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Investigation of the Presence of Different Animal Species within Processed Meat and Meat Products using PCR Procedures and Development of Risk Models based on Consumer Health

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Summary- Fraudulent imitation and adultery of meat and meat products are fooling the consumers, jeopardizing their health, economical situation and potentially causing harm to religious beliefs. The aim of this project was to search for the existence of such fraudulent imitations and adulteries within processed meat products across different sale points (them being markets) found within 11 municipalities of the Marmara Regionusing PCR procedures. According to the findings gathered during the study, 25 of the collected samples (4.54%) contained poultry DNA, 5 of them (0.90%) contained house fly DNA, 6 of them (1.09%) contained sheep DNA, 2 of them (0.36%) contained cockroach DNA, 2 of them (0.36%) contained horse DNA and 4 of them (in chicken sausages/ 0.72%) contained bovine DNA as foreign species. Again our findings showed that, for samples not suitable for human consumption in relation to their Escherichia coli parameter of total coliform bacteria quantity, highest value was found within beef salami and chicken sausage.

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Summary- Fraudulent imitation and adultery of meat and meat products are fooling the consumers, jeopardizing their health, economical situation and potentially causing harm to religious beliefs. The aim of this project was to search for the existence of such fraudulent imitations and adulteries within processed meat products across different sale points (them being markets) found within 11 municipalities of the Marmara Regionusing PCR procedures. According to the findings gathered during the study, 25 of the collected samples (4.54%) contained poultry DNA, 5 of them (0.90%) contained house fly DNA, 6 of them (1.09%) contained sheep DNA, 2 of them (0.36%) contained cockroach DNA, 2 of them (0.36%) contained horse DNA and 4 of them (in chicken sausages/ 0.72%) contained bovine DNA as foreign species. Again our findings showed that, for samples not suitable for human consumption in relation to their *Escherichia coli* parameter of total coliform bacteria quantity, highest value was found within beef salami and chicken sausage. Such findings show significant differences between unadulterated/non-fraudulent products that are not suitable for human consumption and adulterated/fraudulent products, in terms of microbiological risks that can be brought upon the consumer. In the light of these findings, it can be said that adultery and fraudulent imitation can end up seriously jeopardizing the consumer health.

Keywords: meat, meat products, adultery, fraud, pathogens, DNA typing, PCR.

I. Introduction

Having access to sufficient quantities of food which is produced in a high quality and trustworthy environment while guaranteeing its safety is a fundamental right for the well physical, mental and psychological development of every human being. Even though the application of food safety is one of the most prioritized policies of the European Union (EU), when it comes to the management of the quality of meat and meat products throughout the whole process starting at the barn, ending on the table, solely the labeled information cannot actually guarantee the food safety (1). That's why, it's vital for meat and meat products to be checked in order to determine from which animals they are produced from, to validate the labeled information found on their packages, to detect substances that can harm the consumer health(carcass products high in BSE, undesirable fats, illegal addition of animal species into meat products, insect and rodent contamination of the same products because of the lack of proper hygiene, etc.). In the Notification entitled "Instructions for the application of the Notification on meat and meat products" issued in our country on February 2013 (2), the following statement can be found: "Species, as mentioned in its corresponding article in the Notification, can only be mixed with themselves. For example, chicken-turkey mixture or a calf-sheep mixture." which has thus rendered illegal to mix different animal species in meat and meat products.

Listeria monocytogenes is an important gram-positive, facultative anaerobic microorganism that is being frequently isolated from nearly all food products and that can cause sporadic and epidemic infections. As it can live and thrive in active soil, it can survive in vegetables, dairy and dairy products, potable or waste water, as well as poultry meat and poultry products. In turn, this infectious agent can be transmitted to humans or other animals via fecal-oral route (3). Main causes of human listeriosis are pasteurized/non-pasteurized dairy and dairy products, meat and meat products, poultry meat and poultry products, poultry fodders, vegetables and contaminated waters (4). Patients with suppressed immune system because of diseases such as HIV, hepatitis or cancer, as well as pediatric and geriatric cases along with pregnant women form the primary risk group for the human listeriosis.

Escherichia coli are aerobic/facultative aerobic microorganisms that can be found within the normal flora of the intestinal system of humans and warm-blooded animals. Even though some coliform groups as well as some *E.coli* strains are harmless, these

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aforementioned agents can also possess pathogenic strains. Total coliform bacteria quantity and the presence of *E.coli* is being reported as an indicator of poor hygienic conditions and fecal contaminations (5). Among the main sources of the contamination of aforementioned microorganism groups are; willingly or unwillingly introducing foreign animal tissues that weren't subjected to the obligatory food safety inspection system, tissues that come from the same species but shouldn't be put in meat products (such as renal or lung tissues), toilets with poor hygienic conditions and the end consumer or the food production personnel who don't follow the hygienic necessities after using the toilet.

When meat and meat products, all having an important role in human consumption, are acquired from healthy animals and processed within appropriate conditions, they are regarded as microbiologically safe. Unless necessary precautions are taken during elevation and slaughtering, meat and meat products might end up causing serious health problems among the consumers. Also, fraudulent imitation and adulteration done in order to decrease cost and thus increase profit margin may lead to the introduction of undesirable animal species (horse, donkey, pig, etc.) in meat and meat products. Furthermore, in establishments processing than one meat product (mainly establishments processing cattle and poultry meats under the same roof), tissues belonging to foreign animals might unwillingly get introduced into these processed meats. Moreover, in some cases of adultery of meat and meat products, unwanted tissues not coming from a foreign animal (nail, kidney, brain, lung, etc.) might be added willingly or somehow end up unwillingly contaminating these said products.

The aim of this project is to search for the existence of fraudulent imitation and adultery within processed meat products across different sale points (them being markets) found within 11 municipalities of the Marmara Region (Edirne, Tekirdağ, Kırklareli, İstanbul, Kocaeli, Yalova, Sakarya, Bursa, Bilecik, Balıkesir and Çanakkale) using PCR procedures.

II. Materials and Methods

a) Sample collection

Over the course of this study, across 11 different municipalities of the Marmara Region (Edirne, Tekirdağ, Kırklareli, İstanbul, Kocaeli, Yalova, Sakarya, Bursa, Bilecik, Balıkesir and Çanakkale), from a total of 5 different meat product types (Beef Salami, Beef Garlic Flavoured Sausage, Chicken Sausage, Bresaola, Braised Meat), a grand sum of 550 samples were gathered (50 from each municipality, in each municipality 10 samples for each meat product type). The gathered samples, which were put in transportation boxes that were rendered sterile according to the rules of asepsis and antisepsis, were brought to our university inside transportation containers with 4°C inner temperature. Samples were kept at -20°C until the analyses. Detailed information on collected samples is shown on Table 1.

Table 1: Detailed information on the sample collection program.

Tablo 1: Örnek toplama programı hakkında detaylı bilgi

REGION	MUNICIPALITY	SAMPLE NAME	SALE POINT	TOTAL SAMPLE COUNT
Marmara	Edirne	10 of each salami, garlic flavoured sausage, sausage, Bresaola and braised meat sample, 50 samples from each municipality and from all of the municipalities, a total of 550 samples.	Markets	50
Marmara	Tekirdağ			50
Marmara	Kırklareli			50
Marmara	İstanbul			50
Marmara	Kocaeli			50
Marmara	Yalova			50
Marmara	Sakarya			50
Marmara	Bursa			50
Marmara	Bilecik			50
Marmara	Balıkesir			50
Marmara	Çanakkale			50
TOTAL				550

b) Microbiological Analyses

- *E. coli:* From swabsticks containing the growth medium which comes from where the sampling was made, passages have been made, in accordance with asepsis conditions, into TBX agar growth medium that was previously prepared and poured into petri dishes. The petri dishes were then incubated for 24 hours in 44°C. Following this incubation period, typical colonies that formed were counted. About 98% of *E. coli* serotypes contain the enzyme β-D glucuronidase. This

enzyme, rarely found in other bacteria, breaks down its substrate Methylumbilliferyl- β-D glucuronide (MUG), products of which are fluorescent under UV light (6). That's why, while swabbing, a chromogenic growth medium containing MUG (besides TBX Agar) was also used.

- *L. monocytogenes:* 25 gr of the sample was put in 225 ml BLEB, incubated for 4 hours in 30C. Next, selective agents and 25mg/L natamycin were added to the medium and incubated for 48 hours in 35C. At the end of the 48th hour of the incubation, a passage has been made to CLAB, which is one of the numerous selective agars for *L. monocytogenes*. Cultures were purified by making passages from colonies suspected of containing *List. spp.* to a TSA containing Yeast Extract. Suspect isolates were identified according to their following properties: gram staining, catalase, movement, dextrose, maltose, rhamnose, mannitol and xylose fermentation, aesculin fermentation, nitrate oxidation. Furthermore, CAMP test was made using S. aureus in order to detect whether the isolates possess the CAMP factor (6).

c) DNA Extraction

The DNAs of all the isolates were extracted via the commercial DNA extraction kit, in accordance with the kit protocol. The extracts were stored in -20°C to be used later on as target DNA during PCR procedures.

d) PCR

On Table 2 is shown species specific primer sets used during the PCR procedure.

Table 2: Species specific primer sets used during the PCR procedure (7-11).

Tablo 2: PCR prosedüründe kullanılan türe özgü primer setleri (7-11)

Species Name	Primer Direction	Sequence
Pork	Forward	5'-CTTGCAAATCCTAACAGGCCTG-3'
Pork	Reverse	5'-CGTTTGCATGTAGATAGCGAATAAC-3'
Poultry	Forward	5'-TCTGGGCTTAACTCTCATACTCACC-3'
Poultry	Reverse	5'-GGTTACTAGTGGGTTTGCTGGG-3'
Cattle	Forward	5'-CCCGATTCTTCGCTTTCCAT-3'
Cattle	Reverse	5'-CTACGTCTGAGGAAATTCCTGTTG-3'
Sheep	Forward	5'-CCTTATTACACCATTAAAGACATCCTAAGGT-3'
Sheep	Reverse	5'-GGGTCTCCAGTAAGTCAGGC-3'
Horse	Forward	5'-CAGCCAATGCGTATTCGTACTCT-3'
Horse	Reverse	GTGTTCCACTGGCTGTCCG-3'
Donkey	Forward	5'-CATCCTACTAACTATAGCCGTGCTA-3'
Donkey	Reverse	5'-CAGTGTTGGGTTGTACACTAAGATG-3'
Cockroach	Specific	5'-GTGGAACTGGCTGGACTT-3'
Cockroach	Specific	5'-GAGACATGTGTAATCAGG-3'
House fly	Specific	5'-CACAAGGATCGCTTCAAG-
House fly	Specific	5'-TGTTGGTATCATTGTCGG-3'

Besides species specific primers, PCR procedures have been made on colonies that were microbiologically isolated and evaluated as suspicious in order to identify (i) *E. coli*, one of the most important food pathogen which jeopardizes consumer health, (ii) *L. monocytogenes*, which can be isolated and identified in 7 to 10 days and also can be hard to identify due to all the different chemical tests made during its identification process. These two aforementioned food pathogens and the primer sets we have used for them can be found on Table 3.

Table 3: Primer sets designed according to the different serotypes used in our study and their properties (10, 12-16).

Tablo 3: Çalışmamızda kullanılan farklı serotipler için hazırlanmış primer setleri ve onların özellikleri (10, 12-16)

Primer No	Sequence (5' – 3')	Target Gene / Amp (bp)	Target microorganism
1	GCTGATTTAAGAGATAGAGGAACA	*actA* / 827	*L. monocytogenes*
2	TTATGTGGTTATTTGCTGTC	*actA* / 827	*L. monocytogenes*
3	CAATTTTCGTGTCCCCTTCG	*23S* / 450	*Escherichia coli*
4	GTTAATGATAGTGTGTCGAAAC	*23S* / 450	*Escherichia coli*

The real-time PCR procedure is as follows:

- 50-100 mg of tissue from samples were sliced or crushed to bits and then were put in microcentrifuge tubes.
- 400 μL of SH solution was added into the samples in microcentrifuge tubes and mixed via vortex.
- To the homogeneous-looking mixture were added 8 μL of proteinase K and 40 μL of SLS solution. After mixing well enough, the mixture was kept under 60°C for 2 hours for the cells to open up.
- Following the 60°C incubation, 300 μL of SP solution was added to the mixture which was then stirred via vortex for 30 seconds.
- The mixture was centrifuged at 12000 rpm for 30 minutes. The supernatant was then moved into an empty tube.
- 500 μL of isopropanol was added to the supernatant, stirred via vortex and then incubated under -20°C for 1 hour.
- Following the incubation, the mixture was centrifuged at 12000 rpm for 20 minutes. The supernatant was thrown away.
- After adding 0.5 mL of ethanol to the pellet remaining at the bottom of the tube, the pellet was dissolved by gently vortexing and then centrifuging at 12000 rpm for 5 mins.
- The ethanol was thrown away and the sedimenting DNA was left to dry.
- With the ethanol completely evaporated, on the remaining pellet was added 150 μL of SE solution and then it was kept overnight for the DNA to dissolve under room temperature.
- The dissolved DNA was measured via UV spectrophotometer and was diluted to reach a concentration of 50 ng/μL.

Afterwards, the following heat cycle protocol was executed,

1. 95°C for 10 minutes
2. 95°C for 10 seconds
3. 60°C for 15 seconds

2nd and 3rd steps were repeated 35 times in a cycle.

III. Results

a) Foreign species identification and detection of fraud and adultery

In this study, a total of 550 samples of processed meat was collected from different sale points (supermarkets, markets, local bazaars etc. / being local brands, if present), found within 11 municipalities of Marmara Region (Edirne, Tekirdağ, Kırklareli, İstanbul, Kocaeli, Yalova, Sakarya, Bursa, Bilecik, Balıkesir and Çanakkale) and from these collected samples, existence of voluntary and involuntary (in establishments processing meats of different species, improper equipment use/surfaces/personnel borne improper procedure applications…) fraud and adultery was researched using PCR procedures. These aforementioned fraud and adultery applications were analyzed by taking into account 8 different animal species (pork, poultry, cattle, sheep, horse, donkey, cockroach and house fly). Details concerning the collected samples and findings are shown on Table 4.

Table 4: Detailed information on sample collection program.

Tablo 4: Örnek toplama programı hakkında detaylı bilgiler.

REGION	MUNICIPALITY	SAMPLE NAME	SALE POINT	TOTAL SAMPLE COUNT	POSITIVE SAMPLE COUNT	FOREIGN ANIMAL SPECIES
Marmara	Edirne	Beef Salami	Market	10	1 (10%)	Poultry
		Beef Garlic Flavoured Sausage	Market	10	2 (20%)	Poultry [×2]
		Chicken Sausage	Market	10	1 (10%)	House Fly
		Bresaola	Market	10	0	-----
		Braised Beef	Market	10	1 (10%)	Sheep

Marmara	Tekirdağ	Beef Salami	Market	10	2 (20%)	Poultry [×2]
		Beef Garlic Flavoured Sausage	Market	10	0	-----
		Chicken Sausage	Market	10	1 (10%)	Cockroach
		Bresaola	Market	10	0	-----
		Braised Beef	Market	10	1 (10%)	Poultry
Marmara	Kırklareli	Beef Salami	Market	10	0	-----
		Beef Garlic Flavoured Sausage	Market	10	2 (20%)	House Fly,Poultry
		Chicken Sausage	Market	10	1 (10%)	Sheep
		Bresaola	Market	10	0	-----
		Braised Beef	Market	10	0	-----
Marmara	İstanbul	Beef Salami	Market	10	2 (20%)	Poultry, House Fly
		Beef Garlic Flavoured Sausage	Market	10	3 (30%)	Sheep, Poultry [×2]
		Chicken Sausage	Market	10	1 (10%)	Horse
		Bresaola	Market	10	0	-----
		Braised Beef	Market	10	1 (10%)	Cockroach
Marmara	Kocaeli	Beef Salami	Market	10	0	-----
		Beef Garlic Flavoured Sausage	Market	10	1 (10%)	Sheep
		Chicken Sausage	Market	10	1 (10%)	Cattle
		Bresaola	Market	10	0	-----
		Braised Beef	Market	10	0	-----
Marmara	Yalova	Beef Salami	Market	10	0	-----
		Beef Garlic Flavoured Sausage	Market	10	0	-----
		Chicken Sausage	Market	10	0	-----
		Bresaola	Market	10	0	-----
		Braised Beef	Market	10	1 (10%)	Poultry
Marmara	Sakarya	Beef Salami	Market	10	1 (10%)	Poultry
		Beef Garlic Flavoured Sausage	Market	10	2 (10%)	Poultry [×2]
		Chicken Sausage	Market	10	0	-----
		Bresaola	Market	10	0	-----
		Braised Beef	Market	10	0	-----
Marmara	Bursa	Beef Salami	Market	10	3 (30%)	Poultry [×3]
		Beef Garlic Flavoured Sausage	Market	10	2 (20%)	Poultry [×2]
		Chicken Sausage	Market	10	1 (10%)	Cattle
		Bresaola	Market	10	0	-----
		Braised Beef	Market	10	1 (10%)	House Fly
Marmara	Bilecik	Beef Salami	Market	10	2	Sheep
		Beef Garlic Flavoured Sausage	Market	10	0	-----
		Chicken Sausage	Market	10	1	Cattle
		Bresaola	Market	10	0	-----
		Braised Beef	Market	10	1	Poultry
Marmara	Balıkesir	Beef Salami	Market	10	1 (10%)	Poultry
		Beef Garlic Flavoured Sausage	Market	10	2 (20%)	Sheep, Poultry
		Chicken Sausage	Market	10	3 (30%)	Cattle, House Fly [×2]

		Bresaola	Market	10	0	-----
		Braised Beef	Market	10	1 (10%)	Poultry
Marmara	Çanakkale	Beef Salami	Market	10	1	Poultry
		Beef Garlic Flavoured Sausage	Market	10	2	Poultry
		Chicken Sausage	Market	10	1	Horse
		Bresaola	Market	10	0	-----
		Braised Beef	Market	10	1	Poultry
TOTAL				550	48 (8.72%)	

- Brandless or local brand products make up 100% of the adulterated and fraudulent samples.
- None of the samples (0%) were contaminated with pork and donkey meat.
- 25 products (4.54%) had poultry DNA as foreign species.
- 5 products (0.90%) had house fly DNA as foreign species.
- 6 products (1.09%) had sheep DNA as foreign species.
- 2 products (0.36%) had cockroach DNA as foreign species.
- 2 products (0.36%) had horse DNA as foreign species.
- 4 products (0.72%) had cattle DNA as foreign species.

b) Microbiological analyses

All of our samples were analyzed according to 2 food pathogens (*Escherichia coli* and *Listeria monocytogenes*) which can seriously harm consumer health. Table 5 shows analysis details of the collected samples during the study, in relation with the chosen food pathogens.

Table 5: Analysis details of the collected samples in relation with the chosen food pathogens.

Tablo 5: Seçilmiş gıda patojenleri bakımından toplanmış örneklerin analiz bilgileri.

Microbiological parameter	Sample name	Positive sample count (from adulterated/ fraudulent samples)	Positive sample count (from unadulterated/non-fraudulent samples)	Positive sample count (total)
Escherichia coli	Beef salami	12 / 13 (92.3%)	21 / 97 (21.6%)	33 / 110 (30%)
	Beef Garlic Flavoured Sausage	14 / 16 (87.5%)	19 / 94 (20.2%)	35 / 110 (31.8%)
	Chicken Sausage	8 / 11 (72.7%)	9 / 99 (9.1%)	17 / 110 (15.5%)
	Bresaola	0 (0%)	0 (0%)	0 (0%)
	Braised Beef	4 / 8 (50%)	13 / 102 (12.7%)	17 / 110 (15.5%)
Listeria monocytogenes	Beef Salami	0 (0%)	0 (0%)	0 (0%)
	Beef Garlic Flavoured Sausage	0 (0%)	0 (0%)	0 (0%)
	Chicken Sausage	0 (0%)	0 (0%)	0 (0%)
	Bresaola	0 (0%)	0 (0%)	0 (0%)
	Braised Beef	0 (0%)	0 (0%)	0 (0%)

Table 6: Statistical analysis results of the PCR results obtained in our study, in accordance with the ISO 16140 evaluation parameters.

Tablo 6: Çalışmamızda elde edilen PCR sonuçlarının ISO 16140 değerlendirme parametrelerine göre istatistiksel analiz sonuçları

	Relative accuracy (%)	Relative specifity (%)	Relative sensitivity (%)	False negative ratio (%)	False positive Ratio (%)
E. coli	88.90	97.34	97.62	1.18	0.0
L. monocytogenes	-----	-----	-----	-----	-----

- As L. monocytogenes wasn't found in any of the samples, it wasn't evaluated.

Table 7: DNA nano-drop measure details of some of the inspected samples which are positive for foreign species contamination (showing one example for each sample containing foreign species).

Tablo 7: İncelenen örneklerden yabancı tür tespiti pozitif olan örneklerden bazılarının (her bir farklı yabancı tür içeren örnekten birer adet numunenin gösterilmesi olarak) DNA nano-drop ölçüm detayları

DNA type	ng/µl	A260	A280	260/280	260/230	Constant	Cursor Pos.	Cursor abs.	340 raw
Horse	1822.46	36.952	18. 691	1.76	1.82	50.00	230	19.002	3.499
Sheep	2786.21	51.203	24.266	1.89	1.92	50.00	230	26.782	4.001
Poultry	3893.03	72.089	37.668	2.07	1.99	50.00	230	36.988	3.600
Cattle	3055.92	62.580	31.352	2.09	2.01	50.00	230	31.616	5.900
House Fly	3211.87	66.873	34.002	2.13	1.87	50.00	230	32.043	4.999
Cockroach	3100.21	65.660	33.992	2.12	1.43	50.00	230	31.234	5.203
Pork (negative for all the samples)	3343.455	71.650	37.231	1.72	1.89	50.00	230	36.902	5.453

- *Even though no pork DNA was found within any of the samples of our study, pork nano-drop measures were also included since it is important in our country for religious reasons.*

Table 8: Evaluation of group differences between adulterated and unadulterated products in relation with their negative effects on consumer health, using microbiological parameters (According to Pearson Chi Square method). The results obtained on this table shows the group differences between the total number of confirmed unadulterated products and adulterated meat products.

Tablo 8: Tağşiş yapılan ve tağşiş yapılmayan et ürünleri arasındaki grup farklılıklarının tüketici sağlığını riske etmesi açısından analiz edilen mikrobiyolojik parametreler için sınanması (Pearson Chi Square yöntemine göre). Tablodaki sonuçlar tağşiş yapılmadığı tespit edilmiş tüm örneklerin toplamı ve tağşiş yapılmış et ürünleri arasındaki grup farklılıklarını yansıtmaktadır.

	Microbiological parameter	Related variable	Value	Asymp. Sig
Pearson Chi Sq	*Escherichia coli*	Adultered samples / All of the unadultered samples	9.653	.000
Pearson Chi Sq	*Listeria monocyotgenes*	Adultered samples / All of the unadultered samples	-----	-----

- The microbiological load on adulterated samples is statistically significantly higher than it is on unadulterated samples. For every group (them being adulterated and unadulterated samples), group differences were made according to the samples that are positive on microbiological parameter. For these microbiological parameters, samples which didn't show any growth were omitted.
- As L. monocytogenes wasn't found in any of the samples, it wasn't evaluated in this table.

Table 9: Evaluation of group differences between adulterated and unadulterated products in relation with their negative effects on consumer health, using microbiological parameters (According to Pearson Chi Square method). The results obtained on this table shows within the products not suitable for human consumption the group differences between adulterated and unadulterated meat products.

Tablo 9: Tağşiş yapılan ve tağşiş yapılmayan et ürünleri arasındaki grup farklılıklarının tüketici sağlığını riske etmesi açısından analiz edilen mikrobiyolojik parametreler için sınanması (Pearson Chi Square yöntemine göre). Tablodaki sonuçlar insan tüketimine uygun olmayan tüm örneklerin toplamı içerisinden tağşiş yapılan ve tağşiş yapılmayan ürünler arasındaki grup farklılıklarının sısnanmasını yansıtmaktadır.

	Microbiological parameter	Related variable	Value	Asymp. Sig
Pearson Chi Sq	*Escherichia coli*	Adultered samples / Unadultered samples	11.562	.000
Pearson Chi Sq	*Listeria monocyotgenes*	Adultered samples / Unadultered samples	-----	-----

- Values marked with red are statistically significant since they are lower than $P<0.005$.
- In values marked with red, the positive relationship correlation for adulterated products is positive. Adulterated meat products, compared to unadulterated meat products, are significantly harmful to the consumer health when microbiological parameters are taken into account.
- For every group (them being adulterated and unadulterated samples), group differences were made according to the samples that are positive on microbiological parameter. For these microbiological parameters, samples that didn't show any growth were omitted.
- As L. monocytogenes wasn't found in any of the samples, it wasn't evaluated in this table.

IV. Discussion

Even though the application of food safety is one of the most prioritized policies of the European Union (EU), when it comes to the management of the quality of meat and meat products throughout the whole process starting at the barn, ending on the table, solely the labeled information cannot guarantee the food safety (17, 18). Fraudulent imitation and adultery of meat and meat products are fooling the consumers, jeopardizing their health, economical situation and potentially causing harm to religious beliefs.

According to the findings gathered during the study, 25 of the collected samples (4.54%) contained poultry DNA, 5 of them (0.90%) contained house fly DNA, 6 of them (1.09%) contained sheep DNA, 2 of them (0.36%) contained cockroach DNA, 2 of them (0.36%) contained horse DNA and 4 of them (in chicken sausages / 0.72%) contained bovine DNA as foreign species. No pork DNA was found in the collected samples. 100% of the adulterated or fraudulent samples are made up from openly sold brandless or local brand products. Adultery and fraudulent imitation was not found in samples collected from brands producing and marketing nationwide or internationally. According to the results, it could be seen as a high probability that firms producing meat products either without any brand or under a local brand license are processing more than one species of animals and end up accidentally mixing up tissues belonging to different animal species. Another possible cause would be the staff working at the aforementioned firms lacking anytraining on proper hygiene which leads to the mechanical contamination of meat products due to the lack of training or attention. Another possibility is the thought that these aforementioned firms are willfully executing adultery and fraudulent imitation in order to make profits.

In one study conducted in the United States, Hsieh et al. (19) reported that in 90% of the minced meat samples contained poultry meat introduced willingly or unwillingly and therefore adulterated meat was being marketed. Türkyılmaz et al. (20) found that within 121 meat and meat products analyzed using AGID method, 3 of them (2.5%) contained equidae meat, 2 of them (1.7%) contained pork meat. As a result of the study of 223 samples, Türk et al. (21) has found that 16 of the samples (7.1%) contained pork meat, 12 of them (5.3%) contained equidae meat and 6 of them (2.6%) contained a mixture of pork-equidae meat. Within 410 samples of meat and meat products acquired in Bursa and Istanbul, Günşen et al. (22) has found, using ELISA method, that 14 of these samples (3.41%) contained horse meat. Results in our study are lower in relation to the detected species when compared to the aforementioned studies. In addition to the results obtained by these previously mentioned researchers, in our study, in 2 samples (0.36%) cockroach DNA and in 5 samples (0.90%) house fly DNA was detected. The presence of cockroach and house fly DNA in results makes us think that in their corresponding manufacturers, poor hygiene conditions are present, food safety regulations are not applied and these manufacturers are inefficient when it comes to the general cleaning, disinfection, staff hygiene and self-care.

Throughout literatures in our county and around the world, the causes for the acquisition of different results on this subject would be the different physical

conditions of the sales points along with presence or lack of the application of food safety protocols, deficiencies in processing and/or usage of the same equipment for establishments processing more than one species of animal meat, intentional or unintentional application of adultery and fraudulent imitation and staff's lack of knowledge on applied procedures. It's thought that, at the root of the results obtained in our study lies the deficiencies of the inspection of food safety systems as well as staff's lack of knowledge.

According to the results obtained in this study, 102 of the samples (18.5%) were found to be positive for *E. coli* and therefore not suitable for human consumption. One of the most remarkable findings in our study would be the fact that a significant number of *E. coli* positive samples come from those which were adulterated and fraudulent (Table 5). As explained above, in establishments having really poor hygienic conditions (most of them producing adulterated and fraudulent products), our results show that poor toilet hygiene can also be present. Another possible risk factor is that personnel infected with *E. coli* can easily transmit the bacteria to their surroundings (places such as homes, public transportations, public toilets, local bazaars with lots of people in it, malls, cinemas, schools, etc.).

For samples that weren't "suitable for human consumption" according to the *E. coli* parameter, highest value was 3.7×10^4 cob/gr, whereas lowest was 1.2×10^2 cob/gr. According to the results obtained, for samples not suitable for human consumption in relation to their *E. coli* parameter of total coliform bacteria quantity, highest value was found within beef salami (in which poultry DNA was found) and chicken sausage (in which house fly and cockroach DNA was found). There are studies which report that poultry meat does also contain *E. coli*. In a study conducted in Egypt, Abdul-Raouf et al. (23) studied *E. coli* O157:H7 in various foods. In this study, from samples gathered from slaughterhouses, supermarkets and barns, 3 out of 50 samples (6.0%)of minced bovine meat and 2 out of 50 samples (4.0%) of poultry meat contained *E. coli*. In a study conducted by Doyle and Schoeni (24, 25), from samples gathered from cattle, sheep, pork and chicken meat, *E. coli* O157:H7 was tried to be isolated. At the end of the study, *E. coli* O157:H7 was found in 3.7% of cattle meat, 2% of sheep meat, 1.5% of pork meat and 1.5% of chicken meat. The agent was detected in chicken wing samples and again in another study, within chicken nugget samples, *E. coli* O157:H7 serotype was found (24). One of the main reasons of this difference would be that water activity (a_w) in poultry meat is higher when compared to other butchered meats. It is thought that high water activity levels directly influence the total coliform bacteria and *E. coli* parameters. The results also show us that samples containing cockroach and house fly DNA also contain high amounts of *E. coli*. As mentioned in above paragraphs, flies and cockroaches can transmit, as a primary or secondary contamination source, a high quantity of bacteria, parasite, protozoa and virus to its environment by physical contact.

These insects originating mainly from toilets are thought to transmit *E. coli* to meat products mechanically. Another reason for these aforementioned findings would be the deficiencies in application of hygiene protocols within establishments that produce and sell meat products. Even though during our study, neither establishment hygiene nor critical control points (CCP) within establishments were inspected, in establishments from which samples containing high quantity of *E. coli* and total coliform bacteria were gathered, by external inspection, we can conclude that they are lacking minimum hygiene applications. A different reason for this would be the possibility that these previously mentioned high quantities of *E. coli* and total coliform bacteria were already present inpoultry meat.

In our study, *L. monocytogenes* was one of the investigated parameters. Nevertheless, none of the samples contained *L. monocytogenes*.

Another parameter investigated in our study was the difference in potential risks to the consumer between adulterated/fraudulent products and unadulterated/non-fraudulent products. For this reason, a two-way relationship analysis was done using the Pierson Chi Square method. One of the relationship analyses was made to evaluate the relationship analysis between adulterated/fraudulent products and unadulterated/non-fraudulent products. Another relationship analysis was made to investigate the statistical significance between adulterated/fraudulent products and unadulterated/non-fraudulent products both not suitable for human consumption. According to the results obtained in our study, for both of the relationship analyses, statistically significant differences were found on the basis of *E. coli*. For this microbiological parameter which is significant when it comes to the consumer, possible risks were found in favor of adulterated and fraudulent products (among all the products not suitable for human consumption, adulterated and fraudulent ones were found to contain statistically significantly higher quantities of risk factors on the basis of *E. coli*). Since in none of the samples *L. monocytogenes* was detected, relationship analyses were not done on this factor.

In our country and throughout the world, adultery and fraudulent imitation either occurs willfully and illegally in order to increase profits or accidentally, in establishments processing different species of animal meat, by keeping the production of different animal species on the same space or lack of staff training, poorly executed food safety applications or quality management. Especially, adultery and fraudulent imitation done to increase profits brings with itself

serious microbiological risks that can endanger consumer health. Since such willful adultery and fraudulent imitation is executed illegally, inspection and control procedures don't work on them which can create innumerous microbiologically critical control points during processing. Furthermore, no ante-mortem or post-mortem inspections are done on foreign animal borne meats as well as slaughtered animals. Additionally, control over the processes of extraction of internal organs, meat mincing, packaging and transportation remains impossible. Not identifying microbiological, parasitic, chemical risks throughout the whole process of the arrival of meats to customers can end up creating innumerable risk factors. In our study, *L. monocytogenes* was in none of the adulterated or fraudulent meats. When it comes to *E. coli*, it's found in significantly more adulterated/fraudulent meats than unadulterated/non-fraudulent meats. Our findings show significant differences between unadulterated/non-fraudulent products that are not suitable for human consumption and adulterated/fraudulent products, in terms of microbiological risks that can be brought upon the consumer. In the light of these findings, it can be said that adultery and fraudulent imitation can end upseriously jeopardizingthe consumer health.

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