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Research Article

Identification of Meat Species by Polymerase Chain Reaction (PCR) Technique*

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Abstract: The origin of horse, dog, cat, bovine, sheep, porcine, and goat meat was determined by the polymerase chain reaction (PCR) technique, using species-specific primers. Test mixtures of meat were prepared by adding 5%, 2.5%, 1%, 0.5%, and 0.1% levels of pork, horse, cat, or dog meat to beef, sheep, and goat meat. Samples taken from those combinations were analyzed by PCR for species determination. Mitochondrial DNA (mt DNA) fragments of 439, 322, 274, 271, 225, 212, and 157 bp for horse, dog, cat, bovine, sheep, porcine, and goat meat, respectively, were amplified. PCR was conducted at 30 cycles for mixtures at the 5%, 2.5%, 1%, and 0.5% level, while at 35 cycles for mixtures at the 0.1% level. The results indicated that meat species were accurately determined in all combinations by PCR. It is concluded that PCR can be useful for fast, easy, and reliable control of adulterated consumer meat products.

Key Words: Meat species, mt DNA, PCR

Polimeraz Zincir Reaksiyon (PCR) Yöntemi ile Et Türlerinin Belirlenmesi

Özet: Araştırmada at, köpek, kedi, sığır, koyun, domuz ve keçi etine ait spesifik primerler kullanılarak Polimeraz Zincir Reaksiyon (PCR) yöntemi ile etlerde tür tayini yapıldı. Sığır, koyun ve keçi etlerinin her birine % 5, % 2,5, % 1, % 0,5 ve % 0,1 oranlarında ayrı ayrı domuz, at, kedi ve köpek etleri karıştırılarak tür tespiti yapıldı. Tür tespitinde at, köpek, kedi, sığır, koyun, domuz ve keçiye ait sırasıyla 439, 322, 274, 271, 225, 212 ve 157 bp'lik mitokondriyal DNA (mtDNA) parçaları çoğaltıldı. PCR işlemi; % 5, % 2,5, % 1 ve % 0,5 oranındaki et karışımları için 30, % 0,1 oranındaki et karışımları için ise 35 siklusta yapıldı. Sonuç olarak, PCR yöntemi ile kolayca, kısa zamanda ve güvenilir olarak bütün et karışımlarında tür tespiti yapıldı. Böylece et türlerinin orijini tespit edilerek halkın aldatılması engelleneceği gibi toplumun tüketmediği hayvan etleri diğer yöntemlere göre daha kolay, hızlı ve güvenilir bir şekilde saptanabilir.

Anahtar Sözcükler: Et türleri, mtDNA, PCR

Introduction

Methods used for identification of species of origin of raw meat include sensory analysis, anatomical differences, histological differentiation of the hair that may possibly exist in the meat, properties of tissue fat, and level of glycogen in muscle tissue, as well as electrophoresis and DNA hybridization (1-4). Most of these methods have been reported to have limitations in use due to problems in specificity (i.e. sensory analysis, glycogen level, histological differentiation, properties of tissue fat, and immunological methods), complexity (i.e. electrophoresis and DNA hybridization), high cost (i.e. DNA hybridization), and some requirements for baseline data about the differences in protein compositions (i.e. isoelectrofocusing) (5-7). There is a need for the development of a more accurate, fast, and easy-to-use method due to the limitations of the existing methods mentioned above (5).

Developments in molecular biology have facilitated identification of plant, bacteria, and animal species with high accuracy (8-14). Polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP), and random amplified polymorphic DNA (RAPD) techniques have been frequently used for identification of meat species (15-19).

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In the present study, the identification of different meats was determined by PCR, using species-specific primers. In addition, the sensitivity of PCR to identify particular meats in mixtures of meat was determined.

Materials and Methods

Meat samples

Muscle tissue samples from beef, goat, sheep, pig, horse, cat, and dog were used. Meat samples were stored at –20 ± 1 °C until analyzed.

Test meat mixtures

The samples of meat were minced and prepared separately by adding 5%, 2.5%, 1%, 0.5%, and 0.1% (w/w) pork, horse, cat, or dog meat to each of the beef, sheep, and goat meat samples. The mixtures of meat were prepared in a total weight of 250 g. Following mixing, a 2-g portion of each sample was taken separately from 5 different areas of each test mixture. DNA was extracted from each meat sample and used for PCR analysis.

DNA extraction from meats and meat mixtures

DNA was extracted from meat samples as described by Koh et al. (20), though with a slight modification. The sample was homogenized using 4 ml of TNES solution (20 mM Tris, (pH 8.0), 150 mM NaCl, and 10 mM EDTA) in a 15-ml polypropylene tube. A 750-µl aliquot of the resulting homogenate was then transferred into a 1.5-ml Eppendorf tube and 10 µl of proteinase K (200 mg/ml) and 50 µl of 10% SDS were added. The mixture was shaken vigorously and kept for 8 h at 58 ºC in a water bath. A 250-µl volume of 6 M NaCl was added to the resulting mixture and it was centrifuged at 11,600 ×g for 5 min. A 500-µl portion of the aquatic phase of the sample was then transferred into a separate Eppendorf tube and 300 µl of a phenol-chloroform-isoamyl alcohol (25:24:1) mixture was added, followed by vigorous shaking and centrifugation at 11,600 ×g for 5 min. A 400-µl portion of the upper layer was then transferred into another tube and 300 µl of chloroform was added, followed by mixing and centrifugation. A 300-µl portion of the upper phase was then taken and 400 µl of absolute ethanol at –20 ºC and 40 µl of sodium acetate were added prior to vortexing and storing the sample at –20 ºC for 8 h for precipitation of DNA. The resulting mixture was then centrifuged at 11,600 ×g for 10 min and then the liquid phase was removed. A 400-ml volume of 70% ethanol was added to the pellet, followed by centrifugation at 11,600 ×g for 5 min for washing of the DNA. Finally, ethanol was removed and the tube containing DNA was held at room temperature for 30 min for further removal of the residual ethanol via evaporation. The pellet, which was the extracted DNA, was diluted with 100 µl of sterile dH_2O and used for PCR reaction.

Primers

PCR primers for the amplification of bovine, sheep, porcine, goat, and horse meat were designed as described by Lahiff et al. (21) and Matsunaga et al. (5). Species-specific primers (Table) for the detection of dog and cat were designed from sequence information available in the GenBank database (cat: NC_001700,; dog: NC_002008). All primers were obtained from Integrated DNA Technologies, Inc, (Coralville, IA, USA).

Polymerase Chain Reaction (PCR)

The 50-µl reaction mixture was prepared in an Eppendorf tube containing 5 µl of 10 × PCR buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl, 0.1% Triton X-100), 5 µl of 25 mM $MgCl_2$, 250 µM deoxynucleotide triphosphate (dNTP), 0.25 µl of Taq DNA polymerase (Promega, Madison, WI, USA), 20 pmol of each primer, and 5 µl of target DNA. The thermocycler was programmed for 30-cycle PCR. PCR was optimized with different annealing temperatures. The optimal annealing temperature was 58 °C for all primers. Each cycle included holding at 94 ºC for 45 s, at 58 ºC for 45 s, and at 72 ºC for 90 s. For 0.1% meat mixtures, we used 35-cycle PCR amplification.

Electrophoresis was run on agarose gel (1.5%) at 100 V for 2 h on a 15-µl portion of the amplified DNA fragments. The resulting gel was stained with ethidium bromide (0.5 µg/ml), visualized using a UV transilluminator, and photographed with a Polaroid 322 camera and T667 film. The experiments were conducted in triplicate.

Results

Mitochondrial DNA (mt DNA) fragments of 439, 322, 274, 271, 225, 212, and 157 bp of horse, dog, cat, bovine, sheep, porcine, and goat meat, respectively, were amplified (Figure 1). None of the primer pairs used cross-

Table. PCR oligonucleotide primers.

		Position	Accession number
Bovine	5'- GCCATATACTCTCCTTGGTGACA- 3'	8107/8127	J01394
	5'- GTAGGCTTGGGAATAGTACGA- 3'	8377/8357	
Sheep	5'- TTAAAGACTGAGAGCATGATA- 3'	71/91	AF039171
	5'- ATGAAAGAGGCAAATAGATTTTCG- 3'	295/272	
Porcine	5'- GCCTAAATCTCCCCTCAATGGTA- 3'	93/115	AF039170
	5'- ATGAAAGAGGCAAATAGATTTTCG- 3'	304/281	
Cat	5'- CATGCCTATCGAAACCTAACATAA- 3'	11101/11124	NC_001700
	5'- AAAGAAGCTGCAGGAGAGTGAGT- 3'	11373/11351	
Dog	5'- GATGTGATCCGAGAAGGCACA- 3'	8821/8841	NC_002008
	5'- TTGTAATGAATAAGGCTTGAAG- 3'	9142/9121	

	Reference
Goat	
5'- GACCTCCCAGCTCCATCAAACATCTCATCTTGATGAAA- 3'	(Matsunaga et al., 1998)
5'- CTCGACAAATGTGAGTTACAGAGGGA- 3'	
Horse	
5'- GACCTCCCAGCTCCATCAAACATCTCATCTTGATGAAA- 3'	(Matsunaga et al., 1998)
5'- CTCAGATTCACTCGACGAGGGTAGTA- 3'	

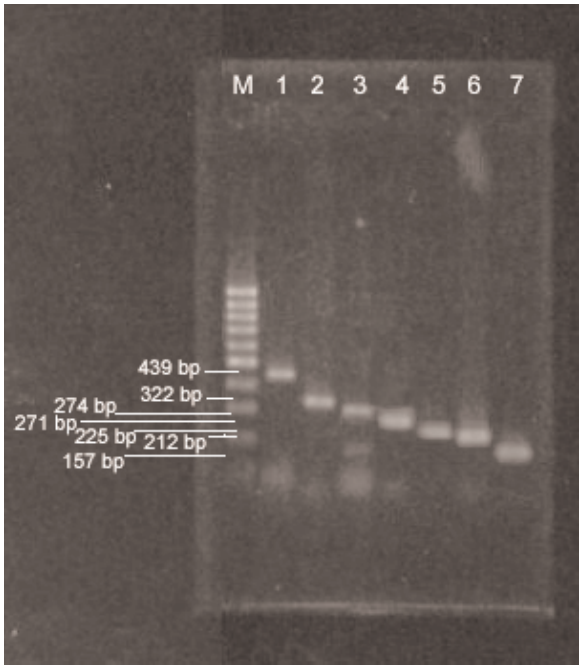

Figure 1. Agarose gel analysis of PCR product amplified with species-specific primers.
M: molecular marker (100 bp); 1: horse meat; 2: dog meat; 3: cat meat; 4: beef; 5: lamb; 6: pork; 7: goat meat.

reacted with DNA of other species. Test mixtures of meat at 5%, 2.5%, 1%, and 0.5% levels were identified after an amplification of 30 cycles, while identification failed for 0.1% mixtures (Figure 2). However, 0.1% mixtures were identified with 35 amplification cycles (Figure 3).

Discussion

Species identification of meat and meat products is important because of health, ethical, and economic reasons. Wintero et al. (22) compared immunodiffusion, immunoelectrophoresis, isoelectric focusing, and DNA-hybridization for determining species of meat. They concluded that DNA hybridization was more reliable and sensitive than other methods, though it was complicated and time-consuming. Similarly, the high cost and complexity associated with this technique have been reported by other researchers (19,20).

Meyer et al. (7) detected 0.5% pork in beef using the duplex PCR technique. Their results revealed that PCR was the method of choice for identifying meat species in muscle foods. Meyer et al. (19) detected 0.01% soy

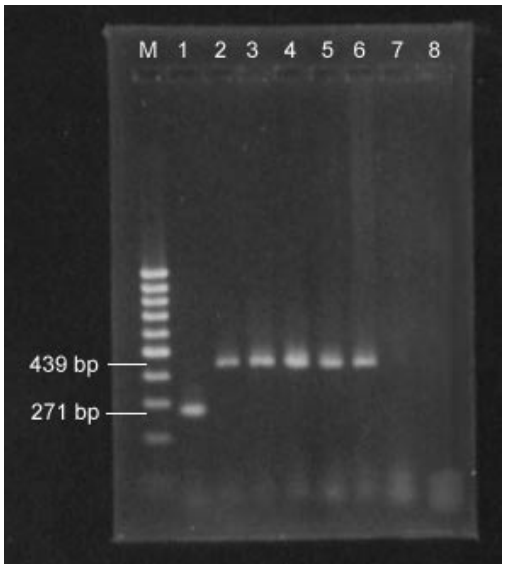

Figure 2. Agarose gel analysis of PCR products from mixtures of beef-horse meat with horse-specific primer (30 PCR cycles)
M: molecular marker (100 bp); 1: 100% beef (beef-specific primer is used to indicate the presence of beef); 2: 100% horse meat (positive control): 3: 5% horse meat in beef; 4: 2.5% horse meat in beef; 5: 1% horse meat in beef; 6: 0.5% horse meat in beef; 7: 0.1% horse meat in beef; 8: 100% beef (negative control: horse-specific primer is used to indicate the absence of horse meat).

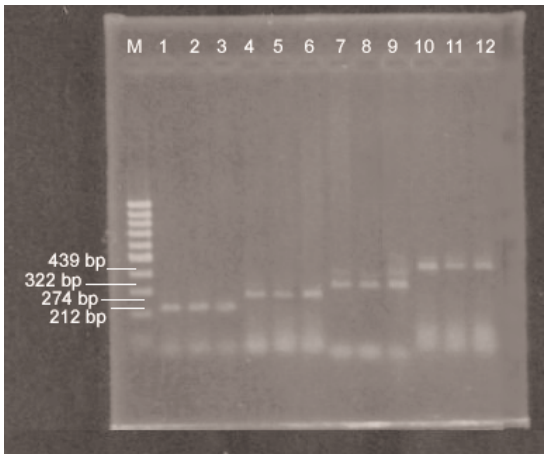

Figure 3. Agarose gel analysis of PCR products from meat mixtures at 0.1% level (35 PCR cycles)
M: molecular marker (100 bp); 1: 0.1% pork in beef; 2: 0.1% pork in lamb; 3: 0.1% pork in goat meat; 4: 0.1% cat meat in beef; 5: 0.1% cat meat in lamb; 6: 0.1% cat meat in goat meat; 7: 0.1% dog meat in beef; 8: 0.1% dog meat in lamb; 9: 0.1% dog meat in goat meat; 10: 0.1% horse meat in beef; 11: 0.1% horse meat in lamb; 12: 0.1% horse meat in goat meat.

protein in processed meat products using the nested-PCR technique. Partis et al. (23) detected 1% pork in beef using RFLP, whereas Hopwood et al. (17) detected 1% chicken in lamb using PCR.

Results of the present study supported the findings published by Meyer et al. (6,7), Hopwood et al. (17), and Partis et al. (23), who reported that PCR could be used for identification of meat mixes at 1% and 0.5% levels. Our results suggested that the number of PCR cycles used for amplification played an essential role in identification of meat in mixes < 0.5%. Therefore, in cases where a very low level of meat is suspected of being mixed into the main meat batch, the meat batch should be homogenized before sampling, multiple samples should be taken, and the number of PCR amplification cycles should be increased (i.e. 35).

In meat plants processing more than one species of meat, it may be inevitable that one species of meat may be contaminated with another during meat operations, such as cutting and grinding via knives, grinders, choppers, and cutting boards. PCR analysis of such samples may result in positive results for a violation due to its high sensitivity (3,6), even though contamination was unintentional and at a very low level. Therefore, precaution should be exercised when interpreting the results of species identification by PCR and analysis of multiple samples should be taken from each lot for an objective evaluation.

These results might be useful for effective control of adulterated consumer meat products and violations of labeling requirements for meat products. PCR species determination can also be used to monitor ruminant feeds for any beef tissue, which has been banned in many countries in an effort to control the spread of bovine spongiform encephalopathy.

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