
- Mark, W. and Sandy, P. (2004): Food forensics:using DNA technology to combat misdescription and fraud. TRENDS in Biotechnology, 22(5):222-226
- Parties, L., Croan, D., Guo, Z., Clark, R., Coldham, T. and Murby, J. (2000): Evaluation of a DNA fingerprinting methods for determination the species origin of meats. Meat Sci., 54:369-376.
- Mane, B., Mendiratta, S. and Tiwari, A. (2009): polymerase chain reaction assay for identification of chicken in meat and meat products. Food Chem., 116:806-810.
- Ebbehoj, K. and Thomsen, P. (1991): Differentiation of closely related species by DNA hybridization. Meat Sci., 30:359-366.
- Hunt, D. and Parker, H. (1997): Identification of the species of origin of raw and cooked meat products using oligonucle- otide probes. Food Chem., 60:437-442.
- Arsalan, A., Ilhak, O. and Calicioglu, M. (2006): Effect of method of cooking on identification of heat processed beef usinig polymerase chain reaction (PCR) technique. Meat Sci., 72:326-330

Chen, Y., Ya-Jun, W., Liang, X. and Qian, Z. (2005):
Species- specific polymerase chain reaction

amplification of camel (camelus) DNA Extracts. J
of AOAC. International, 88(5):1394-139

11/20/2011