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Studying the Presence of Adultery, Fraudulent Imitation and Food Pathogens within Processed Meat Products (Such as Salami, Sausage, Braised Meat) using DNA Typing and PCR Procedures*

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Abstract- Adulteration of meat with cheaper ambiguous meats of different origin during preparation of meat products is a common practice in many countries. Because meat adulteration and mislabeling are illegal and raise many health, religious, cultural and economic issues. In this study, 500 ready to eat raw meat samples (minced meat, lahmacun ingredients, kebap, stew and meatball samples / 100 samples for each type) were collected from different types of plants that were located in Istanbul. The samples were explored if they had different animal originated DNA residues (pork, chicken, cattle, sheep, horse, donkey, cat, dog, mouse, cockroach and house fly) by PCR procedures. According to the results, total of 52 samples were determined as adulterated and different originated animal DNA samples were found (chicken, horse and sheep DNA residues). It was concluded that to apply total quality management and food security systems are very important to decrease the risk factors for both products and the public health.

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Özet- Et ve ürünlerinde taklit ve tağşiş uygulamaları gerek kar amacını yükseltmek amacı ile illegal bir biçimde yapılmakta, gerekse birden fazla et ürünü işleyen işletmelerde kaza / yetersiz hijyen ve san istasyon uygulamaları sonucu meydana gelebilmektedir. Et ve ürünlerinde taklit ve tağşişler ekonomik, dini inançlar, sağlık, kültürel, tüketiciyi aldatma yönünden önemli sorunlara yol açabilmektedir. Bu çalışmada 500 adet tüketime hazır halde satışa sunulmuş olan çiğ et örneği (kıyma, lahmacun iç malzemesi, kebap, köfte ve sulu yemeklerde kullanılmak üzere hazırlanmış etler olmak üzere) İstanbul'da bulunan farklı satış noktalarından toplanılmış ve söz konusu örneklerde 9 adet farklı hayvana ait (domuz, tavuk, sığır, koyun, at, eşek, kedi, köpek, fare, hamamböceği ve ev sineği olmak üzere) DNA örnekleri PCR prosedürleri kullanılarak araştırılmıştır. Elde edilen sonuçlara göre 52 adet örnekte farklı hayvan türlerine ait (tavuk ,at ve koyun olmak üzere) DNA kalıntıları saptanmıştır. Sonuç olarak özellikle et ve ürünlerini üreten işletmelerde toplam kalite yönetimi ve optimal hijyen uygulamalarının kontrollü bir biçimde uygulanmasının taklit ve tağşiş uygulamalarının minimize edilebileceği sonucuna varılmıştır.

Anahtar sözcükler: *PCR, tür tayini, tüketime hazır et ürünleri.*

I. Introduction

The composition of food is a major concern of consumers today. In the case of adulterated meat product consumption, several factors including economic, food safety (allergy) and moral reasons (religious belief), trigger such apprehensions. Among these concerns, consumers are most sensitive because of religious factors and do not tolerate even trace amounts of adulteration of meat products with forbidden meats like pork. Hygiene and right labeling notified on the label of any food stuff are very important criteria especially for public health.

Although food safety practices is one of the top priority policies of European Union, the information on the labels of meat and meat products does not provide food safety guarantee for the period "from the stable to table" (1,2).

According to the latest "Meat and Meat Products Manifest announced in our country in February 2013 (3), production of meat products containing meat from different animal species has been banned.

Meat and meat products are species-wise safe if they are acquired from healthy animals and processed under hygienic conditions. However, in the frauds and adulterations which are used in order to cut down the costs and increase the profits, meat from inappropriate animals (horse, donkey, and hog) might be mixed in the aforementioned meat and meat products. Besides, in facilities which process several animal products (like facilities processing both cattle and poultry), foreign animal meat might be indeliberately adulterated in the meat products. Besides, due to poor hygienic standards, there may be a possibility of meat and meat products to be adulterated by the wastes and/or tissues of mice and / or insects.

Before the introduction of DNA (Deoxyribonucleic Acid) typing method, methods such as Ouchterlony method, SDS-PAGE, ELISA, isoelectric

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focusing (IEF), immune diffusion tests, chromatography, mass spectrophotometry analysis, HPLC analysis based on fractioning hemoglobin or fatty acids have been used to specify the animal type in meat and meat products. Some of these are based on protein analysis and immunological tests (4,5). However, in case of cooked and processed meat products, heat and continuity of temperature causes the denaturation of type-specific proteins and this decreases the reliability of these methods. PCR (Polymerize Chain Reaction) procedures based on DNA isolation are relatively more stable and are considered to be the most reliable method to specify the animal species of meat and meat products, especially for the short primary strands consisting of specific locus in heat treated products (6).

This study aimed to examine various meat and meat products (kebaps, lahmacun ingredients, minced meat, stews, various meat balls etc.) which are presented in various sales points (restaurants, butcher shops, groceries etc.) in Istanbul region, to determine their ingredients through DNA typing method and to specify the different animal tissues / residuals in these products.

II. Materials and Methods

a) Specimen Handling

Random sampling method has been used in this study. From 500 different sales points in the Istanbul region (250 sales points from Asian side and 250 sales points from European side), 500 meat and meat product samples have been collected. As required by the asepsis and antisepsis norms, samples have been placed in sterile containers and transferred to the laboratory in these containers which have +4°C internal heat.

Table 1 : Detailed information about specimen handling

Tablo 1 : Örnek toplama programına ait detay bilgiler

Region	Sample name	Sales point	Total number of samples
İstanbul Europe	Lahmacun ingredients	Kebap shop/restaurant	50
İstanbul Europe	Minced Meat	Butcher shop	50
Istanbul Europe	Kebap	Kebap shop/pedlar wrap point	50
İstanbul Europe	Meat balls	Restaurant	50
İstanbul Europe	Stews	Restaurant	50
Istanbul Asia	Lahmacun ingredients	Kebap shop/restaurant	50
İstanbul Asia	Minced meat	Butcher shop	50
Istanbul Asia	Kebap	Kebap shop/pedlar wrap point	50
İstanbul Asia	Meat ball	Restaurant	50
İstanbul Asia	Stew	Restaurant	50
TOTAL			500

b) DNA Extraction

DNA of all the isolates are extracted using commercial DNA extraction kits and in accordance with kit protocol. Extracts have been kept at –20°C, to be used as target DNA in PCR process.

c) PCR

50-100 mg tissue from the meat samples have been put into a microcentrifuge tube as small pieces. 400 µL solutions SH has been added and blended with vortex. 8 µL Proteinase K and 40 µL solution SLS have been added to the mixture. After blending properly, the mixture has been kept waiting for two hours at 60°C, in order for the cells to stretch. After the incubation at 60°C, 300 µL Solution SP has been added and blended with vortex for 30 seconds. The mixture has been centrifuged at 12.000 rpm for 30 minutes. The supernatant has been transferred to a clean tube and 500 µL isopropanol has been added.

Table 2 : Type-specific primer sets used in PCR procedure (15,16,17,18,19).

Tablo 2 : PCR prosedüründe kullanılan türe spesifik primer setleri (15,16,17,18,19)

Type	Primer Direction	Sequence
Pork	Forward / Reverse	5'-CTTGCAAATCCTAACAGGCCTG-3'/5'-CGTTTGCATGTAGATAGCGAATAAC-3'
Chicken	Forward / Reverse	5'-TCTGGGCTTAACTCTCATACTCACC-3'/5'-GGTTACTAGTGGGTTTGCTGGG-3'
Cattle	Forward / Reverse	5'-CCCGATTCTTCGCTTTCCAT-3'/5'-CTACGTCTGAGGAAATTCCTGTTG-3'
Sheep	Forward / Reverse	5'-CCTTATTACACCATTAAAGACATCCTAAGGT-3'/5'–GGGTCTCCAGTAAGTCAGGC-3'
Horse	Forward / Reverse	5'-CAGCCAATGCGTATTCGTACTCT-3'/5'-GTGTTCCACTGGCTGTCCG-3'

Donkey	Forward / Reverse	5'-CATCCTACTAACTATAGCCGTGCTA-3'/5'-CAGTGTTGGGTTGTACACTAAGATG-3'
Cat	Forward / Reverse	5'-CATGCCTATCGAAACCTAACATAA-3'/5'-AAGAAGCTGCAGGAGAGTGAGT-3'
Dog	Forward / Reverse	5'-GATGTGATCCGAGAAGGCACA-3'/5'-TTGTAATGAATAAGGCTTGAAG-3'
Mice	Forward / Reverse	5'-CCAAGTCGACATGCACRTGTATACATAGTAAC–3'/5'TTATGTAAAACGACGGCCAGT-3'
Cocroach	Forward / Reverse	5'-GTGGAACTGGCTGGACTT-3'/5'-GAGACATGTGTAATCAGG-3'
Fly	Forward / Reverse	5'-CACAAGGATCGCTTCAAG-3'/5'-TGTTGGTATCATTGTCGG-3'

After blending with vortex, the mixture has been incubated for an hour at -20 ℃. Then, it has been centrifuged at 12.000 rpm for 20 minutes. Supernatant has been removed. The remaining pellet has been gently vortexed by 1 ml 70% ethanol and has been distributed, then centrifuged at 13.000 rpm for 5 minutes. Ethanol has been removed and the subsided DNA has been left to dry. After ethanol completely vaporized, 150 µL Solution SE has been added to the pellet and kept waiting for one night at room temperature, in order for the DNA to dissolve. The dissolved DNA has been measured with UV Spectrometers and diluted to the point of 50 ng/µL concentration. After that, heat treatment protocol has been applied for 10 seconds at 95°C and 15 seconds at 60°C. The second and third steps are repeated for 5 times as 3 cycles (7,8,9,10,11).

III. Results

18 (3.6%) of the samples showed chicken DNA, 33 (6.6%) of them showed sheep DNA and 1 (0.2%) of them showed horse DNA. None of them showed pork, donkey, cat, dog, mice, cockroach and fly DNA. The detailed refraction of the results can be seen in Table 3. The positive results have been determined through Real-time PCR procedures.

Table 3 : Extraneous DNAs (other than cattle DNA) determined ın the samples

Tablo 3 : Toplanılan örneklerde tespit edilen yabancı türlere ait (sığır DNA'sı dışında olmak üzere) DNA kalıntıları

Region	Sample (RAW)	Sales point	Extraneous DNA	DNA positive samples
Istanbul Europe - İstanbul Asia	Lahmacun ingredients	Kebap shop	Chicken	11
İstanbul Europe - İstanbul Asia	Minced meat	Butcher shop	Chicken	5
İstanbul Europe	Kebap	Kebap shop	Chicken	2
İstanbul Europe - İstanbul Asia	Kebap	Kebap shop	Sheep	30
İstanbul Europe	Minced meat	Butcher shop	Sheep	3
Istanbul Asia	Minced meat	Butcher shop	Horse	1
TOTAL				52

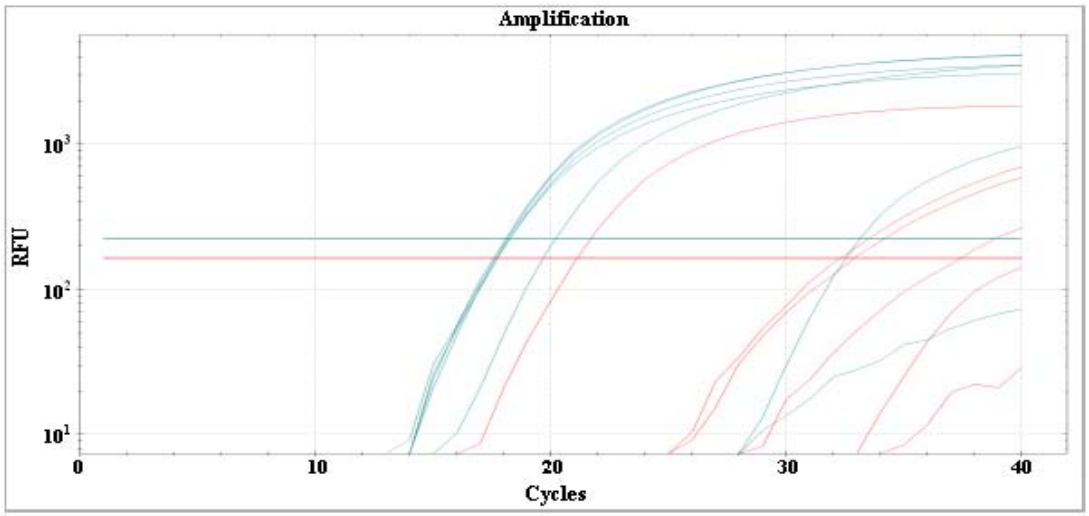

Figure 1 : Real time PCR Horse DNA amplification samples

Şekil 1 : Real time PCR at DNA'sına ait ampflikasyon örneği

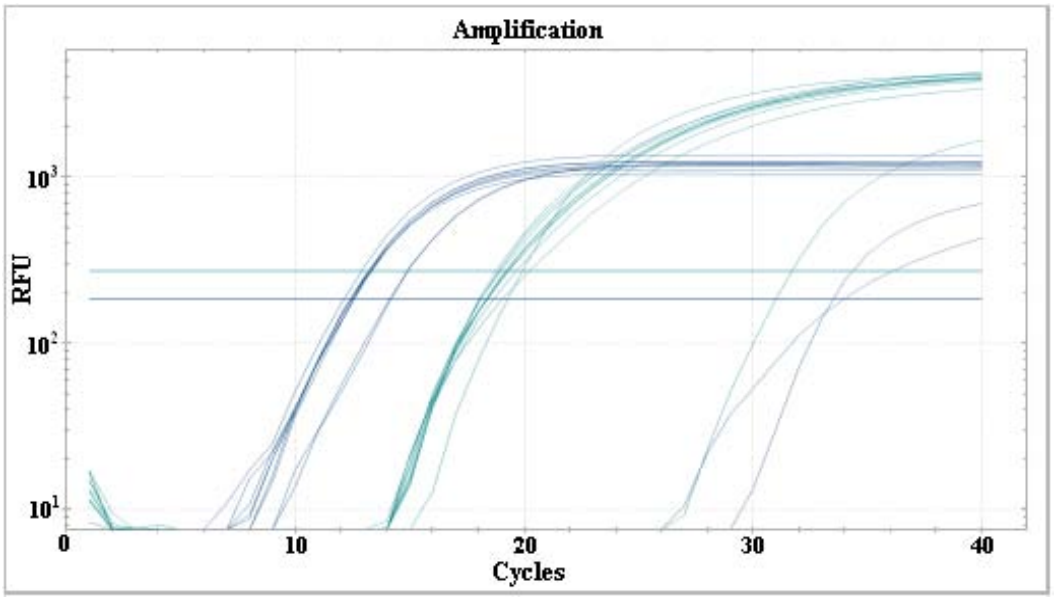

Figure 2 : Real time PCR Sheep DNA amplification samples

Şekil 2 : Real time PCR koyun DNA'sına ait ampflikasyon örneği

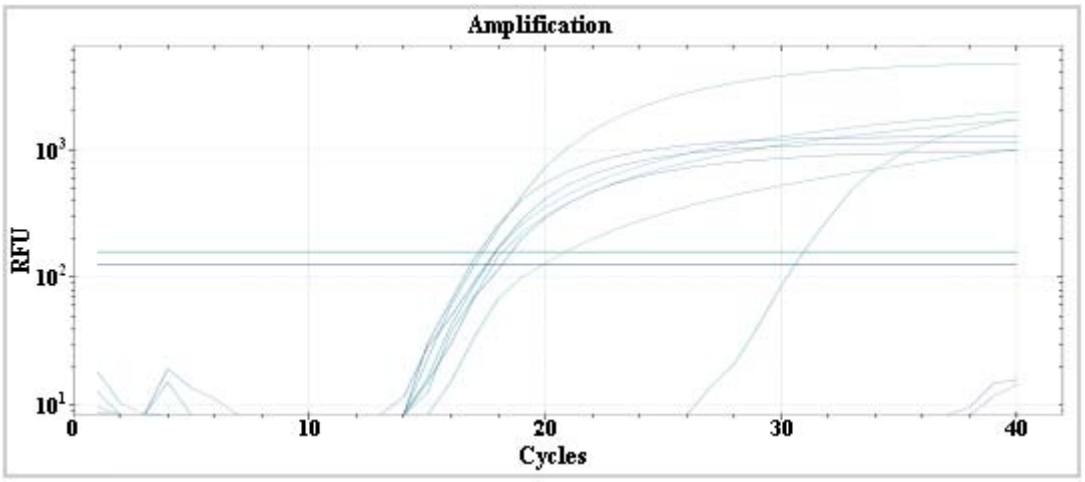

Figure 3 : Real time PCR Chicken DNA amplification samples

Şekil 3 : Real time PCR tavuk DNA'sına ait ampflikasyon örneği

IV. Discussion

The nutritious choices are determined by life styles, religious beliefs, cultures, diets and health conditions. Pursuant to community health, customs, traditions and beliefs, to determine the source of animals of the consumed meat and meat products has been one of the main research subjects for food scientists (12). In many countries, food fraud and adulteration in food products, especially in meat and meat products are done either deliberately in order to increase the profit margin or involuntarily as a result of not following the food safety standards, especially in facilities which process more than one animal species.

A study conducted in USA (United States of America) has analyzed raw minced meat and determined 15.9% of the samples to be containing extraneous animal DNAs . Hsieh et al. (13) has conducted another study in USA in 1996 and reported that 90% of the minced meat samples has been adulterated with poultry, either deliberately or unintentionally. Turkyılmaz et al. (14), studied 121 meat and meat product samples using the AGID method and determined horse meat in 3 (2.5%) of them and pork meat in 2 (1.7%). Turk et al. (15), studied 223 samples and determined pork meat in 16 (7.1%), horse meat in 12 (5.3%) and mixture of pork and horse meat in 6 (2.6%). The results of our study in general examination are lower than the results of Hsieh et al. (16), similar to those of Turkyılmaz et al. (14) and Turk et al. (15) The different results which have been reported in world and Turkey literature may originate from many reasons, such

as the physical conditions of the sales points, whether the food safety products have been applied or not, the differences in the supervision processes, the deficiencies of the facilities which process more than one animal species and/or usage of the same equipment, the deliberateness of adulterations and the staff's lack of information about the procedures.

In this study, the highest extraneous DNA in the bovine meat samples was sheep DNA (6.2%). 96% (30 of the 31 mutton positive samples) of these positive samples have been collected from kebap shops. Since mutton meat is used commonly in kebap shops, mixture of bovine and mutton meat can be a microbiological threat to consumers.

Out of the 500 samples collected, 68 (13.6%) were determined to be risky for human consumption according to the plate count parameter. 39 (57.4%) of these "risky" samples contain meat from different animal species. On the other hand, 29 (42.6%) of these samples contained only one type of meat. Plate count is an indicator of not only food hygiene but also of the tools used in production, food contact surfaces and hands of the staff who contact food. If the plate count is high, it may mean that food, contact surfaces, tools and hands may be carrying potential pathogens and saprophytes.

In a study conducted to determine the food intolerance reactions, 22% of the subjects showed food intolerance and if the foods causing the intolerance are consumed again, the reactions repeated themselves in 15% of the subjects (17,18). Food intolerance may cause chronic inflammatory diseases such as chronic headache, abnormal weight gain, abnormal weight loss, dermatological problems, autoimmune diseases, fibromyalgia, migraine, stomach diseases, bowel diseases such as inflammatory bowel disease (IBD), malabsorptions, rheumatic diseases, shortness of breath, asthma, depression, anxiety, Type 2 diabetes, hypertension, metabolic syndrome, hypothyroidism, chronic rhinitis, eczema, acne, edematous eyelids, urinary diseases, Crohn's disease, cardiovascular diseases (19,20). Literature shows intolerance against food of animal origin. The intolerance, which is determined to be more common in males can cause the abovementioned clinical symptoms and some of them can be life threatening. According to WHO (World Health Organization), half of the world population has food intolerance and 1 billion people have been diagnosed with it. WHO predicts that by the year 2015, the count would reach 2.5 billion (21).

Whether done deliberately in order to increase the profit margin or accidentally by the facilities which process meat from more than one animal species, adulteration is an illegal practice which deceives the consumer in the sense of health, religion, culture and economy. Another point to be kept in mind is that adulterated meat and meat products pose a greater microbiological risk for consumer health as well. DNA typing also used in our study is a very efficient way of detecting foreign meat species in meat and meat products.

Whatever the reason of the adulteration maybe, it results in deficient hygiene conditions and this is a serious threat for the facility, staff and product and consumer health. Besides, microorganisms which reproduce in meat and meat products because of hygiene deficiency can quickly develop single or multi resistance to antibiotics through complex genetic interactions. Our study shows that adulterated products pose a statistically meaningful higher risk for consumer health than unadulterated products. Total quality management systems and food safety practices should be applied together with the official inspection of the state authorities; programs to raise consumer awareness and continuous training programs for the staff responsible for food production should also be carried into effect. All these would be beneficial to reduce the incidence of the adulteration practices.

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