Identification of Horse Meat and Beef using a Polymerase Chain Reaction Based Method with *Cytochrome b* Gene

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Introduction

Adulteration of meat and meat products are taking place in many parts of the world and it is believed that numerous such incidents have even occurred in Sri Lanka as well. Demanding meat and meat products are being adulterated with cheaper or unconventional meat types (eg. dog, horse or rat meat) with phenotypic similarities. This situation created a scenario where the food analyst from many developing countries needs to pay special attention to identify meat. A recent disclosure in UK about an adulteration of beef with equine meat created a paranoid situation, which drastically affected genuine brands produced by large companies and international food chains (Thomson, 2013). Hence, the adulteration must be prevented to ensure traceability of meat from farm to form. In general, molecular methods facilitate accurate and more reliable analysis of meat adulteration. Compared to nuclear DNA, application of mitochondrial DNA (mtDNA) in meat identification experiments provides many advantages, such as maternal inheritance and ubiquitous natures (Kvist, 2000). Thus, mtDNA can be used when the evidentiary material supply is limited. Among the Mitochondrial genes mitochondrial cytochrome b (*Cyt b*) gene has often used to detect the source of meat (Farias, *et al*., 2001). Hence, the aim of this study is to establish qualitative polymerase chain reaction method to detect horse meat adulteration in beef using mitochondrial DNA *Cyt b* region.

Methodology

Beef and horse (*Equus ferus caballus*) meat samples were collected from slaughter house at Dematagoda and from Faculty of Veterinary Medicine and Animal Science, at University of Peradeniya, respectively. Genomic DNA was extracted following the protocol as described in Abdel-Rahman *et al.* (2009) with modifications. First, 600 mg of tissue was homogenized in 6000 μL STE buffer and 48 μL of 10% SDS and 24 μL of proteinase K (10 mg/mL) was added. Then, the mixture was incubated at 37 °C overnight. After that, DNA was purified by equal volumes of phenol–chloroform–isoamylalcohol (25:24:1) and chloroform–isoamylalcohol (24:1), successively. Then, DNA was precipitated by adding chilled ethanol in the presence of 3 M sodium acetate. The resulting pellet was washed with 70% ethanol, air-dried and subsequently dissolved in 80 μL of TE buffer.

Species specific primers were designed (horse forward (HF) - ATC ATC ACA GCC CTG GTA GTC GTA CAT, horse reverse (HR) - ATG TGG AGG GTG GGG ATG AGT GCT A, cattle forward (CF) - CAT CGG CAC AAA TTT AGT CG and cattle reverse (CR) - GAG CTA GAA TTA GTA AGA GGG CC) to amplify mitochondrial *Cyt b* gene of cattle and horse. The PCR products were electrophoresed on 2% agarose gel containing 0.5 μg/mL Ethidium bromide and were visualized and imaged using a UV trans-illuminator (Gel Dox XP+ system, BioRad) and gel documentation system (Image Lab ™ 3.0, BioRad) to distinguish the species origin.

Furthermore, to investigate the detection limit of the PCR system, DNA was extracted from 600 mg of beef which was mixed separately with 1%, 5% and 20% of horse meat.

Results and Discussion

MtDNA evolve at a much faster rate than nuclear DNA. At the same time different regions of the mitochondrial genome would evolve at different rates. Therefore, mtDNA maintain variable regions but with a certain level of conservation. Similarly, mitochondrial *Cyt b* gene contains both slow and rapid evolving regions with conservative and variable regions. The evolution of the *Cyt b* gene is prevented due to the functional restrictions in the conservative regions (Farias, *et al.*, 2001). Therefore, *Cyt b* gene is used to identify horse meat from beef as the sequence variability in *Cyt b* gene between different species is extremely high.

PCR amplification of horse and cattle DNA produced bands of 370 bp and 250 bp fragments at 2% agarose gel electrophoresis (Figure 1). Therefore, the size difference in amplified fragments can be used effectively to distinguish the two meat species. Furthermore, the specificity of primers was confirmed by having a band for horse specific primers in beef and vise versa (Figure 1).

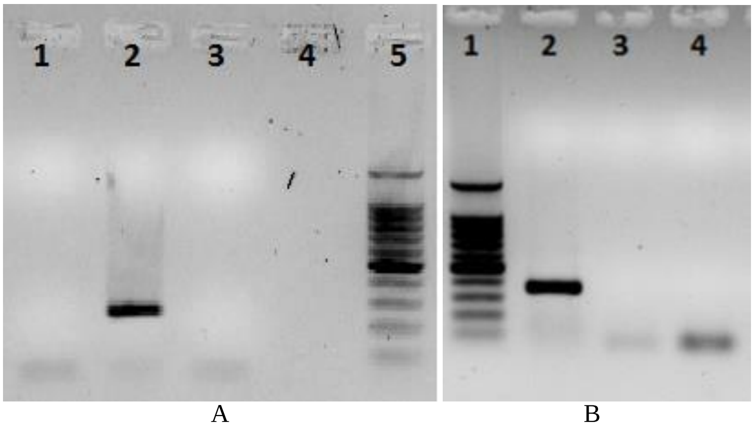

Figure 1. PCR amplification of 100% cattle DNA with cattle specific primers and 100% horse DNA with horse specific primers. A: PCR amplification using cattle specific primers. Lane 1, 100% horse DNA; Lane 2, 100% cattle DNA; Lane 3, negative control with no DNA template; Lane 5, 100 bp ladder. B: PCR amplification using horse specific primers. Lane 1, 100 bp ladder; Lane 2, 100% horse DNA; Lane 3, 100% cattle DNA; Lane 4, negative control with no DNA template.

Furthermore, HR and HF primers were used to check the sensitivity of the PCR assay by mixing 1%, 5% and 20% of horse meat in beef. The sensitivity of the assay using horse specific primers is great as it can detect an adulteration even up to 1% horse meat in beef (Figure 2). Further, the results also confirm the specificity of horse specific primers by providing single bands at cattle and horse meat mixtures (Figure 2). Moreover, non template controls were included in every batch of PCR assay to confirm the absence of contaminations.

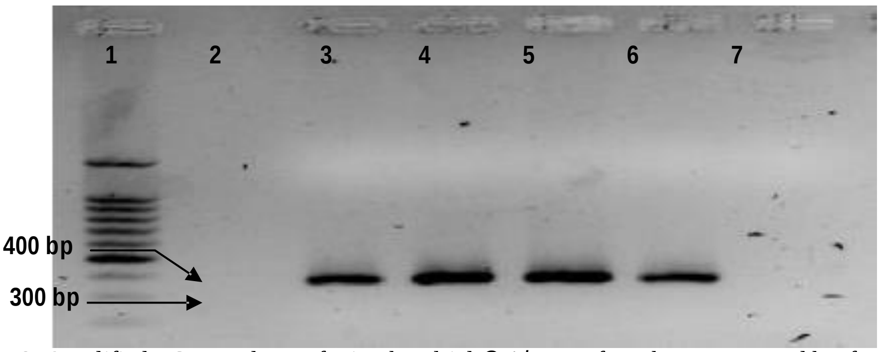

Figure 2. Amplified PCR products of mitochondrial *Cyt b* gene from horse meat and beef samples using horse specific primers. Lane 1, 100 bp ladder. Lane 2, negative control. Lane 3, 1% horse meat in beef. Lane 4, 5% horse meat in beef. Lane 5, 20% horse meat in beef. Lane 6, 100% horse meat. Lane 7, 100% beef.

Therefore, the assay has a high potential to be used as a molecular based method to verify horse meat adulteration in beef and therefore to control the contamination of raw beef and beef products such as sausages, burgers, minced meat and etc., with horse meat.

Conclusion

This study reveals a successful qualitative PCR based method to detect horse and cattle meat types. Importantly, PCR assay with horse specific primers have a detection level up to 1% of horse meat adulterated by beef.

References

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