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Research Article

Applicability of Polymerase Chain Reaction for the Detection of Meat Species in Dried Animal Tissues (pet chews)

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ABSTRACT

Polymerase chain reaction technique was employed to identify the meat species of dried animals tissue like trachea, oesophagus, ligamentum nuchae, penis, ears obtained from meat processing plants which are used as pet chews for feeding of pet animals. The simultaneous detection of both the species cattle and buffalo at a time was achieved by conducting duplex polymerase chain reaction. Using mitochondrial sequences, species specific primers of cyt b gene was used for cattle and buffalo primer at 274 bp and 425 bp respectively. All the samples screened were tested positive for buffalo species which could be due to the ban of cow slaughter and it's processing in India.

Key words: *Pet chews, Cyt b gene, Cattle, Buffalo.*

INTRODUCTION

Exports of animal products represent an important and significant contribution to the Indian Agriculture sector. The export of animal products includes buffalo meat and its processed products. The country has exported 1350563.48 MT of buffalo meat products to the world for the worth of Rs.26033.83 Crores/ 4036.89 USD Millions during the year of 2017-18. The demand for Indian buffalo meat in international market has sparked a sudden increase in the meat exports. Buffalo meat dominated the exports with a contribution of over 89.08% in total animal products export from India[1]. With the rapid increase in buffalo meat processing, there would be increased availability of byproducts which can be processed further as pet food supplements for feeding of dogs and cats which will boost up the revenue for meat processors. The pet population in India is increasing at a robust pace, where majority of the pet owners are adopting dogs due to the need for security and companionship. In addition, rising number of affluent and growing middle class families are also increasingly adopting pets. As a result, the demand for pet food is rising, mainly in urban centres of India.

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The reluctance of cattle meat and its products by different groups of people due to the religious sentiments associated with it and also due to the ban of cow slaughter and export of its products in India is yet another reason for considering cattle meat and its products in both human and animal food as fraudulent substitution. So there is a need to develop effective techniques to check the meat species incorporated in pet foods.

Meat proteins and DNA molecules have been used as species specific biological markers for meat species identification. However, proteins lose their biological activity after an animal dies, and their presence and characteristics depend on cell types and processing can alter the structure and stability of protein. Furthermore, most of them are heat labile. Thus, for meat species identification, DNA analysis would be preferable to protein analysis. A number of studies have addressed meat species identification in the recent past and attempts were made to differentiate closely related meat species[9,4].

Moreover, PCR assay has no problem of cross reaction with closely related species and requires less time[3]. So, an attempt has been done to screen the meat species present in dried pet chews using polymerase chain reaction technique.

MATERIAL AND METHODS

Pet chew samples comprising of dried trachea, dried penis, dried oesophagus, dried ligamentum nuchae and dried ears were procured from commercial meat processing plant where the byproducts are dried sterilized and sold as pet chews. The meat samples of cattle and buffalo for positive controls were procured from local slaughter house. The collected samples are transported hygienically to the laboratory with proper packing.

DNA was extracted from 25 mg of each sample with QIAamp DNA mini kit (QIAGENE). The quality and quantity of the DNA extracted from samples were determined by spectrophotometry, using a Nanodrop one^{C} spectrophotometer (Thermo Scientific, USA).

Species specific primers for cattle and buffalo were procured as per Matsunaga *et al.*[5] and Narendra Babu *et al.*[7] listed in table-1.

Table-1

Species	Sequence	Reference
Universal forward	5’-GAC CTC CCA GCT CCA TCA AAC ATC TCA TCT TGA TGA AA-3’	Matsunaga *et al.*[5]
Cattle reverse	5’CTA GAA AAG TGT AAG ACC CGT AAT ATA AG-3’	-do-
Buffalo reverse	5’- CCA ATG TAT GGG ATT GCT GAG AG-3’	Narendra Babu *et al.*[7]

PCR amplification was carried out using a master thermal cycler (Eppendorf, Germany). Duplex PCR reaction was carried out by simultaneous use of both cattle and buffalo species primers. PCR reactions were carried out in a volume of 25 µl with 12.5 µl master mix, 4 µl template DNA, 2 µl of universal forward and 1 µl each of cattle reverse and buffalo reverse primer and 4.5 µl of nuclease-free water to adjust the volume.

The PCR programme used for amplification of target genes is as follows: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds and extension at 72°C for 30 seconds. Then final extension was conducted at 72°C for 10 minutes. The amplicons were separated by agarose gel electrophoresis (2% prepared in 0.5X TBE buffer (Tris-borate-ethylenediaminetetraacetic acid) at 50 V for 1.5 and stained with ethidium bromide.

RESULTS AND DISCUSSION

Identification of species in meat products by PCR amplification of specific genes has received particular attention in recent years. The PCR method could give reliable results and also could work with processed samples where the DNA would still be amenable to

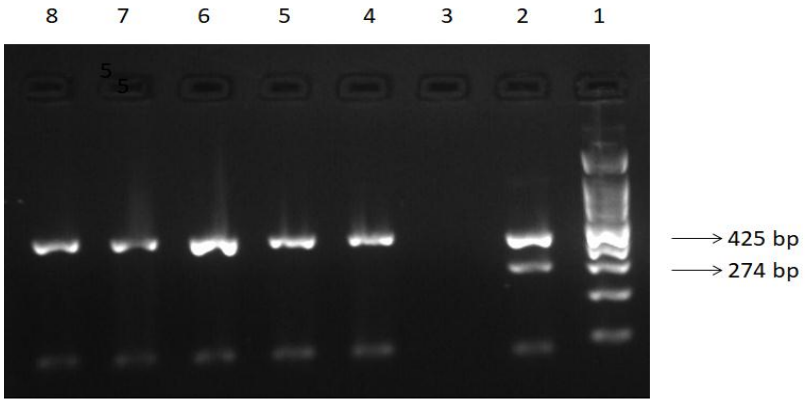
PCR amplification even after subjecting to heat during processing of the food products[8]. The present study proved the statement mentioned above with the reproducible result obtained.

The results showed that extracted DNA was suitable for PCR amplification. The purity (purity=A260/A280) and yields of the total DNA extracted were found to be satisfactory ranging between 1.72±0.01 and 1.83±0.02 and yield between 74.27±0.57 and 152.0±0.32 ng/µl respectively.

The Polymerase chain reaction (PCR) amplified products (Samples 1,2,3,4 and 5) run in agarose gel (2%) electrophoresis analysed in gel documentation system revealed mitochondrial DNA cytochrome b gene with 425 bp (figure 1). The results were comparable with the results of Gupta *et al.*[2] who concluded that cattle and buffalo meat could be reliably identified and differentiated using duplex PCR at optimized conditions, detecting up to 1 pg adulteration in cattle-buffalo meat mixture.

Similarly, Munira *et al*, 2016 concluded that mitochondrial *Cyt b* gene could be used as an excellent molecular marker for detecting species origin of meat as the conventional and duplex PCR revealed expected different specific amplified PCR products of buffalo and cattle species respectively, for pure and mixed sample of those species.

Plate 27



PCR amplification of Dried pet chews

Lane 1	**:**	**100 bp DNA Ladder**
Lane 2	**:**	**Positive control**
Lane 3	**:**	**Negative control**
Lane 4-8	**:**	**Dried trachea, dried penis, dried ear, dried oesophagus and dried ligamentum nuchae samples**

CONCLUSION

The present study indicates that DNA based method provides a reliable species specific identification. The PCR method offers fast, sensitive and specific in species identification. It describes a simple and promising method for meat species identification in meat products which can be applied by research bodies and quality control laboratories for authentication of species present in meat and meat products.

The results revealed the presence of buffalo meat species in all the dried pet chews which indicates that there is no adulteration with cattle meat species. This is a clear indication of ban of cow slaughter and processing in India and its exports to other countries.

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