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Mislabeling in Cooked Sausage is a Seriously Increasingly Problem in Food Safety

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Abstract:

BACKGROUND: Identifying the animal species origin in meat and meat products is important for preventing adulteration and protecting consumers in terms of health and religious convictions. Species-specific polymerase chain reaction (PCR) is known as a suitable method for identifying meat species.

OBJECTIVES: This study aimed to use a species-specific PCR assay for the detection of mislabeling in cooked sausage meats as adulterants by use of multiplex PCR.

METHODS: A total of 114 samples including sausage labeled containing 40%, 55% and 70% red meat of 10 different brands were collected from various markets and supermarkets. Following genomic DNA extraction from cooked sausages which were claimed to be made of red meat, multiplex PCR was performed to detect adulteration in processed food.

RESULTS: According to the analysis, 60 sausage samples showed that they consist of only meat from chicken (52.6%), 48 sausage samples consist of meat from beef and chicken (42.1%) and only 5.3% of the examined sausages were prepared with the meat of beef (6 samples).

CONCLUSIONS: This high rate of undeclared chicken meat in sausage samples is most probably due to achieving more profit. Our results indicated that the meat species substitution occurs often in processed meats like sausages, which indicates the need of more governmental controls.

Keywords:

Adulteration, Mislabeling, Multiplex PCR, Polymerase Chain Reaction (PCR), Sausage

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Introduction

Mislabeling of meat source in sausage is a concern of consumers (Han, Oh, & Cho, 2017).

Determination of the animal source of meat in processed mixed meat products is an important issue for official food control laboratories (Köppel, Ruf, & Rentsch, 2011).

To provide consumers accurate information about the products they purchase, identification of source of species meat in food is becoming a very important issue concerning the assessment of food composition (He et al., 2015). In many studies, adulteration or fraudulent labeling were reported and chicken tissue was the most frequently detected undeclared animal species in processed food (Mehdizadeh et al., 2014). The main reason for the substitution of cheaper chicken flesh or fat for more expensive beef and mutton constituents is economic. Another potential source could be the use of mechanically deboned meat (MDM) (Keyvan, ÇİL, KUL, BİLGEN, & İrelİ, 2017). The MDM is mostly produced from chicken carcasses and can be included in sausages as a cheap protein source (Keyvan et al., 2017).

To detect meat of animal species in mixed samples and processed foods, numerous analytical methods have been developed based on protein and DNA analyses. Detection of proteins in food can be considered as one of the optimal methods for detection of the source of animal meat in processed food. Different methods were presented by investigators worldwide. Peptide mass fingerprinting (PMF) (Pappin, Hojrup, & Bleasby, 1993) and peptide fragmentation fingerprinting (PFF) (Saez, Sanz, & Toldra, 2004) belong to the most used methods. High-resolution mass spectrometry method (Ruiz Orduna, Husby, Yang, Ghosh, & Beaudry, 2015) was also described. The methods based on the proteins can detect only the used species-specific proteins in meat which are used in food, whereas the methods based on the detection of species-specific DNA can detect different sources of materials harboring DNA used in food. The methods based on DNA such as PCR (Keyvan et al., 2017), nested PCR (Unajak et al., 2011), RFLP-PCR(Abdel-Rahman, El-Saadani, Ashry, & Haggag, 2009), multiplex PCR (Ghovvati, Nassiri, Mirhoseini, Moussavi, & Javadmanesh, 2009; Kitpipit, Sittichan, & Thanakiatkrai, 2014), Reverse Line Blot (Shayan et al., 2018) and Taqman or SYBR GREEN real-time PCR (Safdar & Abasıyanık, 2013) were previously described for detection of food fraud.

In comparison to the methods based on proteins, DNA based methods are fast, inexpensive and more reliable (Luo et al., 2008; Yin et al., 2009). Polymerase Chain Reaction technique has been used for specific identification of chicken (Gallus gallus) adulteration in different meat products (Dalmasso et al., 2004; Ghovvati et al., 2009). Multiplex PCR is a widespread molecular biology technique for amplification of multiple targets in a single PCR experiment. In a multiplexing assay, more than one target sequence can be amplified by using multiple primer pairs in a reaction mixture. As an extension to the practical use of PCR, this technique has the potential to produce considerable savings in time and cost.

The aim of this study was applying multiplex PCR method as a sensitive and spe-

cific tool to detect adulteration in sausage samples produced by different companies in supermarkets.

Materials and Methods

Samples: A total of 114 samples including sausage labeled containing 40% (38 samples), 55% (38 samples) and 70% red meat (38 samples) of 4 different production batches from 10 different brands were collected from supermarkets from 5 areas of Tehran (north, south, east, west and the city center) and stored at -20 ºC until used.

DNA extraction: DNA extraction from different sausage samples was done by using a DNA extraction kit (MBST, Tehran, Iran). Briefly, 50 mg of samples were mixed thoroughly with 180 µl lysis buffer and incubated for 10 min at 55 ºC. Afterward, 20 µl proteinase K was added to the solution and then tubes were incubated (Stuart UK) for 60 min at 58 ºC to degrade the proteins. After this, a volume of 580 µl of binding buffer was added to tubes and incubated for 10 min at 70 ˚C. Subsequently, a volume of 440 µl ethanol (100%) was added to the solution and after vortexing (Yellowline USA), the complete volume was transferred to the MBST-column. MBST column was first centrifuged (SIGMA Germany) and washed twice with 500 µl washing-buffer. Finally, DNA was eluted from the carrier with elution buffer. DNA was than analyzed on 0.8% agarose gel and visualized using ethidium bromide under UV condition in a Bio-rad GelDoc 1000 gel documentation system (USA). Afterward, extracted DNA was stored at -20 ºC until the subsequent analysis.

Oligonucleotide primers: According to Kitpipit et al. (2014), the species specific primers for cattle, sheep, horse, pig, chicken and ostrich were used for multiplex PCR (Table 1).

Multiplex-PCR: In order to simultaneously identify individual animal species, 50 to 100 ng DNA extracted from sausage was used. The multiplex PCR was performed in a final volume of 50 µl (BIORAD T100 USA). The amplification was done with the following program: an initial denaturation step at 95 °C for 5 min, 35 cycles consisting of denaturation at 94 °C for 30 sec, annealing at 60 °C for 30 sec, extension at 72 °C for 30s, and final extension at 72 °C for 10 min. Subsequently, the PCR products were analyzed on 2 % agarose gel and visualized using ethidium bromide under UV condition.

Determination of the sensitivity of the test: In the preliminary phase of this investigation, primer specificity was assessed with DNA extracted from raw meats. For detection of a cross-reaction, the primer set of each species was analyzed by all DNA species separately in simplex PCR as described above. Additionally the extracted DNA from sausage was diluted (1:1, 1:10, 1:100, 1:1000, 1:10000) and used for PCR amplification. Furthermore, 50 ng DNA from different species was mixed (beef and sheep), (beef, sheep, and chicken), (beef, sheep, pig, chicken and horse) and amplified by multiplex PCR as described above.

Results

DNA was successfully extracted from meat prepared from chicken, pig, beef, horse, sheep and ostrich and 114 samples of processed meat products as sausages (Fig.1). The results indicated that extracted DNA with quality measured by spectrophotometer (Eppendorf, Germany 22331) with ratio of OD260/OD280 between 1.7 and 1.9

Table 1. Details of primer sequence, melting temperature, PCR product size, and reference are shown.

Meat species	Primer name	Sequences (50–30)	Gene	Product size	References
pig	Sus-F1	5′-GAA AAA TCA TCG TTG TAC TTC AAC TAC A-3′	cyt b	100 bp	Lopez-Andreo, Lugo, Garrido-Pertierra, Prieto,and Puyet (2005)
	Sus-R1	5′-GGT CAA TGA ATG CGT TGT TGA T-3′			
Lamb	Ovi-F2	5′-GAA AAA CCA TCG TTG TCA TTC AAC T-3′	t-Glu – cyt b	119 bp	Lopez-Andreo et al. (2005)
	Ovi-R2	5′-AAA TAT TTG ATG GAG CTG GGA GA-3′			
Chicken	Gal-F3	5′-AGC AAT TCC CTA CAT TGG ACA CA-3′	cyt b	133 bp	Zhang, Fowler, Scott, Lawson, and Slater (2007)
	Gal-R3	5′-GAT GAT AGT AAT ACC TGC GAT TGC A-3′			
Ostrich	Str -F4	5′-CCC TTT AAA GAC ATC TGG TAT TGT GAG-3′			
	Str-R4	5′-TAA ATT GTA GGC TCT CTG GGG TTC-3′	12s rRNA	155 bp	Rojas et al. (2011)
Horse	Equ-F5	5′-CGT TTG ATC TGT CCT TAT TAC GGC A-3′	COI	253 bp	Kitpipit T, Sittichan K, Thanakiatkrai P (2014)
	Uni-R	5′-CCG AAT GGT TCY TTT TTY CCY GAG TAG TA-3′			
Cattle	Bos-F6	5′-CAT CAA CTT CAT TAC AAC AAT TAT CAA CAT AAA G-3′			
	Uni-R	5′-CCG AAT GGT TCY TTT TTY CCY GAG TAG TA-3′	COI	311 bp	Kitpipit T, Sittichan K, Thanakiatkrai P (2014)

was adequate for PCR amplification. PCR analysis of DNA extracted from meat of mentioned animals resulted in PCR products clearly visible as single bands of expected size (133 bp chicken, 100 bp pig, 311 bp beef, 253bp horse, 119 bp sheep, 155 bp ostrich) on agarose gel (Fig.1) with the exception of primers prepared for detection of chicken meat, with which sometimes cross reactivity exists. All positive and negative controls, which were run alongside each separate PCR, gave the expected results. The multiplex PCR with DNA extracted from each animal meat showed the expected PCR product. In multiplex PCR, the primers designed for detection of chicken meat showed rare cross reactivity (Fig. 2). Multiplex PCR analysis of sausages with 40% red meat (38 samples) showed that 27 samples had only chicken and 11 samples consist of meat from beef and chicken. No sample with only meat from beef could be detected (Table 2). The analysis of sausages (38 samples) with 55% red meat showed that 5 samples had only meat from beef, 18 samples consist of meat from chicken and 15 samples had meat from beef and chicken (Fig. 2). The analysis of sausages (38 samples) with 70% red meat showed that 1 sample had only meat from beef, 15 samples consisted of meat from chicken and 22 samples had meat from beef and chicken (Fig.

Table 2. DNA extracted from sausages purchased from different companies was analyzed by multiplex PCR. * Note: "+" presence; "–" absence.

Sample No.	Labeled as	Meat percentage	Cow	Chicken	Sheep	Pig	Ostrich	Horse
1	red meat	55%	-	+	-	-	-	-
2	red meat	55%	-	+	-	-	-	-
3	red meat	55%	-	+	-	-	-	-
4	red meat	55%	+	-	-	-	-	-
5	red meat	55%	+	-	-	-	-	-
6	red meat	55%	+	-	-	-	-	-
7	red meat	55%	+	+	-	-	-	-
8	red meat	55%	+	-	-	-	-	-
9	red meat	55%	+	+	-	-	-	-
10	red meat	55%	+	+	-	-	-	-
11	red meat	55%	-	+	-	-	-	-
12	red meat	55%	-	+	-	-	-	-
13	red meat	55%	+	+	-	-	-	-
14	red meat	55%	+	+	-	-	-	-
15	red meat	55%	-	+	-	-	-	-
16	red meat	55%	-	+	-	-	-	-
17	red meat	55%	-	+	-	-	-	-
18	red meat	55%	-	+	-	-	-	-
19	red meat	55%	-	+	-	-	-	-
20	red meat	55%	-	+	-	-	-	-
21	red meat	55%	-	+	-	-	-	-
22	red meat	55%	+	+	-	-	-	-
23	red meat	55%	+	+	-	-	-	-
24	red meat	55%	+	+	-	-	-	-
25	red meat	55%	+	+	-	-	-	-
26	red meat	55%	+	+	-	-	-	-
27	red meat	55%	+	+	-	-	-	-
28	red meat	55%	+	+	-	-	-	-
29	red meat	55%	-	+	-	-	-	-
30	red meat	55%	+	+	-	-	-	-
31	red meat	55%	-	+	-	-	-	-
32	red meat	55%	-	+	-	-	-	-
33	red meat	55%	+	+	-	-	-	-
34	red meat	55%	+	-	-	-	-	-
35	red meat	55%	+	+	-	-	-	-
36	red meat	55%	-	+	-	-	-	-
37	red meat	55%	-	+	-	-	-	-
38	red meat	55%	-	+	-	-	-	-
39	red meat	70%	+	+	-	-	-	-
40	red meat	70%	+	+	-	-	-	-
41	red meat	70%	+	-	-	-	-	-
42	red meat	70%	+	+	-	-	-	-
43	red meat	70%	+	+	-	-	-	-
44	red meat	70%	-	+	-	-	-	-

Table 2.

Sample No.	Labeled as	Meat percentage	Cow	Chicken	Sheep	Pig	Ostrich	Horse
45	red meat	70%	-	+	-	-	-	-
46	red meat	70%	-	+	-	-	-	-
47	red meat	70%	-	+	-	-	-	-
48	red meat	70%	-	+	-	-	-	-
49	red meat	70%	+	+	-	-	-	-
50	red meat	70%	-	+	-	-	-	-
51	red meat	70%	+	+	-	-	-	-
52	red meat	70%	+	+	-	-	-	-
53	red meat	70%	-	+	-	-	-	-
54	red meat	70%	+	+	-	-	-	-
55	red meat	70%	-	+	-	-	-	-
56	red meat	70%	-	+	-	-	-	-
57	red meat	70%	+	+	-	-	-	-
58	red meat	70%	+	+	-	-	-	-
59	red meat	70%	+	+	-	-	-	-
60	red meat	70%	-	+	-	-	-	-
61	red meat	70%	-	+	-	-	-	-
62	red meat	70%	-	+	-	-	-	-
63	red meat	70%	-	+	-	-	-	-
64	red meat	70%	+	+	-	-	-	-
65	red meat	70%	+	+	-	-	-	-
66	red meat	70%	+	+	-	-	-	-
67	red meat	70%	+	+	-	-	-	-
68	red meat	70%	+	+	-	-	-	-
69	red meat	70%	-	+	-	-	-	-
70	red meat	70%	+	+	-	-	-	-
71	red meat	70%	-	+	-	-	-	-
72	red meat	70%	+	+	-	-	-	-
73	red meat	70%	+	+	-	-	-	-
74	red meat	70%	+	+	-	-	-	-
75	red meat	70%	+	+	-	-	-	-
76	red meat	70%	+	+	-	-	-	-
77	red meat	40%	-	+	-	-	-	-
78	red meat	40%	-	+	-	-	-	-
79	red meat	40%	-	+	-	-	-	-
80	red meat	40%	-	+	-	-	-	-
81	red meat	40%	-	+	-	-	-	-
82	red meat	40%	+	+	-	-	-	-
83	red meat	40%	+	+	-	-	-	-
84	red meat	40%	+	+	-	-	-	-
85	red meat	40%	+	+	-	-	-	-
86	red meat	40%	+	+	-	-	-	-
87	red meat	40%	+	+	-	-	-	-
88	red meat	40%	-	+	-	-	-	-

Table 2.

Sample No.	Labeled as	Meat percentage	Cow	Chicken	Sheep	Pig	Ostrich	Horse
89	red meat	40%	+	+	-	-	-	-
90	red meat	40%	+	+	-	-	-	-
91	red meat	40%	-	+	-	-	-	-
92	red meat	40%	-	+	-	-	-	-
93	red meat	40%	-	+	-	-	-	-
94	red meat	40%	-	+	-	-	-	-
95	red meat	40%	-	+	-	-	-	-
96	red meat	40%	-	+	-	-	-	-
97	red meat	40%	-	+	-	-	-	-
98	red meat	40%	-	+	-	-	-	-
99	red meat	40%	-	+	-	-	-	-
100	red meat	40%	-	+	-	-	-	-
101	red meat	40%	-	+	-	-	-	-
102	red meat	40%	-	+	-	-	-	-
103	red meat	40%	-	+	-	-	-	-
104	red meat	40%	-	+	-	-	-	-
105	red meat	40%	+	+	-	-	-	-
106	red meat	40%	-	+	-	-	-	-
107	red meat	40%	-	+	-	-	-	-
108	red meat	40%	+	+	-	-	-	-
109	red meat	40%	-	+	-	-	-	-
110	red meat	40%	-	+	-	-	-	-
111	red meat	40%	-	+	-	-	-	-
112	red meat	40%	+	+	-	-	-	-
113	red meat	40%	-	+	-	-	-	-
114	red meat	40%	-	+	-	-	-	-

2). In different production batch of sausages with 40% red meat it could be shown that 5 companies used only chicken meat in their sausages labeled as red meat. Five other companies used meat from chicken or beef and chicken in their production labeled also as red meat. Seven companies used no beef meat alone for production of sausages labeled 55% red meat. One company used only meat from chicken in all tested batch sausages labeled 55% red meat. None of the 10 companies labeled the sausages with 70% red meat, used beef meat alone. They used meat from either chicken or chicken and beef for the mentioned batches. Seven companies used only meat from chicken for production of sausages labeled 70% red meat. In none of the sausages, could the meat from horse, sheep, ostrich and pig be detected.

Discussion

Every consumer has the right to know about the source of meat prepared in the foods like sausages and this is important also for food safety and consumer demands to protect the consumer from various health risks, as well as due to many considerations like nutritional and religious reasons. To prevent losing consumer confidence in the food products, the control of food quality, safety and analysis of food components

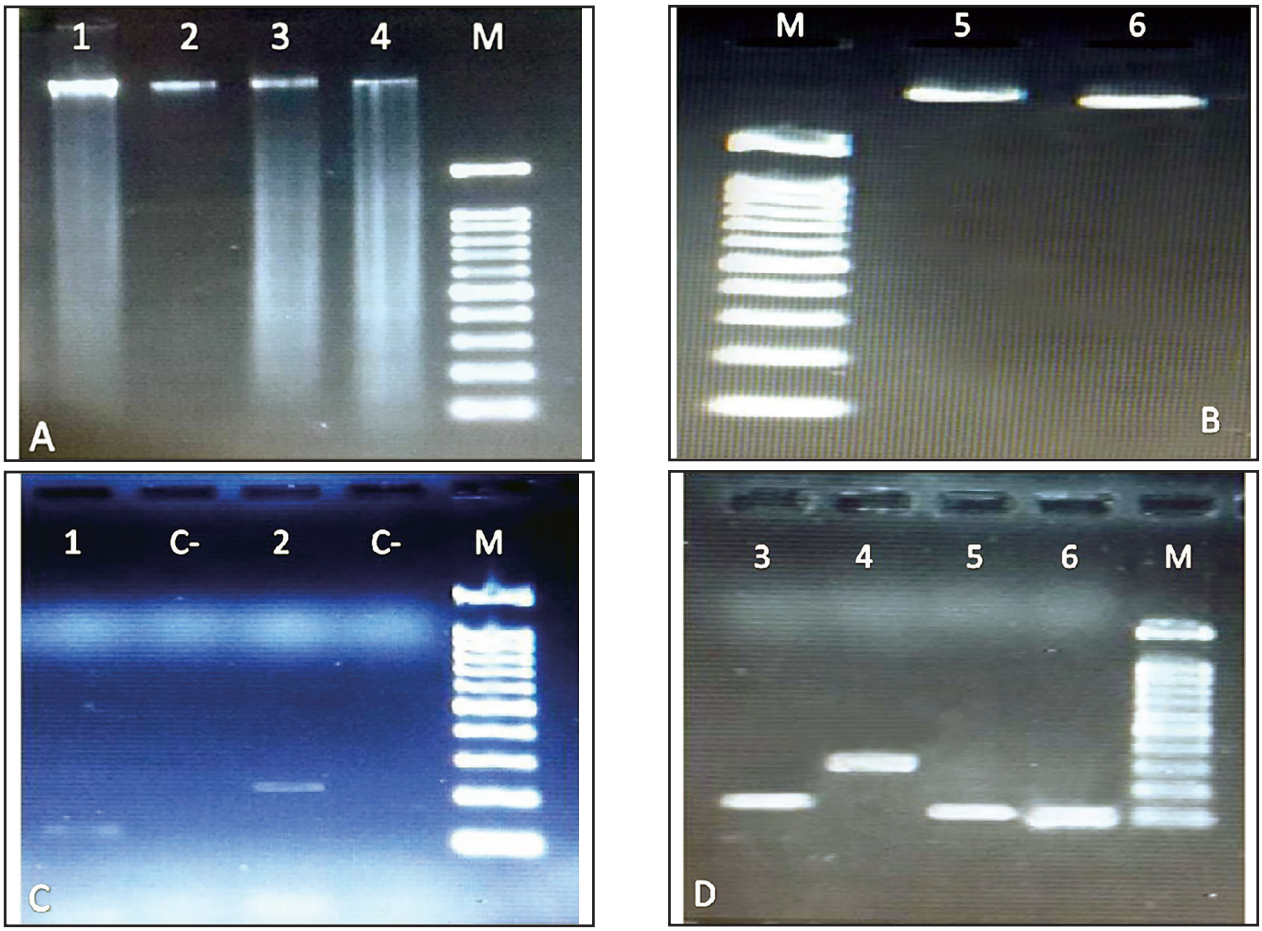

Figure 1. DNA was extracted from meat prepared from different species and analyzed on 1% agarose gel. lane 1–6 were of ostrich, cattle, pig, sheep, horse and chicken respectively. M was 100 bp DNA marker (A and B). DNA extracted from meat of different animals was amplified using animal specific primers and analyzed on 2% agarose gel. Lanes 1-6 are PCR with DNA extracted from chicken, horse, ostrich, cattle, sheep and pig. C- is control negative. M is 100 bp DNA marker (C and D).

is essential. For this aim, many different methods were reported. Many investigators used different methods for the analysis of meat origin. Some of these analyzed the meat products with methods based on protein analysis like immunological assays or based on chromatography(Armstrong, Leach, & Wyllie, 1992; Hsieh, Johnson, Wetzstein, Green, 1996; Zerifi, Labie, & Benard, 1991), on Peptide mass fingerprinting (PMF) (Pappin et al., 1993) and on peptide fragmentation fingerprinting (PFF) which are expensive (Saez et al., 2004).

The methods based on DNA such as PCR (Keyvan et al., 2017), nested PCR (Unajak et al., 2011), RFLP-PCR (Abdel-Rahman et al., 2009), multiplex PCR (Ghovvati et al., 2009; Kitpipit et al., 2014), Reverse Line Blot (Shayan et al., 2018) and Taqman or SYBR GREEN real-time PCR (Safdar & Abasıyanık, 2013) were previously described for detection of food fraud. In the present study multiplex PCR with species specific primers as described by Kitpipit et al. 2014 was used. The used multiplex PCR was designed for detection of meat from chicken, beef, sheep, horse, pig and ostrich. Single PCR of DNA extracted from meat prepared from the mentioned animals with corresponding species specific primers showed the expected PCR products. Only rarely in multiplex PCR with DNA extract-

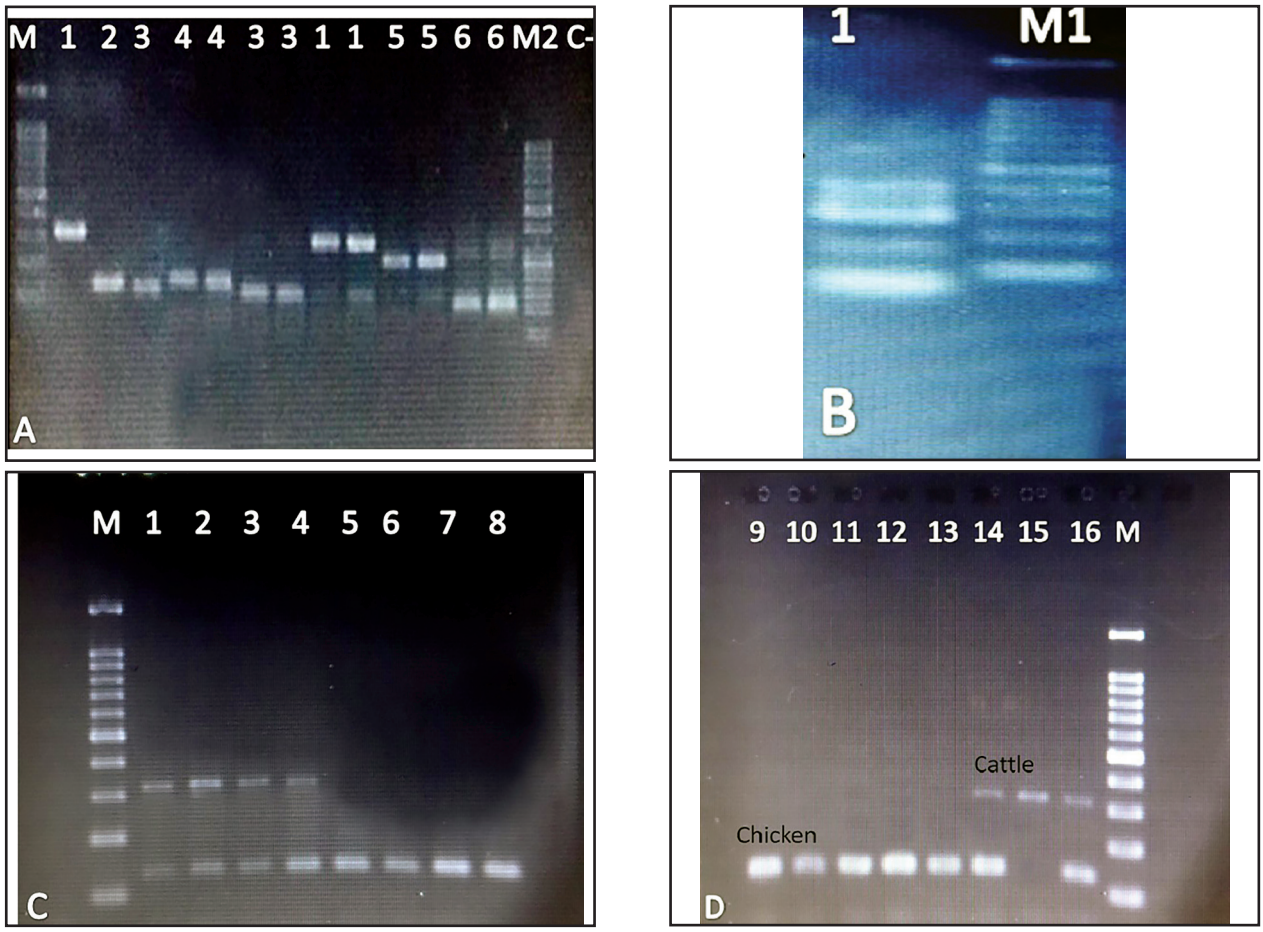

Figure 2. DNA extracted from different animals was amplified by multiplex PCR and analyzed on 2% agarose gel. A and B: lanes 1=cattle, 2= chicken, 3= sheep, 4= ostrich, 5= horse and 6= pig. In the case of cattle, sheep, ostrich, horse and pig, the amplification was done in double approaches. M1= 100 bp DNA marker, M2= 50 bp DNA marker, C- is control negative.C: Mix of DNA from above mentioned animals was amplified by multiplex PCR. Lane 1= Mix of DNAs. D and E:Multiplex PCR analysis of DNA extracted from sausages purchased from different companies. M is 100 bp DNA marker.

ed from chicken could another PCR product resembling a PCR product with 311bp in length be observed.

In the present study 114 samples from 10 producing companies were analyzed with the above mentioned multiplex PCR. The results showed that the information about the source of meat labeled on the products was not correct. Alone in 60 samples, the producers have used only the cheaper chicken tissues like meat or gizzard instead of red meat. In 108 samples out of 114 samples (94.7%) mislabeling was detected. Similar results were reported by Mehdizadeh et al., 2014 in Tehran who recorded (94.4%) of their samples contained undeclared chicken meat. (Mousavi et al., 2015) found mislabeling detected by species specific PCR in 47.2% of their analyzed samples collected from the same city as the collection samples was performed in the present study. Nejad, Tafvizi, Ebrahimi, & Hosseni, 2014 reported that they have detected DNA from poultry also in sausages labeled with only red meat in Tehran. The mislabeling seems to be not locally (country) limited. In Turkey, the adulteration was reported about 50% in sausages and 30.3% in fermented sausages respectively (Özpiner, Tezmen, Gokce, & Tekiner, 2013). The mislabeling was also re-

ported in another Islamic country, Malaysia, which was about 78.3% in the commercial meat product (Chuah et al., 2016). The mislabeling of food products was also reported from Europe (Colombo, Marchisio, Pizzini, & Cantoni, 2002; Kane & Hellberg, 2016; Miller, Jessel, & Mariani, 2012; Quinto, Tinoco, & Hellberg, 2016).

In all of the studies, adulteration or fraudulent labeling was probably due to the substitution of cheaper animal tissue instead of expensive labeled animal meat. A further reason that should not be forgotten is accidental cross-contamination. Because of improper handling and the use of shared equipment, sometimes spice contamination can occur during processing (Keyvan et al., 2017). In conclusion, our results and most of the other studies indicated that the meat species substitution occurs often in processed meats like sausages, which indicates the need of more governmental controls.

Acknowledgments

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Conflicts of interest

The author declared no conflict of interest.

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برچسب نادرست بر روی بسته بندی‌های سوسیس یکی از مشکلات روز افزون و مهم در تقلب در فراورده‌های غذایی

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چکیده

زمینه مطالعه: تشـخیص نوع گونه حیوانی گوشـت و محصولات گوشتی جهت مبارزه با تخلفات و محافظت از حقوق مصرف کنندگان در ارتباط با موضوع سلامتی و باورهای مذهبی بسیار مهم است. روش تکثیر مکرر با استفاده از آغازگرهای اختصاصی هر گونه حیوانی به عنوان روش مناسب شناخته شده است.

هدف: هدف از مقاله حاضر اسـتفاده از این چنین روش جهت تشـخیص برچسب نادرست به عنوان تقلب در سوسیس با روش multiplex PCR می‌باشد.

روش کار: در این مطالعه ۱۱۴ نمونه سوسیس که با برچسب‌های ۴۰، ۵۵ و ۷۰ ٪ گوشت از نوع گوشت قرمز نشان‌دار شده بودند و از ۱۰ شرکت مختلف در سطح تهران، تهیه شدند. پس از استخراج DNA از سوسیس‌های استخراج شده با روش multiplex PCR تکثیر داده شدند.

نتایج: نتایج نشان دادند که در ۶۰ سوسیس (۵۲/۶٪) فقط DNA مربوط به مرغ قابل ردیابی بود. در ۴۸ نمونه سوسیس (۴۲/۱٪) DNA از گاو و مرغ و در ۶ نمونه (۵/۳٪) DNA مربوط به گاو قابل تشخیص بودند. استفاده از گوشت مرغ در سوسیس که با برچسب گوشت قرمز ارزه شده بود، به احتمال زیاد به علت قیمت ارزان‌تر این گوشت در مقایسه با گوشت قرمز می‌باشد که از این طریق بتوان به سود بالاتری رسید.

نتیجه گیری نهایی: نتایج این مطالعه نشان می‌دهند که جایگزینی گوشت‌گران توسط گوشت ارزانتر در فراورده‌های دامی بسیار به چشم می‌خورد، لذا کنترل بیشتر اورگان‌های نظارتی دولتی بسیار حائز اهمیت می‌باشد.

واژه‌های کلیدی:
تقلب، برچسب اشتباه، multiplex PCR، واکنش زنجیره‌ای پلیمراز، سوسیس

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