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ORIGINAL ARTICLE

Authentication of camel meat using species-specific PCR and PCR-RFLP

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Abstract In India and some of the African countries, one of the unconventional meats receiving the latest attention in meat adulteration is camel meat. So, the objective of this study was to develop a species-specific PCR based on mitochondrial cytochrome b (CYTB) gene and a PCR-RFLP assay of mitochondrial 12S rRNA to identify camel meat in suspected samples. Known sample of camel meat, samples suspected to be from illegally slaughtered camel and known samples of cattle, buffalo, sheep, goat, pork and chicken were used in the study. DNA were extracted from all samples following spin column method and PCR amplification were carried out using both CYTB and 12S rRNA gene primers. The CYTB gene amplification produced amplicon with a size of 435 bp without any non-specific spurious amplification towards other species studied. Further, the 12S rRNA PCR products were analysed both by sequencing and by RFLP using enzyme *Alu*I. On BLAST analysis the 448 bp sequence obtained from suspected samples showed > 99% sequence homology to previously reported *Camelus dromedaries* (accession no: AM 9369251.1). On *Alu*I digestion of the 448 bp product from both known and suspected camel samples, a specific RFLP pattern with three distinct products of 90, 148 and 210 bp size were evident, which were significantly different from the pattern of cattle, sheep, goat, chicken and buffalo. Further, after in-house validation, this cost effective and rapid method of camel meat identification is placed into practice for regular screening of vetero-legal samples in the lab.

Keywords Camel · Meat authentication · Species-specific · PCR-RFLP · 12S rRNA · CYTB gene · Vetero-legal

Introduction

The ever-increasing demand for meat due to an increasing trend in consumption make the fraudsters to search for unconventional meat, to adulterate with high-quality meat. The driving force behind any adulteration is the revenue maximization, either by using a low-cost ingredient to substitute a more expensive one, or by removing the valued component (Ioannis and Nikolaos 2005). The adulteration of high-value commercial meat, such as mutton, with low-value meat, such as chicken, pork and beef are done for financial gains, which results in high motivation of the traders to adulterate (Nischella et al. 2016; Mahajan et al. 2011).

In India and some of the African countries, one of the unconventional meats receiving the latest attention of adulteration is camel meat. However, the slaughter of camel and sale of camel meat is prohibited in India (FSSAI 2006). The total number of camels in the world is 25 million, and India possesses 0.25 million camels mostly in the Indian states of Rajasthan, Haryana and Gujarat (Indian Livestock Census 2019). The camel population in India is decreased by 37.1% from 0.40 million animals reported in the last census. Hence, conservation of this animal is important to maintain the proper ecosystem in the deserts. One of the conservation systems is based on the detection

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of illegally slaughtered and sold camel meat in the Indian market so that potential fraudsters can be identified and controlled.

However, the detection of camel meat is not easily done by physical and anatomical methods (Singh and Neelam 2011). Once the taxonomic features are removed from the meat, it is difficult to identify the species of the meat visually. Authentication methods can be categorized into the areas where fraud is most likely to occur and a lot of research has already been carried out using various biomarkers (like DNA, proteins) to detect the species and sex origin of meat. The alternative, and most accepted methods of detection of meat adulteration, are those based on DNA, such as PCR assay. These methods over the last two decades allowed the regulatory laboratories to develop reliable and accurate protocols for meat authentication (Ballin 2010).

In meat specification, either nuclear (nDNA) or mitochondrial DNA (mtDNA), genes have been targeted through PCR analyses. The mtDNA analyses were commonly used in the species-specific PCR assay, especially for food (Meyer et al. 1994; Matsunaga et al. 1999; Che et al. 2007; Girish et al. 2004; Sahilah et al. 2011; De et al. 2011; El-Morshedy et al. 2011; Vaithiyanathan and Kulkarni 2016). Unseld et al. (1995) found a high copy number of mtDNA in the cells. Besides, it remained intact during food processing thereby minimizing DNA degradation and does not contain any introns. The published methods clearly indicate that PCR using mtDNA offers both the desired sensitivity and the specificity for detection of adulteration of meat and meat products. This includes cytochrome b (CYTB) gene, 12S rRNA and D loop genes.

One of the alternative methods of detection of adulteration based on DNA is RFLP (Restriction Fragments Length Polymorphism) analysis in conjunction with PCR assay (Patil et al. 2015, Mahajan et al. 2011; Girish et al. 2005) to identify meat species. The cleavage of the small amplified DNA fragment with a specific restriction enzyme reveals polymorphism between species (Patil et al. 2015, Mahajan et al. 2011; Girish et al. 2005). Earlier researchers have used the mt 12S rRNA gene primer, mt D loop gene primer, CYTB gene primer and mt cytochrome oxidase subunit I gene primer for PCR assay followed by restriction digestion to generate species-specific polymorphism to identify meat of camel, cattle, buffalo, sheep, goat, chicken, dog at the species level (Farag et al. 2015; Patil et al. 2015; Haider et al. 2012; Mahajan et al. 2011; El-Morshedy et al. 2011; Girish et al. 2005).

Only few published reports are available on DNA based methods for camel meat identification from suspected samples of illegally slaughtered animals. Therefore, a study was designed to develop a simple and robust method of species-specific PCR assay and PCR-RFLP assay, to detect camel meat from suspected samples.

Materials and methods

Meat samples

Known samples of meat from cattle (ox), buffalo, sheep and goat were collected from municipal slaughterhouse of Hyderabad, Telangana, India, where the animals are slaughtered following Halal procedure. Pork samples were collected from registered retail meat shops of Hyderabad. Chicken meat was collected from broiler birds, which are procured from local market of Hyderabad and slaughtered scientifically in the experimental slaughter house of ICAR-NRC on Meat, Chengicherla, Hyderabad, Telangana state, India (FSSAI Registration No: 23618029000451). The samples were transported into the Meat Species Identification Laboratory (MSIL) of ICAR-National Research Centre on Meat, Hyderabad, and preserved at − 20 °C immediately. The camel (*Camelus dromedarius*) meat used in this experiment was obtained from autopsy samples of ICAR-National Research Centre on Camel, Bikaner, Rajasthan, India. Unknown samples received from law enforcement agencies of Government of India, which are suspected to be from illegally slaughtered camel were used to validate the new methodology developed for camel meat authentication.

DNA extraction

DNA was extracted from all known as well as unknown samples of meat in an accredited environment (ISO/IEC 17025: 2005), following the method of Ivanova et al. (2012) with minor modifications.

Composition of reagents

Vertebrate lysis buffer (VLB)—100 mM NaCl, 50 mM Tris–HCl pH 8.0, 10 mM EDTA pH 8.0, 0.5% SDS. Binding buffer (BB)—6 M GuSCN, 20 mM EDTA pH 8.0,10 mM Tris–HCl pH 6.4 and 4% Triton X-100 was pre warmed at 56 °C to dissolve. Binding mix (BM)—50 mL of ethanol (96%) was thoroughly mixed with 50 mL of BB (stable at 20 °C for 1 week). Protein wash buffer (PWB)—70 mL of ethanol (96%) was thoroughly mixed with 26 mL of BB (stable at 20 °C for 1 week). Wash buffer (WB)—ethanol (60%), 50 mM NaCl, 10 mM Tris–HCl pH 7.4 and 0.5 mM EDTA pH 8.0 (stored at − 20 °C).

Method protocol

A 100 mg of meat sample was mixed with 200 µL of VLB in the pre-distributed zirconium beads in the 2.0 mL micro tube. The samples were homogenized in homogenizer (Bead bug, Benchmark scientific, USA) for 3 min followed by overnight incubation at 56 °C in a water bath (Genei laboratories Pvt. Ltd., India). After centrifugation (Model no: 5430R, Eppendorf, Germany) at 10,000 rpm for 5 min, clear supernatant was collected in a fresh micro tube and gently added 100 µL of BM buffer followed by slight vortex (Model: Spinix, Tarsons, India) and pipetting it in the spin column (SRL, Mumbai, India). They were then centrifuged at 11,000 rpm for 5 min and discarded and replaced with new collecting tube. Then, 180 µL of PWB added in column and centrifuged at 11,000 rpm for 2 min and discarded and replaced with new collecting tube. The spin column was washed twice with 300 µL of WB by centrifugation at 14,000 rpm for 5 min and the centrifugation was repeated one more time. Spin column with collecting tubes were kept at 56 °C in a heating block (Major Science, USA) for 30 min to evaporate the residual ethanol. Finally, 70 µL of pre warmed nuclease free water added for elution and incubated for 5 min at room temperature followed by centrifugation at 11,000 rpm for 5 min. Once again, the eluted DNA were incubated in dry bath at 60 °C for 10 min before taking absorbance reading in Nano spectrophotometer (Biospec Nano, Shimadzu, Japan) and using them in PCR assay.

Designing of oligonucleotide primer pair

Species-specific primer pair for detection of camel meat DNA was designed based on mitochondrial cytochrome b (CYTB) gene using the accession number X56281.1 from National Centre for Biotechnology Information (NCBI). Alignments and comparisons of available gene sequences of camel and other species were made before designing the primer pair using NCBI GenBank database. The primer was designed using the online Primer Blast software (NCBI primer blast). The primer pair designed was synthesized from Bioserve Biotechnologies India, Pvt. Ltd. (Hyderabad, India). The details of species-specific primer pair used in the present investigation are given below:

L378 (forward: 5′-AGCTTTCATGGGCTACGTCC C-3′)
H812 (reverse: 5′-TCCGGCTTGATATGTGGTGG-3′)

Species-specific PCR assay

In the present study, the primers mentioned above were used for camel specific PCR assay standardization. Each PCR amplification reaction was set in a volume of 25 µL with 2.5 µL of 10X PCR buffer (New England Biolabs, USA) with following composition (100 mM Tris–HCl, pH 9.0, 15 mM MgCl2, 500 mM KCl and 0.1% gelatin), 0.5 µL of 10 mM dNTP mix (Chromous Biotech, Bangalore), 0.5 µL (20 pmol) each of forward and reverse primers (Bioserve Biotechnologies India, Pvt. Ltd., Hyderabad, India), 1U of Taq DNA polymerase (New England Biolab, USA) and 50 ng of purified DNA. Volume was made up to 25 µL by adding nuclease free water (SRL, Mumbai, India). Reaction conditions on a gradient thermocycler (Eppendorf, Germany) were as follows: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 0.5 min, annealing at 54.3 °C for 0.5 min and extension at 72 °C for 0.75 min. Then, final extension was done at 72 °C for 10 min. Amplified products were analyzed by electrophoresis in 2% agarose gel with ethidium bromide (0.5 µg/mL) staining and images were captured through gel documentation system (Alpha Image, USA).

Validation of the species-specific PCR method

The species-specific PCR assay designed for authentication of camel meat has been validated following the Codex Alimentarius guidelines (CAC/GL 74-2010). The validation has been performed as in the same way as the method is intended to be used for routine sample analysis.

Specificity

Experimental evaluation of the specificity of the assay has been performed using known samples of target as well as non-target animal species. Animal species which are closely related and commonly used in food were used to evaluate the ability of the method to distinguish target and non-target animal species. Sufficiently high concentrations of DNA from all the animal species studied were tested thrice and each time in triplicate using the primer pair designed. Results at which target species yields positive results and non-target species yield negative results at least 95% of the time were reported.

Sensitivity

Experimental evaluation of the sensitivity of the assay has been performed to establish the range of the method using different concentrations of target animal species DNA. This could establish the reliability of the test to detect a positive sample and at the same time doesn't give rise to false positive.

Absolute limit of detection (LoD_{abs}) Absolute limit of detection for a qualitative assay is defined as the lowest concentration of DNA at which positive samples yields positive results at least 95% of the times (CAC/GL 74-2010). DNA from known samples of camel meat and with initial concentration of 100 ng was diluted serially at 1:10 ratio for five times. PCR reactions were conducted for each dilution in triplicate and the whole experiment was performed thrice. The lowest concentration at which the test yielded positive results with 95% true positive was reported.

Relative limit of detection (LoD_{rel}) Relative limit of detection for the PCR assay has been determined using non-target animal species DNA as background for camel DNA. A 100 ng camel DNA solution with equal amount of background DNA (buffalo) were diluted serially at 1:2 ratio for 11 times. PCR reactions were conducted as the previous cases and results with 95% true positive were reported.

PCR-RFLP assay of 12S rRNA gene

PCR amplification of 12S rRNA gene sequences were carried out using the primer pair described by Kocher et al. (1989) and the primer composition is as follows:

Forward-5′-CAA ACT GGG ATT AGA TAC CCC ACT AT-3′
Reverse-5′-GAG GGT GAC GGG CGG TGT GT-3′

PCR conditions used were similar to that of species-specific PCR (except for temperature of annealing at 55.4 °C) and the results were recorded.

Sequencing and identification of restriction site

Products of 12S rRNA gene amplifications were sequenced with plus strand using Big Dye Terminator v3.1 Cycle Sequencing Kit (Life Technologies, USA) and an Applied Biosystems 3730 × 1 genetic analyser (Life Technologies, USA) at DNA sequencing facility of Bioserve Biotechnologies (India) Pvt Ltd, Hyderabad. The sequences obtained were queried in Gene Bank using the basic local alignment search tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and the top species matches were recorded. Further the sequences were analysed for restriction sites using the online restriction mapper site (http://restrictionmapper.org/). Restriction enzyme with unique restriction patterns with respect to camel species was selected for further analysis.

Restriction fragment length polymorphism

PCR amplicons of the mt 12S rRNA gene were subjected to restriction enzyme digestion with enzyme *Alu*I, following the procedure of Mahajan et al. (2011) and Girish et al. (2005). Briefly, enzyme-buffer mix was prepared by mixing 2 µL of restriction enzyme (ThermoFisher Scientific, USA) with 8 µL of the respective buffer (ThermoFisher Scientific, USA). Reaction mix was prepared by mixing 10 µL PCR products with 2 µL of enzyme buffer mix. Volume was made up to 20 µL with nuclease free water (SRL, Mumbai, India) and incubated overnight at 37 °C. Reactions were conducted as the previous cases and results with 95% true positive were reported.

Field sample analysis using the assay developed

During the study, in our laboratory we encountered a unique problem of 16 unknown meat samples of different species to resolve. These samples were submitted by the law enforcement agency who requested us to detect the species origin of the samples. Hence the developed protocol of species-specific PCR and PCR-RFLP for authentication of camel meat has been utilized for the purpose.

Results and discussion

In the present study, a species-specific PCR assay was developed and validated for authentication of camel meat targeting mitochondrial Cytochrome b (CYTB) gene. Further, a PCR-RFLP assay that produces unique and reproducible camel specific digestion pattern from amplified products of 12S rRNA gene digested using *Alu*I enzyme was also standardized.

Species specific PCR assay

A species-specific primer pair was designed targeting mitochondrial CYTB gene for authentication of camel meat without any cross reactivity towards other meat animal species like cattle, buffalo, sheep, goat, chicken and pig. In order to optimize the PCR assay, a gradient PCR was performed to ascertain the optimal annealing temperature for primers. It was observed that the designed primer pair produced a 435 bp amplicon in the temperature range of 50–60 °C studied. Optimal annealing was observed at temperature of 54 °C and without any non-specific amplicons (Fig. 1).

Similarly, various species-specific PCR assay were already standardized for pork (Meyer et al. 1994), beef (Arslan et al. 2006), chicken (Mane et al. 2009), buffalo (Mane et al. 2012) and for pork, beef, sheep, goat, camel

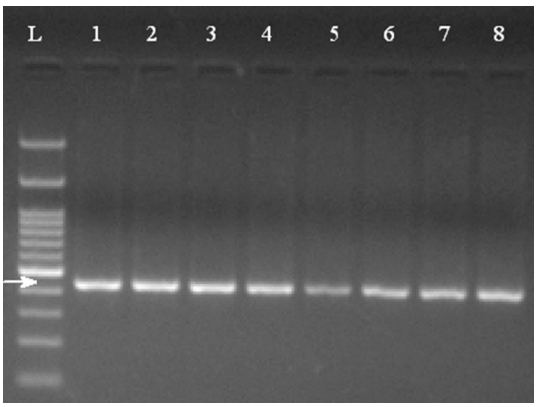

Fig. 1 Agarose gel (2%) electrophoresis of amplified product of DNA from camel meat (435 bp) in camel specific (gradient) PCR assay. (L: 100 bp + ladder, L1, L2, L3, L4, L5, L6, L7 and L8 denotes annealing temperatures of 50, 51.4, 52.9, 54.3, 55.7, 57.1, 58.6 and 60 °C respectively)

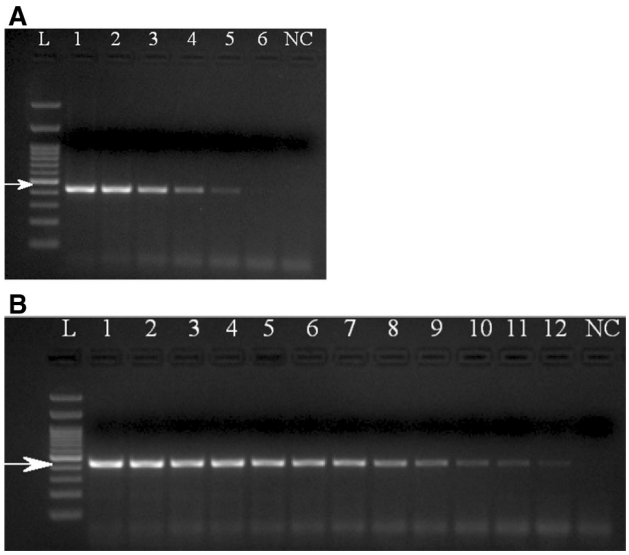

Fig. 2 **a** Agarose gel (2%) electrophoresis of camel specific amplified product (435 bp) in absolute limit of detection assay (L: 100 bp + ladder, L1, to L6 represent serial dilutions of camel DNA with concentrations of 100 ng, 10 ng, 1 ng, 0.1 ng, 0.01 ng and 0.001 ng and NC is negative control). **b** Agarose gel (2%) electrophoresis of camel specific amplified product (435 bp) in the relative limit of detection assay (L: 100 bp + ladder, L1 to L12 represents camel DNA template having background DNA (buffalo) with decreasing concentrations of 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19, 0.09 and 0.045 ng respectively and NC is negative control)

and chicken (Ibrahim et al. 2015). Many mitochondrial sequences have been attempted as amplification targets such as Cytochrome b gene, Cytochrome oxidase I gene, rRNA genes, D-loop genes, ND5 gene by several investigators (Girish et al. 2016, 2007; Karabasanavar et al. 2011). However, targeting CYTB is promising to get differentiation between species due to its sequence variability, which makes it appropriate for comparisons at subspecies, species and genus levels (Romaino et al. 2014). Species specific primers used in the present study yielded an easily differentiable PCR product of 435 bp. The products formed only with the target species and positive results were reported at least 95% of cases.

Validation and performance characteristics of the assay

In the specificity analysis, developed PCR assay amplified and yielded the amplicon with size of 435 bp without any non-specific spurious amplifications at least 95% of positive cases. For other non-targeted animal species studied such as cattle, buffalo, sheep, goat, chicken and pig, no false positive results were reported. This finding showed that the primer pair designed is specific for camel meat species authentication.

The optimized PCR assay was further tested for sensitivity both in terms of absolute limit of detection as well as relative limit of detection, with 95% true positive rate. The results presented in the Fig. 2a showed that the assay can detect up to 10 pg of camel DNA. The lowest concentration of target DNA when the single source DNA with concentration of 100 ng was serially diluted (1:10) was reported as LOD_{abs}. The conventional PCR assay has a sensitivity of 10 pg of DNA (Karabasanavar et al. 2011) and this study too have achieved fairly high LOD_{abs} of 10 pg. It has been reported that the amplification of short fragment of a target DNA produces higher sensitivity (Mendoza-Romero et al. 2004). Matsunaga et al. (1999) reported a sensitivity of 250 pg DNA templates in a PCR assay with a 274 bp product size, Frezza et al. (2003) reported sensitivity of 1 pg for 147 bp product size and Karabasanavar et al. (2011) reported a sensitivity of 1 pg for both 329 and 404 bp products size. El-Morshedy et al. (2011) have reported camel specific PCR assay using mitochondrial D-loop gene and could detect up to 0.05% level of adulteration of camel meat. Haider et al. (2012) and Farag et al. (2015) have reported a camel specific PCR-RFLP assay but the detection level was not reported. In the present study for camel specific PCR with a relatively large amplicon size of 435 bp has been reported with optimum absolute limit of detection required for meat authentication in regulatory laboratories.

The results presented in the Fig. 2b showed that the assay can detect up to 0.045 ng camel DNA at least in 95% cases and reported as relative limit of detection (LOD_{rel}). LOD_{rel} of 0.045 ng for camel DNA was reported when DNA with concentration of 100 ng was serially diluted at 1:2 ratios in the presence of background DNA (buffalo). Quantity of PCR products as visualized in the gels was observed to be directly proportional to the concentration of

camel DNA with a detection threshold of 45 pg. Therefore, the developed camel meat specific PCR assay is robust, since it can detect as low as 45 pg camel DNA, in the presence of background DNA.

PCR-RFLP assay

Universal primer technology (UPT) targeting the mt 12S rRNA gene were used in this study to amplify the DNA template from known meat samples of camel, cattle, buffalo, sheep, goat and chicken. The in silico analysis reported the product size as 448 bp for camel, 440 bp for cattle, buffalo, sheep and goat and 444 bp for domestic chicken. This observation was slightly different from the previous reports where a common PCR product size of 456 bp was reported for cattle, buffalo, sheep, goat and camel (Girish et al. 2004; 2005; Patil et al. 2015; Mahajan et al. 2011). There was no size variation observed in the amplicons as visualized in the agarose gel electrophoresis (Fig. 3a). This is due to the small difference in the PCR product size which is difficult to precisely separate in gels and that could be the reason for reporting a common amplicon size. In the case of chicken, there was an additional non-specific product of 200 bp. The mt sequences are highly conserved in various species of animals and general differences in mt 12S rRNA gene sequences are sufficient for species identification (Prakash et al. 2000). This property has enabled researchers to design universal primers which can amplify corresponding fragments in a wide variety of organisms (Kocher et al. 1989).

Sequencing and identification of restriction site

Sequences of mt 12S rRNA gene from authentic camel meat with 448 bp size were retrieved from the ABI files and subjected for annotation using chromaslite software. The sequences obtained were analysed using BLAST in NCBI. These sequences showed 99% homology to previously reported *Camelus dromedarius* (accession no: AM 9369251.1). The in silico analysis using the sequences obtained in the present study showed that *Alu*I restriction digestion can produce different fragments to differentiate the camel, cattle, buffalo, sheep, goat and chicken species. As per the restriction mapper virtual digestion using *Alu*I, it was reported 3 products (210, 148, 90 bp) for camel, 2 products (350, 90 bp) for cattle, 2 products (239, 199 bp) for sheep and goat and three products (244, 153, 47 bp) for chicken. And buffalo product remained undigested during *Alu*I RE digestion.

Restriction fragment length polymorphism

The endonuclease enzyme, *Alu*I, cut the amplified product of 12S rRNA gene and yielded 210, 148 and 90 bp fragments in camel, while it yielded 350 and 90 bp fragments in cattle (Fig. 3b). In the case of sheep and goat, the products yielded were 239 and 199 bp size. Visualization of this polymorphism patter can be used to differentiate sheep and goat meat from other species whereas differentiation between these two species is difficult to achieve. Further, this enzyme generated 244, 153 and 47 bp products from the amplified product of chicken DNA template. These results are slightly different from the earlier reports on the PCR-RFLP analysis of cattle, buffalo, sheep, goat

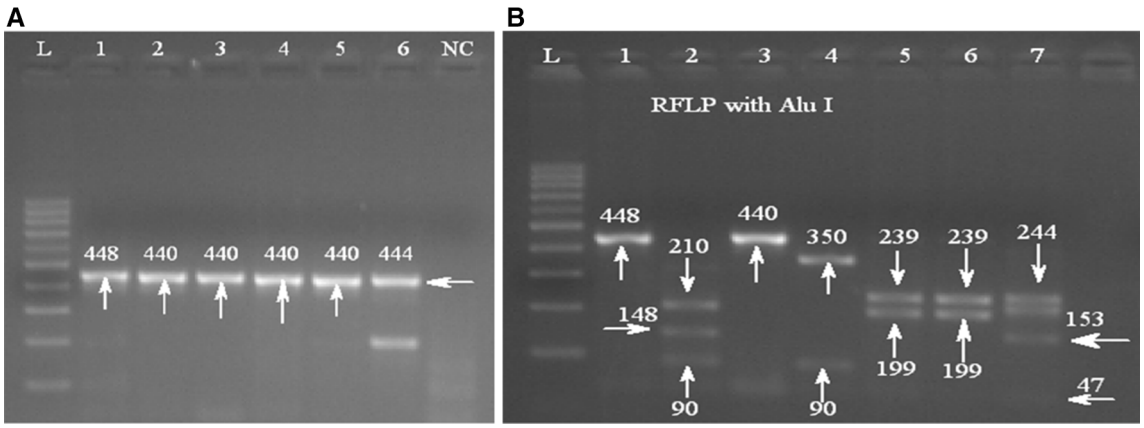

Fig. 3 **a** Agarose gel (2%) electrophoresis of amplified products of mitochondrial 12S rRNA gene. (L: 100 bp + ladder, L1, L2, L3, L4, L5 and L6 represents PCR products from camel, cattle, buffalo, sheep, goat, chicken and NC is negative control). **b** PCR-RFLP analysis of mt 12S rRNA gene after restriction digestion with *Alu*I. Amplified and digested products were analysed by 2% agarose gel electrophoresis. (L: 100 bp + , L1: undigested product of camel DNA (448 bp), L2: camel DNA (210, 148, 90 bp), L3: buffalo DNA (440 bp), L4: cattle DNA (350, 90 bp), L5: sheep DNA (239, 199 bp), L6: goat DNA (239, 199 bp) and L7: chicken DNA (244, 153, 47 bp)

and chicken (Patil et al. 2014, 2015; Girish et al. 2005). Earlier studies carried out using the mt 12S rRNA PCR-RFLP assay have shown the efficacy of these methods in precise identification of cattle, buffalo, sheep, goat, chicken and dog meat (Mahajan et al. 2011; Girish et al. 2005).

With regards to other assays for camel meat identification, Haider et al. (2012) have reported, meat species identification of many animals including camel targeting mt COI gene PCR-RFLP assay using *Hpa*II enzyme. El-Morshedy et al. (2011) have reported the camel meat identification by species specific PCR assay using mt D loop gene primer and PCR-RFLP using *Taq*I restriction enzyme. Farag et al. (2015) have reported CYTB PCR-RFLP assay using four restriction enzymes (*Alu*I, *Hae*III, *Hinf*I and *Taq*I). Thus, the results obtained in the present study using the time tested and the robust mt 12S rRNA PCR-RFLP assay with *Alu*I clearly demonstrate the camel species identification without any ambiguity and it possess potential field application.

Field sample analysis

One of the methods we employed to test the field samples is the forensically important nucleotide sequence (FINS) analysis (Girish et al. 2005) and found that three samples out of 16 were from camel (unpublished data). Further to confirm the authenticity of the samples based on the laboratory policy before reporting to the customer, we utilized the species-specific PCR and PCR-RFLP assay developed and validated in-house. The results of species-specific PCR analysis along with other non-targeted species was reported (Fig. 4). PCR-RFLP assay further confirmed the results reported using camel specific PCR assay (Fig. 5). This approach helps in ensuring the validity of test reports as part of laboratory accreditation and submitting the analysis reports for legal enforcement.

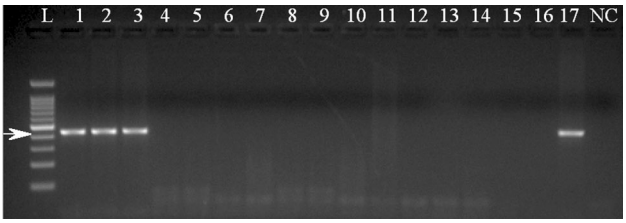

Fig. 4 Agarose gel (2%) electrophoresis of camel specific amplified product (435 bp) in PCR assay of field samples. (L: 100 bp + ladder, L1, L2 and L3 are suspected samples of camel meat, L4, L5, L7, L12, L13, L14: cattle, L6, L8, L9, L11, L15, L16: buffalo, L10: chicken, L17: positive sample of camel meat, NC: negative control)

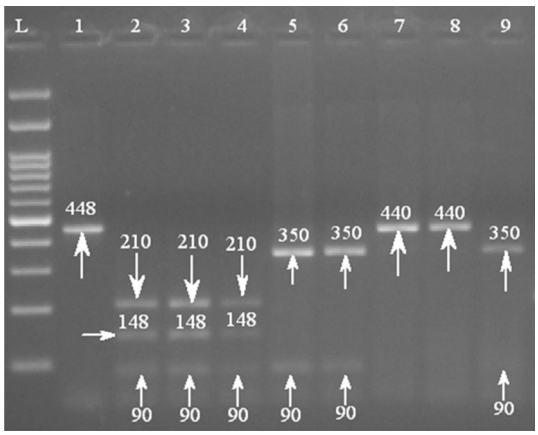

Fig. 5 PCR-RFLP assay of mt 12S rRNA gene of field samples after restriction digestion with *Alu*I. Amplified and digested products were analysed by 2% agarose gel electrophoresis. (L: 100 bp + ladder, 1: undigested product of camel DNA (448 bp), 2, 3, 4: camel DNA (210, 148, 90 bp), 5, 6, 9: cattle DNA (350, 90 bp) and 7, 8: buffalo DNA (440 bp))

Conclusion

Meat species identification is considered important because of food frauds committed by the unscrupulous meat traders/food processors. At present scenario, DNA based methods such as PCR assay and PCR-RFLP techniques are the most preferred for meat species identification because DNA based methods provide sensitivity, accuracy, repeatability and reproducibility. The relative merits of species-specific PCR assay developed in the present study is that, in single step PCR and gel electrophoresis the conclusive identification of camel meat can be done. The developed PCR assay is having LOD_{abs} of 10 pg and LOD_{rel} of 45 pg. This assay does not have cross reactivity towards other species such as cattle, buffalo, sheep, goat, chicken and pork. Similarly, PCR-RFLP assay developed, is relatively cost effective and does not require sequencer for conclusive identification of camel meat and useful in the field application for camel meat species identification.

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