Int.J.Curr.Microbiol.App.Sci (2020) *9*(6): 1197-1203

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 9 Number 6 (2020)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2020.906.149

Identification of Species of Meat Origin using *ATPase* Gene Variability by the Polymerase Chain Reaction

P. P. Bhavsar[1*], M. N. Brahmbhatt[2], J. B. Nayak[2], B. C. Parmar[2], J. H. Chaudhary[2], H. K. Gida[2] and D. M. Paghdar[2]

[1]Department of Livestock Products Technology, [2]Department of Veterinary Public Health and Epidemiology, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, Gujarat, India

**Corresponding author*

Keywords

Animal foods, DNA, meat Species, *ATPase*

Article Info

Accepted:
18 May 2020
Available Online:
10 June 2020

A B S T R A C T

Food authenticity is currently an issue of major concern for food authorities, since incorrect labelling of animal foods may have remarkable negative consequences. To circumvent this problem, DNA based method had been utilised. The present study was carried out for the detection of meat species by using variability in *ATPase Subunit 6 and 8* genes by multiplex PCR. Meat samples from cattle, buffalo, sheep, goat, chicken and dog were utilized for molecular analysis. KAPA express extract kit was used to extract DNA from meat samples. Sequences among mitochondrial *ATPase Subunit 6 and 8 genes* were targeted for species-specific amplifications. The specificity of the primers was checked by using the Basic Local Alignment Search Tool (BLAST) software. The designed primers yielded specific amplification of 65, 107, 144, 186, 200 and 232 bp for Goat, Sheep, Buffalo, Dog, Poultry and Cattle respectively. Further to detect the sensitivity of the assay different level of meat mixture of 16, 8, 4, 2, 1 and 0.1% was formulated by adding pork. The assay successfully identified the presence of meat at the level of 1%. In the current assay, cooked and putrefied meat samples also showed successful amplification of the DNA, so this assay is useful in the detection of meat adulteration.

Introduction

Indian subcontinent covers a very large area with huge diversity in terms of geography, season and religion. All three parameters greatly affect food habits and large geography make it possible for various types of adulteration. This includes the admixture of cheaper ingredients in meat products to earn more profit. Rapid urbanization and higher disposable income led to a hike in the consumption of non-vegetarian food in recent years. Once the meat has been removed from the carcass, it is not always easy to identify the different species visually. More the processed food it is more difficult to identify its origin. It is the step of processing where adulteration and / or contamination can occur.

This harms the health of consumers, as well as undermines national economies and in some cases violates established religious norms for Hinduism and Islam to not include beef and pork in their diet. Beyond socio-religious factors, food allergy due to the consumption of a particular type of meat or meat products has emerged as another major health concern implicating the beef (73%), pork (58%) and chicken (41%) as the most common cause (Ayuso *et al.,* 1999). These factors raised immediate concerns in consumers for proper labelling of meat products (Ballin, 2010).

Cawthorn *et al.,* (2013) have also reported a high incidence of mislabelling of meat products in South Africa. Likewise, Nischala *et al.,* (2016) have reported high incidence of mislabelling of chevon sold in the retail sales units in India.

Quinto *et al.,* (2016) have reported 18.3% of mislabelling in game meat sold in the USA, while Kane and Hellberg (2016) have reported 35% mislabelling in the online specialty meat distributors. Amaral *et al.,* (2017) have reported undeclared pork species in 54% of the analysed samples and 40% of Halal products with traces of pork.

More incidences of imprecise labelling led to concern in consumers thus identification of animal species in meat products is important to maintain consumer trust and food quality (Devine & Dikeman, 2014). Various methods can be utilised for meat tissue identification which are mainly based on protein, lipid or DNA (Aida *et al.,* 2005).

Identification of meat species is mainly conducted using DNA is often preferred mainly because of the thermal stability of DNA, unique nature and the ubiquitous presence of the DNA (Vallejo-Cordoba *et al.,* 2005; Montowska and Pospiech, 2010).

Materials and Methods

Samples

To calibrate the protocol 5 samples from each 6 animals namely cattle, buffalo, sheep, goat, poultry and dog has been obtained from known source. All the meat samples were tested for the assay in raw, heat treated and putrefied form.

Preparation of meat mixtures of different proportion

To determine sensitivity of the assay 6 mix of16%, 8%, 4%, 2%, 1% and 0.1% was formed. by adding meat of 6 species of animals viz. Caprine, Ovine, Bubaline, Bovine, Canine and Poultry as per Table 1.

DNA extraction

All the samples were then subjected for extraction of the DNA by KAPA Express Extract Kit by standard protocol provided by the manufacturer. In brief DNA extractions are performed in 100 µL volumes, and it has been set up as 88 µL PCR-grade water, 10µL10X KAPA Express Extract Buffer, 2 µL 1 U/µL KAPA Express Extract Enzyme and about 2 mm^3 of meat sample in a 200 µL PCR tube and it has been subjected for lysis in thermal cycler 75^0C for 10 min and 95^0C for 5 min for enzyme activation. Mixture was then subjected to centrifugation at 10000 RPM for 2 min to pellet the debris at the bottom. Supernatant of DNA was harvested in a different tube and diluted with 10 mM Tris HCl.

Primer designing

Primers used in this study were designed by targeting *ATPase synthase subunit*6&8 (ATP 6 &8) gene of mitochondrial DNA. The primers are developed by using Primer – 3

software of National Centre for Biotechnology Information. All the primers were subjected to "BLAST" (Basic Local Alignment Search Tool) for checking their species-specific binding during PCR. Details of the primers are given in table 2.

Primer dilution

The primers used in this experiment were supplied in freeze dried form and they were dissolved in 0.3 X TE to obtain desirable concentration to use it in PCR. All the primers were initially dissolved for obtaining final concentration of 20 p mol / µl for PCR.

PCR reaction

PCR reaction was carried out in total 25 µl volume in PCR tube as per the table 3 and table 4 by subjecting Pre denaturation at 94 ^{o} C for 10 min followed by 35 cycles of Denaturation, Annealing and Extension at 94^{o} C for 20 S, 61^{o} for 20 S and 72^{o} for 30 S followed by final extension at 72 ^{o} C for 10 min.

Gel electrophoresis

PCR products were then subjected for agarose gel electrophoresis. Agarose gel was prepared by dissolving 2gm agarose in 100 ml of 1X Tris- borate EDTA (TBE) buffer. After cooling to approximately to 50 ˚C, one drop of ethidium bromide (Thermo Scientic) was added. The gel casting tray, with opened ends sealed with adhesive tape was placed on a horizontal surface. Gel comb was then placed in the designated slots of casting tray. The molten agarose preparation was poured into the tray and left undisturbed for certain period. After solidification of the gel the comb was gently taken out and adhesive tapes were removed. The casting tray along with the set gel was submerged in the submarine electrophoresis tank keeping the wells at the cathode end with electrophoresis buffer (0.5 X TBE) at least 1-2 mm above the upper surface of the gel. PCR products were loaded in the appropriate wells by mixing them in to 6X gel loading dye. Electrophoresis was carried out at 85 V for 60 min and then it has been subjected for gel documentation.

Results and Discussion

During gel documentation the result of uniplex PCR obtained species specific bands of 65, 107, 144, 186, 200 and 232 bp amplicon without any false positive result for Goat, Sheep, Buffalo, Dog, Poultry and Cattle respectively in Figure 1.

In multiplex PCR carried out reaction 6 distinct bands of 65, 107, 144, 186, 200 and 232 bp amplicon was found for 16% meat mixture described in table 1. This assay also amplified bands of desired product length from raw, heat treated and putrefied meat as in Figure 2.

Table.1 Composition of meat mixtures of different proportions

Proportion	Goat %	Sheep %	Buffalo %	Cattle %	Dog %	Poultry %	Pig %
16	16	16	16	16	16	16	4
8	8	8	8	8	8	8	52
4	4	4	4	4	4	4	76
2	2	2	2	2	2	2	88
1	1	1	1	1	1	1	94
0.1	0.1	0.1	0.1	0.1	0.1	0.1	99.4

Table.2 Details of designed primers for species specific multiplex amplification

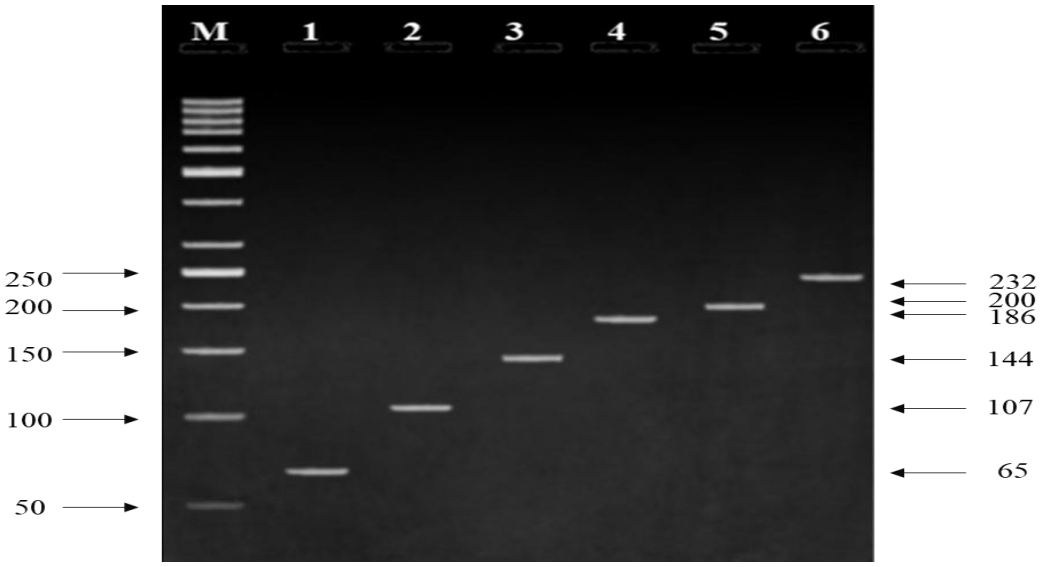
Sr. no.	Name of Primer	Composition of Primers From 5' → 3'	PCR fragment position	Product length
1	Goat F	CGACTTCTACCACAACCCAGAA	NC_005044.2	65
2	Goat R	TTGTTTCTCAAGGGGTGTTATGC	7864 - 7929	
3	Sheep F	CACAACTTCTACCACAACCCAG	EF490456	107
4	Sheep R	AGGGGTAATGAAAGAGGCAAATAGA	7865 - 7972	
5	Buffalo F	TGCCACAGTTAGACACATCAACATGACT	NC_006295.1	144
6	Buffalo R	TGTCTTGGTATTTTTGTTGGTTGTTTTGT	8145 - 8289	
7	Dog F	CGATAACCAAATCTGCTAAAATTGCTGG	EU177862	186
8	Dog R	AATGGAGATTAACCGATTATTGATTAGGCG	7914 - 8100	
9	Poultry F	CAATTAAACCCAAACCCATGATTCTCCA	NC007236	200
10	Poultry R	GATTCCTAGTAGGCAGGGGCTTGAGAAT	9091-9291	
11	Cattle F	AACATGACTGACAATGATCTTATCAATATTCTTGA	DQ480503	232
12	Cattle R	ATAGTAGGCTTGGGAATAGTACGATAAGGGTT	8150 -8382	

Table.3 Composition of PCR mix for uniplex PCR

Ingredient	Volume
Master mix 2X	12.5 µl
Forward primer (10 p mol / µl)	1 µl
Reverse Primer (10 p mol / µl)	1 µl
DNA template	3 µl
Nuclease Free Water	7.5 µl
Total Volume	25 µl

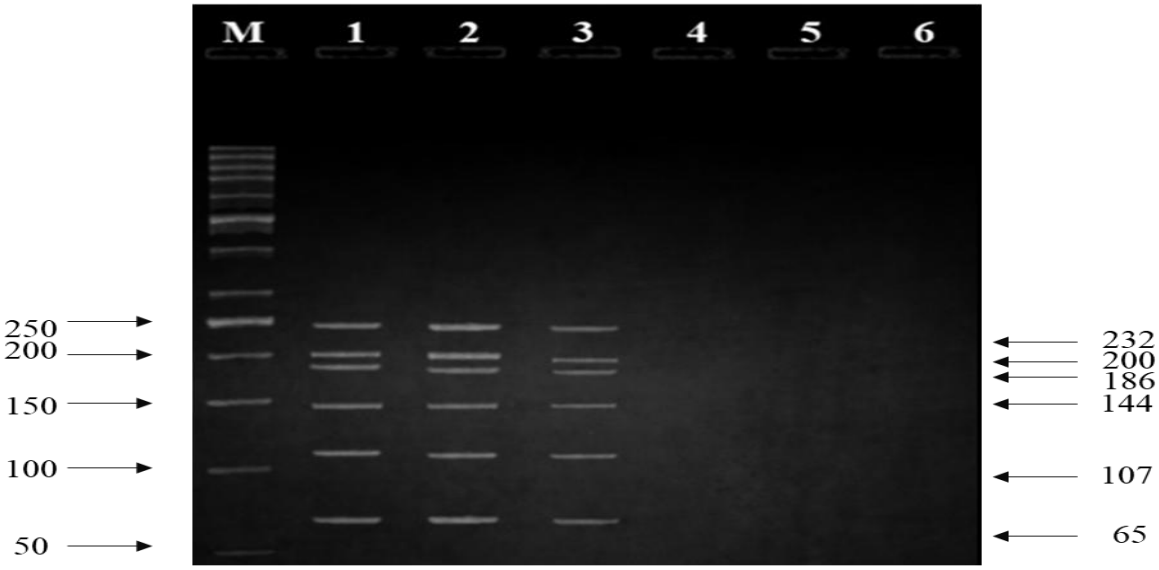
Table.4 Composition of PCR mix for multiplex PCR

Ingredient	Volume
Master mix 2X	12.5 µl
Primers (20 p mol / µl) 0.5µl each primer	6 µl
DNA template	3 µl
Nuclease Free Water	3.5 µl
Total Volume	25 µl



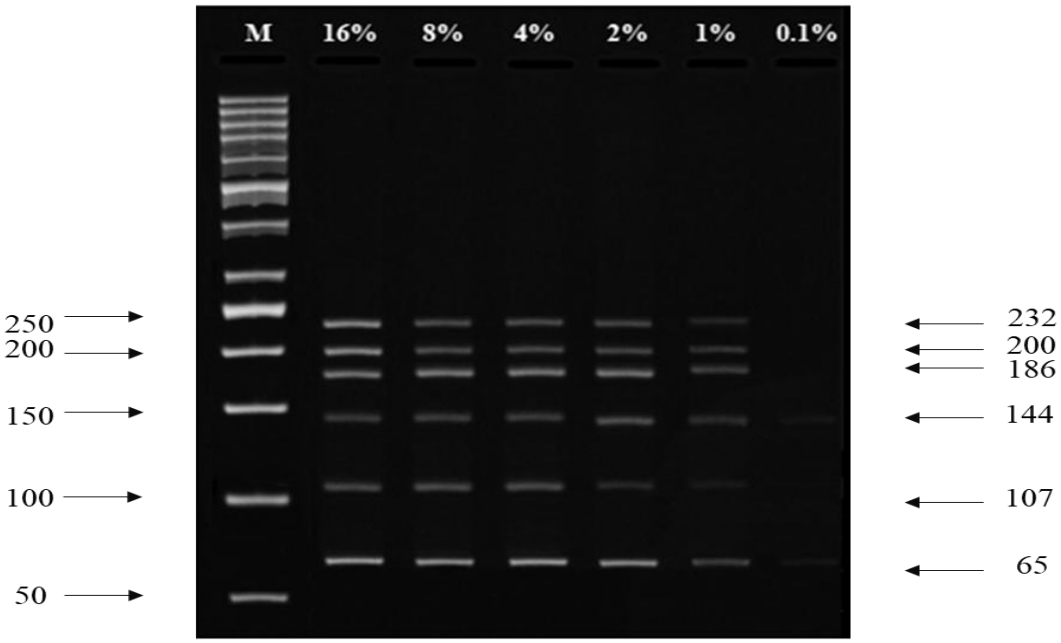
M - Molecular Marker
1 - Goat specific amplicon 65 bp
2 - Sheep specific amplicon 107 bp
3 - Buffalo specific amplicon 144 bp
4 - Dog specific amplicon 186 bp
5 - Poultry specific amplicon 200 bp
6 - Cattle specific amplicon 232 bp

Figure.1 Species specific Uniplex PCR amplification



M - Molecular Marker
1 - Fresh untreated meat
2 - Cooked meat
3 - Putrefied meat
4 - Blank
5 - Blank
6 - Blank

Figure.2 PCR amplification of fresh, heated and putrefied meat



M - Molecular Marker
16% - 16% mix lane
8% - 8% mix lane
4% - 4% mix lane
2% - 2% mix lane
1% - 1% mix lane
0.1% - 0.1% mix lane

Figure.3 Sensitivity determination of PCR assay

Sensitivity of the assay

DNA mixture of various proportions 16%, 8%, 4%, 2%, 1% and 0.1% was subjected for multiplex detection of meat origin by PCR. Primers amplified different amplification size based on the species and simultaneously the detection limit of the assay was also judged. In this study DNA of all the species has been successfully amplified for up to 1%. So this assay can detect meats of animals for up to 1% adulteration (Figure 3).

Hopwood *et al.,* (2000) detected 1% chicken in lamb using PCR. The result of the study is the same for all the species accounted in this study. Mane *et al.,* (2009) identified chicken meat in meat products with similar sensitivity and reported that cooking and autoclaving has no negative effects on poultry DNA fragments. In the current study also successful PCR amplification was made in uncooked, cooked and putrefied meat. Soares *et al.,* (2010) performed PCR and detected 0.1% pork in poultry from the DNA extracted from the binary meat mixtures of pork and poultry while the detection limit of the current assay is 1% for all the species.

Zarringhabaie *et al.,* (2011) established multiplex PCR sensitivity of 10% for cattle, buffalo, sheep and goat meats in their respective binary meat mixtures. In this study sensitivity of the assay is 1% for all the species. Nischala (2016) carried out touchdown duplex PCR assay for detection of mutton and chevon using a common forward primer and species-specific reverse primers for sheep and goat targeting the *cytb* gene. When performed on the binary meat mixtures showed sensitivity of 5% in case of mutton and 10% in case of chevon. In this study, for all cases, the sensitivity of assay is 1%.

References

Aida, A. A., Man, Y. C., Wong, C. M. V. L., Raha, A. R., & Son, R. (2005). Analysis of raw meats and fats of pigs using polymerase chain reaction for Halal authentication. *Meat science*, *69*(1), 47-52.

Amaral, J. S., Santos, G., Oliveira, M. B. P., &Mafra, I. (2017). Quantitative detection of pork meat by EvaGreen real-time PCR to assess the authenticity of processed meat products. *Food control*, *72*, 53-61.

Ayuso, R., Lehrer, S., Tanaka, L., Ibanez, M., Pascual, C., & Burks, A. *et al.*, (1999). IgE antibody response to vertebrate meat proteins including tropomyosin. *Annals of Allergy, Asthma & Immunology*, *83*(5): 399-405. doi: 10.1016/s1081-1206(10)62837-2

Ballin, N. Z. (2010). Authentication of meat and meat products. *Meat science*, *86*(3), 577-587.

Cawthorn, D. M., Steinman, H. A., & Hoffman, L. C. (2013). A high incidence of species substitution and mislabelling detected in meat products sold in South Africa. *Food Control*, *32*(2), 440-449.

Devine, C., & Dikeman, M. (2014). *Encyclopedia of meat sciences*. Elsevier.

Hopwood, A.J., Fairbrother, K.S., Lockley, A.K., Bardsley, R.G.: An actin gene-related polymerase chain reaction (PCR) test for identification of chicken in meat mixtures. *Meat Science*, 1999; *53*: 227-231.

Kane, D. E., & Hellberg, R. S. (2016). Identification of species in ground meat products sold on the US commercial market using DNA-based methods. *Food Control*, *59*, 158-163.

Mane, B. G., Mendiratta, S. K., & Tiwari, A. K. (2009). Polymerase chain reaction assay for identification of chicken in meat and meat products. *Food Chemistry*, *116*(3), 806-810.

Montowska, M., &Pospiech, E. (2010). Authenticity determination of meat and meat products on the protein and DNA basis. *Food Reviews International*, *27*(1), 84-100.

Nischala, S., Vaithiyanathan, S., Ashok, V., Kalyani, P., 2016. Detection of mutton and chevon by PCR assay using cyt b gene primers. In: Proc of 7th Indian meat science association (IMSA) conference held on November 10-12 at GADVASU, Ludhiana, India. pp 311.

Quinto, C. A., Tinoco, R., &Hellberg, R. S. (2016). DNA barcoding reveals mislabeling of game meat species on the US commercial market. *Food Control*, *59*, 386-392.

Singh, V. P., & Neelam, S. (2011). Meat species specifications to ensure the quality of meat: a review. *International Journal of Meat Science*, *1*(1), 15-26.

Soares, S., Amaral, J. S., Mafra, I. and Oliveira, M. B. P. P. (2010). Quantitative detection of poultry meat adulteration with pork by a duplex PCR assay. *Meat Science 85*(3): 531–536.

Vallejo- Cordoba, B., González- Córdova, A. F., Mazorra- Manzano, M. A., & Rodríguez- Ramírez, R. (2005). Caillary electrophoresis for the analysis of meat authenticity. *Journal of separation science*, *28*(9-10), 826-836.

Zarringhabaie, G.E., Pirany, N. and Javanmard, A. 2011. Molecular traceability of the species origin of meats using Multiplex PCR. *African Journal of Biotechnology 10*(73): 15461-16465.

How to cite this article:

Bhavsar. P. P., M. N. Brahmbhatt, J. B. Nayak, B. C. Parmar, J. H. Chaudhary, H. K. Gida and Paghdar. D. M. 2020. Identification of Species of Meat Origin using *ATPase* Gene Variability by the Polymerase Chain Reaction. *Int.J.Curr.Microbiol.App.Sci*. 9(06): 1197-1203.
doi: https://doi.org/10.20546/ijcmas.2020.906.149