Development of PCR Assay for Differentiation of Goat Meat from Dog Meat

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ABSTRACT

A polymerase chain reaction (PCR) assay was developed to differentiate meat of goat from meat of dog. A set of primers which is designed according to the sequence of mitochondrial cytochrom *b*. gene of goat was used and by PCR amplification about 150 bp band was observed only for goat DNA sample. These primers do not produce any amplified products with dog meat DNA sample under the tested reaction condition. A band of 649 was observed for both dog and goat DNA samples when DNA was amplified with Universal primers and that indicated the presence of mitochondrial DNA in the samples. Further, the results indicated that this technique was sensitive enough to differentiate rotten meat, at least five days after killing. However this technique was not sufficiently sensitive to differentiate cooked meat of these two species.

KEY WORDS: Dog Meat, Goat Meat, Meat Identification, PCR

INTRODUCTION

Goat meat, or mutton, is one of the most widely consumed meats in the world. It has an excellent flavor somewhat similar to beef. It has less fat than chicken or any of the red meats commonly consumed in Sri Lanka. This is because goats tend to deposit their fat internally before they deposit it externally. When a goat is slaughtered, this internal fat is removed along with the rest of the "innards". A well-conditioned goat does have a tiny coating of fat over its muscles that help keep the meat from drying out rapidly. Goats do not marble (intersperse fat within their muscles), thus, goat fat along a cut of meat is usually easily trimmed. Although goat meat is low in fat, studies have indicated that this fat contains a higher percentage of cholesterol than chicken or beef fat. However, these same studies have concluded that goat meat is much lower in saturated fats than the rest of the commonly consumed meats including chicken with the skin off. It has an excellent ratio of polyunsaturated to saturated fats making it a very healthy choice of meat. Because of these excellent qualities price of mutton is very high in Sri Lanka (Table 1).

The average price of a kg of goat meat was Rs. 180.00 in 1994. In 2001 it was Rs. 280.00/kg (Anon 2002). The overall increase of price was 55.6%.

Per capita availability of goat meat has been reduced by 25% during the period of 1995-1999 (Food Balance Sheet, 1995-1999, Anon 2000) Production of goat meat is also low compared to the other meat types like chicken, beef or pork. Small population size (Food Balance Sheet, Anon 2002) and poor productivity can be other reasons for non availability of goat meat.

Compared to developed countries and developing countries in the region, the goat meat consumption in Sri Lanka is very much low. However, as the selling price of goat meat is high, adulteration of goat meat in order to obtain higher profits is a common problem.

It is believed that dog meat is being sold as goat meat. There have been many recent newspaper articles reporting such incidences. As stray dogs are available in large numbers and the killing of dogs is not banded in Sri Lanka, there is a high chance of selling dog meat as mutton because of the similar size of both animal species.

Table 1. Prices of meat types in Sri Lanka market in second week of July 2005. (Anon 2005)

Type of meat	Price (Rs/kg)
Beef	174.71
Mutton	309.33
Chicken	
Fresh	189.65
Broiler	166.62
Pork	175.33

There are incidences of selling of cat meat with goat meat too. Although adulteration of mutton with dog or cat meat and selling is an offence, implementation of legal procedures has been constrained by several factors. The most notable constrain is the lack of facilities for rapid and reliable identification of species from which meat is derived and this is, clearly, essential for implementation of legal procedures.

Adulteration of other meats such as beef with buffalo meat is also common in Sri Lanka. In addition, sale of the meats of protected animals such as the animals of the *Cervus* family (spotted deer, sambhur, barking deer and hog deer) and many other wild animals is on increasing trend. Therefore a reliable and rapid diagnostic method is a prerequisite for taking legal action in this respect.

At present, immunological methods such as agar gel precipitation test (AGTP) and Dot blot assay or counter immunoelectrophoresis are being used for the identification of meat (Dissanayaka *et al.*, 2001) These methods are based on the detection of specific meat proteins, which are denatured or destroyed during processing and in the process of rotting. Therefore, the validity of these traditional methods has been highly limited in identifying processed or rotten meat because of poor sensitivity. In Sri Lanka one of the most

important factors is the time period between the killing of animal and the meat sample reaching the laboratory. Majority of the meat samples received are rotten. Further, lack of specific anti serum for goat and dog too has been a serious problem with the conventional diagnostic techniques.

Polymerase Chain Reaction (PCR) method has been successfully used to identify various meats of domesticated animals and meat products (Chikuni *et al.*, 1994, Lee *et al.*, 1994).) This technique has also been established in fish varieties (Unseld *et al.*, 1995). In these studies mitochondrial *cytochrome b* gene has been used and the PCR products have been studied by Restriction Fragment Length Polymorphism (RFLP) analysis. Recently, a multiplex PCR technique was developed for identification of meat of some domesticated animals (Matasunaga *et al.*, 1999). Similarly a PCR method for the identification of meat of *cervus* family (Rajapaksha *et al.*, 2002) and buffalo (Rajapaksha *et al.*, 2003) was developed in Sri Lanka.

The biggest disadvantage of the PCR based finger printing methods is the very high costs involved due to the usage of more expensive enzymes and other chemicals. Other disadvantages are need of highly skilled labor and sophisticated laboratory equipment.

This study was carried out as a continuation of a research project by Veterinary Research Institute, Peradeniya, having the objective of developing PCR based technique for identification of meat of various species of animals. In this study particular attention was made to develop a specific PCR technique to differentiate the meat of goats from that of dogs.

MATERIALS AND METHODS

Deoxyribose nucleic acid samples were collected from dog and goat meat samples, using DNAzole (Life technologies, USA), according to the protocol given by the manufacturers.. Briefly, about 50mg of meat was used to extract DNA and total DNA was dissolved in 250µl of water. One microlitre of DNA was amplified using two universal primers designed from conserved region of the mitochondrial cytochrom b. gene (Matasunanga et al, 1999). This was used as a positive control for the PCR. A set of primers designed according to the published region of the *cytochrom b.* gene of goat was used as specific primers. PCR amplification was conducted in 30µl of 20mM $MgCl_2$, 100 µM dNTP, 75mM Tris-Hcl, 20mM $(NH_4)_2So_4$, 2pmol of each primer and 1 unit of taq polymerase (sigma, st. Louis, Mo USA).

The following oligonucliotide primers were used as universal primers.

5'TAGGCGAATAGGAAATATCATTCGGGTTTGAT3'(p1)

5'CAAATCCTCACAGGCCTATTCCTAGC3' (p2)

Following two primers (synthesized by MWG-Biotech,Ebersberg, Germany.) were used as specific primers to differentiate goat DNA samples.

5'CTCGACAAATGTGAGTTACAGAGGGA3'(p3)

5'GACCTCCCAGCTCCATCAAACATCTCATCTTGATGAA A3' (p8)

After 1 min. of initial denaturation at 95°C, 35 cycles of 94°C for 30s, 52°C for 30s (annealing) and 1min. of 72°C and 5min of final elongation step were run using thermal cycler(Amplitron® II, Thermolyn, USA) PCR machine. For each PCR amplification, a negative control was run to check the contamination. Then, the PCR products (10µl) were run with lambda DNA digested by Hind III as a molecular sized marker in 1% agarose gel stained with ethidium bromide and visualized under UV light.

To study the effect of rotting of meat on sensitivity of the PCR assay, meat samples from goat were kept at room temperature (25°C – 30°C) for 2, 3, 4 and 5 days before the extraction of DNA. Then DNA was amplified with the specific primers under the same reaction condition.

Similarly, DNA was extracted from goat meat boiled for 30mins, which is similar to the normal cooking time. This DNA was amplified with specific primers under the above mentioned PCR reaction conditions and PCR products were run in an agarose gel and visualized under UV light.

RESULTS AND DISCUSSION

Differentiation of meat

When DNA was amplified with P_1 and P_2 (universal primers) a band was observed for both meat samples and there was no band for negative control (Fig.1). This indicates the presence of DNA in the samples. This is also an indication of the accuracy of the DNA extraction method used. Although use of kits *i.e.* DNAzole (Life technologies, USA) is expensive compared to the conventional method of extracting DNA but it has shown promising results. For a large number of samples, use of DNAzole for the extraction of DNA would be more appropriate due to the shorter time period taken. In this study, the DNA quantification was not done. Therefore, it is difficult to compare the efficacy of the DNA extraction of the two methods. However there are advantages and disadvantages in both methods.

By amplification of the same DNA with P_3 and P_8 (goat specific primers) a band of about 150bp was observed only for DNA from the goat meat sample(Fig. 1).Therefore it can be concluded that PCR method can be successfully used to differentiate meat of goat from the meat of dog.

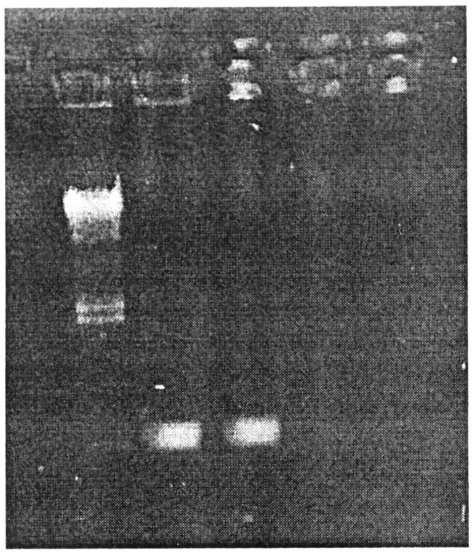
Sensitivity of the PCR assay on rotten meat

Correct size bands were observed when the DNA from all the stages of rotten meat was amplified by specific(P_3/P_8) primers. (Fig. 2)

Sensitivity of the PCR assay on cooked meat

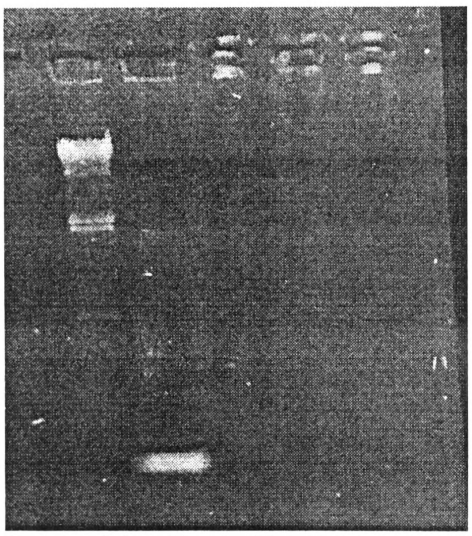
No bands were observed when the DNA extracted from cooked meat was amplified with both sets of primers under the same PCR reaction conditions. As the extraction of DNA from the samples was accurate, theoretically there should be bands with the cooked meat sample too. Generally cooking temperature should not destroy the DNA structure.

(a)



M G D -ve

(b)

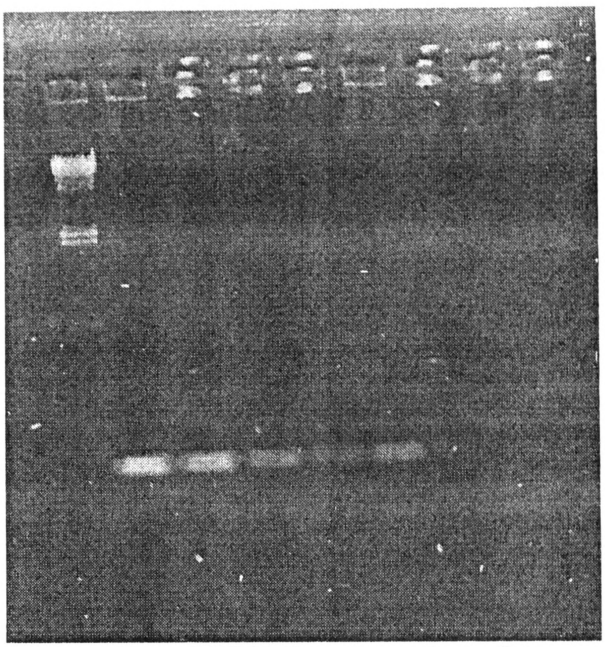


M G D -ve

Fig. 1. One percent agarose gel electrophresis of PCR products of Goat and Dog DNA with universal primers. A band of 646bp is visible for both animal species except negative control (panel a). Panel b shows the products of PCR amplification of DNA of Goat and dog with goat specific primers (P3 and P8). Molecular size marker and animal species are indicated at the bottom of the picture. M=Marker, G=Goat, D=Dog, -ve= Negative control.

Absence of bands with the specific primer may be due to a technical error in the PCR process. Further trials are needed to establish PCR technique to detect DNA from cooked samples.. Under the PCR conditions described here, the goat specific primers (P3 and P8) produced positive results only for the goat and not cross reacted with DNA of dog.

Further research is also needed to establish a technique to differentiate cat meat from that of goat as cat meat is also a possible adulterant for goat meat.



M 1 2 3 4 5 C -ve

Fig. 2. PCR amplification of DNA extracted from decomposed meat and boiled meat with specific primers. Molecular size marker(M), days of process of rotting (1-5), and cooking (C), are indicated at the bottom of the picture.

CONCLUSIONS

In the present study, a simple, sensitive and accurate PCR method to differentiate meat of goat from dog was developed. These results demonstrate the potential use of this technique for the protection of consumer rights of the country. The legal authorities should utilize this facility not only to control adulteration of goat meat with other types of inedible meat but also to control adulteration of other meat as well as killing of many species of wildlife for human consumption.

Although the capital and recurrent expenditures are high in the application of PCR technology to differentiate meat samples, there is a valid reason for a reliable and rapid technique in order to protect the customers as well as exploitation of the valuable indigenous animal genetic resources.

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