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PCR-Based Detection of Horse Meat in Commercially Processed Meat Products

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Abstract: *This study presents a validated PCR-based method for detecting horse meat (Equus caballus) in processed food products. A pair of species-specific primers targeting the COX1 mitochondrial gene was designed and tested for specificity, sensitivity, and robustness. The assay detected horse DNA at levels as low as 0.005 ng/µl, and was effective in various processed food conditions. Applied to commercial products, it confirmed correct labelling and absence of adulteration in non-equine items. The method is cost-effective, sensitive, and suitable for routine food authenticity testing.*

Keywords: *Horse meat, PCR, food adulteration, COX1 gene, mitochondrial DNA, species identification*

• Introduction

Food adulteration involving undeclared meat species poses risks to public health, consumer trust, and regulatory systems. The 2013 horse meat scandal highlighted the need for more reliable detection methods. Traditional assays lack the sensitivity to identify trace animal DNA in processed products. This study addresses that gap by developing a sensitive and specific PCR assay targeting the mitochondrial COX1 gene for equine DNA detection.

• Material and method

- Biological samples: Raw horse meat and 7 commercial meat products (salami, sausages, burgers).
- DNA extraction: MagneSil® Magnetic Purification System, evaluated for purity and yield.
- Primer design: COX1-targeted primers (346 bp), validated in silico (NCBI Primer-BLAST).
- PCR protocol: 35 cycles, GoTaq® Green Master Mix, annealing at 60°C.
- Validation: Specificity (cross-species), sensitivity (serial dilutions), robustness (processing methods).

• Results and discussions

Specificity: Horse DNA exclusively amplified. No cross-reactivity with bovine, porcine, ovine, poultry, plant or fungal DNA.

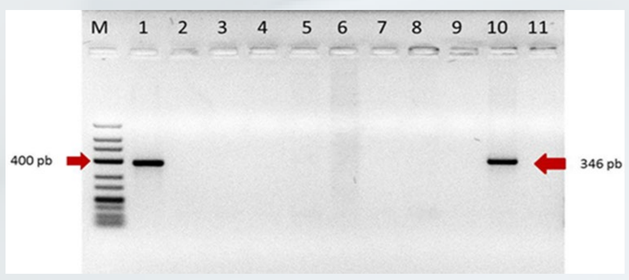

Figure 1. Electrophoresis gel image illustrating specific amplification in horse DNA sample. Negative controls (bovine, porcine, poultry, soybean) show no product.

Sensitivity: Detection threshold at 0.005 ng/µl; detectable down to 0.005% in mixed matrices.

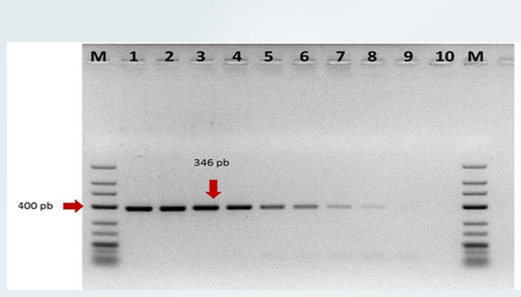

Figure 4. Gel electrophoresis showing results from serial dilutions of horse DNA.

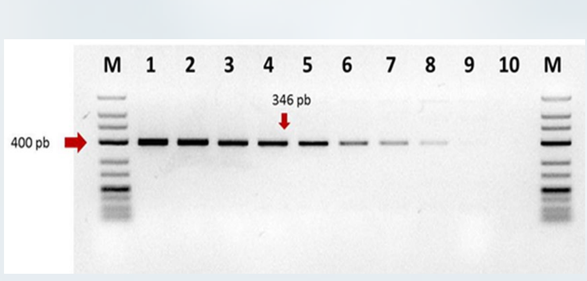

Figure 3. PCR detection of horse DNA in mixed matrices with soybean DNA, down to 0.005% concentration.

Processing tolerance: PCR worked even on boiled, fried, marinated, and dried meat samples.

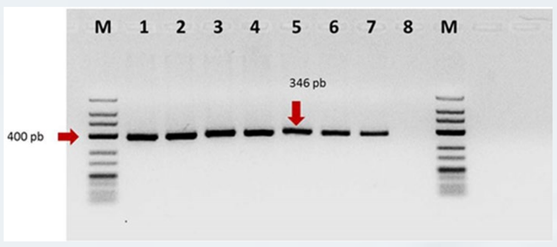

Figure 4. Amplification results from differently processed products: lanes correspond to products boiled, fried, marinated, and dried meat samples.

Commercial testing: Horse DNA correctly detected in labelled products; absent in beef or mixed-meat products.

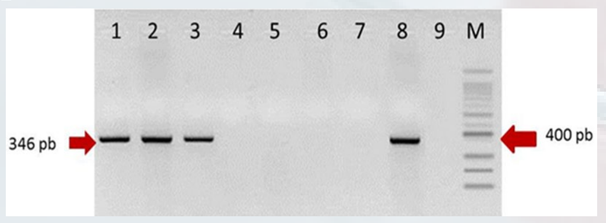

Figure 4. Applicability of the method to different food matrices. Lane M – molecular weight marker (O'Gene Ruler); Lane 1 – reference horse DNA; Lanes 2–7 – processed samples (ground, boiled, dried, marinated, fried in oil, fried in lard); Lane 8 – NTC (no template control).

Implication: This assay provides a robust molecular tool for routine detection of horse meat, enhancing consumer protection and regulatory compliance.

• Conclusions

The developed PCR assay is rapid, specific, and highly sensitive for detecting horse DNA in processed meat products. It performs reliably across food matrices, supporting its use in authenticity testing and food fraud prevention.