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Histological, Bacteriological, and Parasitological Study and Animal Host Origin of Handmade Sausages Marketed in Eight Cities in Iran

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Abstract

The aim of this study was to examine the quality of handmade sausages using histological, bacteriological, and parasitological techniques. During 2023, 19 samples were randomly purchased from the market in eight cities in Iran, i.e., Tehran, Karaj, Tabriz, Hamedan, Borujerd, Mashhad, and Shiraz. Specimens were cut into three parts: i) fixed in 10% buffered formalin solution and sectioned and stained for histology according to the Iranian National Standard No. a6103, ii) minced and cultured on Plate Count Agar (PCA) medium and antibiogrammed, iii) used for genomic DNA extraction and conventional PCR to identify the animal host origin of the sausages and examine for coccidian parasites, *Toxoplasma gondii*, *Sarcocystis* spp., *Neospora caninum* DNA. Results showed that 4 samples were suspected of being parasitic cysts. PCR testing revealed that all four samples contained DNA of the coccidian protozoan parasite, and one sample contained DNA of *Neospora*. Additionally, based on PCR testing, 18 samples contained both chicken and beef meat, while one sample contained lamb meat. In the bacterial analysis, 3 samples contained a significant amount of *Bacillus subtilis*, and one sample contained *Bacillus cereus*. In the histological examination of the collected samples soybean, cartilage tissue, bone tissue, smooth muscle tissue, and dense connective tissue were observed. Therefore, it can be concluded that since there is no sanitary supervision or quality control in the production of handmade sausages, there is a possibility of using inferior meat products with lower nutritional value.

1. Introduction

It can be confidently said that sausages and cold cuts are among the oldest meat products, which were highly regarded by the Greeks. In fact, some sources trace the history of processed meat products back to two thousand and five hundred years ago. In the literature of Greece, there are references to sausages, cold cuts,

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and salami (a city in eastern Greece), and even Homer mentions sausages in his writings. In Iran, the history of making sausages and cold cuts, and the transition from handmade to factory production, dates back to 1928 in Bandar Anzali. In 1958 (1337 in the Iranian calendar), the first large sausage and cold cut factory was established in the southwest of Tehran. According to the Iranian National Standard, meat products must be free of prohibited tissues and organs from livestock and poultry (such as the digestive system of animals and poultry, including the lips of livestock, oral cavities, tongue, esophagus, gizzard of poultry, rumen and crop of poultry, rumens of livestock, milk glands, liver, intestines, rectum, glandular organs like the pancreas, salivary and adrenal glands, lymphoid tissues such as lymph nodes, thymus, spleen, Bursa of Fabricius, tonsils, and lymph follicles, respiratory system organs like the lungs, trachea, and larynx, reproductive system organs like kidneys, bladder, cloaca, mammary glands, meat from the head and face of the animal, heart, tail, skin, bone tissue, epithelial tissue, central nervous tissue, elastic cartilage, and transparent cartilage) (Asadi *et al.*, 2019).

Unauthorized tissues have low nutritional value and, from a hygiene standpoint, have a higher microbial load compared to muscle tissue. Additionally, some plant-based additives in meat products are considered allergens. Considering that the presence of unauthorized tissues in these types of meat products poses a serious threat to consumer health, and that the consumption of some of these tissues is forbidden in Islam and has religious prohibitions, the use of specific diagnostic methods to detect these tissues becomes necessary (Liscio and Hopley, 2016).

Given the high cost of certain food ingredients, producers may substitute them with materials that have lower costs. Fraud can also involve replacing a species with lower nutritional value in place of one with higher nutritional value. Some individuals may have allergies to certain diets, and using them can have irreversible consequences (Olsen and Borit, 2018).

The use of histological methods as a quality control test in the food industry has been employed since 1910. Histological methods are capable of directly identifying and differentiating the components of meat products, allowing for the direct detection of the composition and type of tissue in meat products. These methods can be used to detect tissue fraud. Therefore, histological methods can assist in the quality control of heat-treated meat products and complement the standards and regulations for meat and its products. The most common histological method used for detecting fraud in meat products is the standard tissue staining method (Hematoxylin-Eosin) (Hajimohammadi *et al.*, 2020).

Today, various methods are used for quality control of meat products, and each of these methods has its own specific capabilities. One of these methods is histological techniques. This method allows for the direct detection of a composition and tissue in meat products, and it can be used to identify tissue fraud in food products (Asadi *et al.*, 2023).

Histology can identify animal tissue structures (both authorized and unauthorized), such as muscle, connective tissue, bone, cartilage, and others, in meat products. Histology can also be used to identify non-meat proteins, such as plant-based proteins (wheat, soy, and flour), in meat products. This method has been used in meat products since 1910 (Sadeghi *et al.*, 2011). Muscle and connective tissues, which are the two main factors in evaluating the edibility quality of meat, can be identified by examining histological sections under a microscope. In this way, the higher the total percentage of muscle in a sample, the higher its edible quality will be. There are various techniques for staining cells or tissues, with Hematoxylin-Eosin staining being the most accepted and common method (Veuthey *et al.*, 2014).

In recent years, the production and presentation of meat products in front of customers and their display in stores have become widespread, and in most provinces of Iran, centers with this focus are actively operating. The production of these products in the presence of customers creates a sense of security. Currently, in Iran, if a store wants to sell its produced meat products under a brand, it needs approval from the Ministry of Industry, Mine, and Trade (SMT) as well as the Food and Drug Administration. However, if a store intends to produce meat products without a brand, it only requires a health permit and a license from the Guilds Organization. These meat products must be produced according to the Iranian National Standard No. 2303.

Along with the sense of assurance that the visibility of the production of these products provides to the customer, there may also be associated risks, such as the failure to use preservatives like nitrites (which may lead to botulism), the creation of new opportunities for fraud, and the pursuit of higher profits, among others. Given the widespread use of these products, their high price, and the lack of studies regarding the quality of these products, the present study aims to examine the components of these products using histological methods, measure the bacterial load, determine the animal species used to source the meat, and also conduct parasitological examinations of the collected samples.

2. Materials and Methods

2.1. Sample Collection

From June to February 2023, 19 sausage and cold cut samples were collected from production units in various cities, as outlined in Table 1. The samples were

immediately transferred to the laboratories of the Faculty of Veterinary Medicine, Bu-Ali Sina University, Hamedan, Iran, under the cold chain.

Table 1
Method of sampling handmade sausages from different cities in Iran in the present study

Province	City	Number of samples
Tehran	Tehran	4
Alborz	Karaj	3
East Azerbaijan	Tabriz	3
Hamedan	Hamedan	2
Loresatn	Borujerd	1
Mazandaran	Babol	2
Khorasan Razavi	Mashhad	2
Shiraz	Shiraz	2
Total		19

2.2. Histological Study

For histological examination (including the analysis of unauthorized tissues, percentage of skeletal muscle, connective tissue, and fat tissue), sampling was performed according to standard 6103 and a6103, and the samples were fixed in a 10% buffered formalin solution for at least 48 hours. After fixation, the samples underwent tissue processing and block preparation, and the tissue sections were cut using a microtome (Model DS4055, manufactured by Dideh Sabz, Iran) and stained using the Hematoxylin-Eosin staining method. To assess the percentage of skeletal muscle and connective tissue in the samples, a digital image analysis system was used, which included a light microscope (Medic M-107 BN, China) equipped with a camera (Dino-Late), imaging software (Dino capture V.2), and image analysis software (v.6 Image-Pro Plus). According to standard 6103, 36 sections were prepared for each sample. All tissue sections prepared for each sample were scanned using a slide scanner (PathScan Enabler IV, Plustek- USA), and the resulting images were analyzed with the mentioned software. The average image analysis for each sample was recorded. Since the aim of this study was to evaluate the quality of samples at the national level, and comparisons between provinces and cities were not intended (due to the limited number of production units and, consequently, the low number of samples taken from some cities), statistical comparisons were not made.

2.3. Bacteriological Study

A portion of the samples was sent to the Bacteriology Laboratory of the Faculty for bacterial load assessment and antibiogram testing. For the bacteriological tests, 100 milliliters of buffered peptone water medium were first placed into a sterile wide-mouthed plastic sampling container with a sealed lid (sterilized by autoclaving). Then, under a suitable flame, a sausage (sample) was placed inside the container. The lid was sealed, and the container was thoroughly crushed and shaken well for 2-3 minutes. The resulting solution was then used for microbiological cultivation. For the total bacterial count in each milliliter of washing liquid, the surface plating method was used as described below: Serial dilutions of 0.1, 0.01, 0.001, 0.0001, 0.00001, and 0.000001 were prepared from the washing liquid. Then, 1 milliliter of each dilution (starting from 0.1) was separately transferred to plates containing Plate Count Agar, and the medium was spread using a sterile loop or spreader. Finally, the plates were incubated upside down at 37°C for 24-48 hours. After this period, the plates were examined. The number of colonies on each plate was counted, and the counted number was multiplied by the dilution factor. The average number of colonies from the different dilutions was recorded as the bacterial count per milliliter of washing liquid.
The identification of bacterial isolates was performed using macroscopic and microscopic tests, Gram staining, and biochemical tests, including catalase, sulfide production (SIM), motility, indole, citrate utilization, oxidation and fermentation of sugars (OF), Methyl-Red Voges-Proskauer (MR-VP), starch hydrolysis, casein hydrolysis, and gelatin liquefaction. The antibiotic sensitivity and resistance patterns of the isolates were determined using the disk diffusion method (Kirby-Bauer) based on the Clinical and Laboratory Standards Institute (CLSI) protocol. The results were read and recorded after 18 hours by measuring the diameter of the zone of inhibition and comparing it with available reference tables. For this purpose, antibiotic disks of chloramphenicol, cefixime, penicillin, cefazolin, ampicillin, and vancomycin were used. The procedure was as follows: a bacterial suspension with a turbidity equivalent to a 0.5 McFarland standard was prepared. Then, using a sterile swab, the suspension was inoculated onto a solid Mueller-Hinton agar medium. Sterile forceps were used to place the antibiotic discs on the surface of the medium. The plate was then incubated at 35°C for 18 to 24 hours. To read the results, the diameter of the zone of inhibition around the disc was measured in millimeters using a precise ruler, and the bacterial sensitivity to the antibiotic was reported. Additionally, part of the samples was considered for DNA extraction and determining the animal species used in the production of the products (Table 2). The remaining extracted DNA samples were stored at -80°C for further studies.

2.4. DNA Isolation and PCR Assay

For parasite specimen identification, genomic DNA was extracted from ca. 200 mg of each specimen using a commercial kit (MBST, Tehran, Iran) and examined for the presence of cyst-forming protozoan parasites DNA targeting different genes. Initially all of the

samples were tested with the common apicomplexan primer pair COC1 F: AAGTATAAGCTTTTATACGGCT / COC2 R: CACTGCCACGGTAGTCCAATAC amplifying 270-350 bp fragment of the chromosomal small-subunit (16S) rRNA of Toxoplasma gondii, Neospora caninum, eimeriid coccidia, isosporid coccidia, Sarcocystis, Cryptosporidium and Hammondia (Ho *et al.*, 1996). PCR-positive samples were further tested with species-specific primers targeting B1 gene of Toxoplasma gondii (B22: AACGGGCGAGTAGCACCTGAGGAGA/ B23 GGGTCTACGTCGATGGCATGACAAC, amplicon size ∼115bp) (Bretagne *et al.*, 1993), NC5 gene of Neospora caninum (Np21plus: CCCAGTGCGTCCAATCCTGTAAC / Np6plus: CTCGCCAGTCAACCTACGTCTTCT, amplicon size ∼337 bp) (Müller *et al.*, 1996), mitochondrial cytochrome c oxidase (cox1) gene of Sarcocystis species (SF1: ATGGCGTACAACAATCATAAAGAA / SR5: TAGGTATCATGTAACGCAATATCCAT, amplicon size ∼1100 bp) (Gjerde, 2013). All PCRs were performed with the Taq DNA Polymerase Master Mix RED® (Ampliqon, Odense, Denmark) in a SimpliAmp® thermal cycler (Applied Biosystems, Waltham, MA, USA). In each run, positive DNA control retrieved from a previous study (Khordadmehr *et al.*, 2023) and double-distilled water as negative control were included. The amplified products were detected by electrophoresis on 2 % agarose gels stained with DNA Safe Stain (SinaClon, Tehran, Iran) (Table 3).

3. Results

3.1. Microbiological Results

In four samples, a single colony was observed at a 0.1 dilution. Among these, three samples contained Bacillus subtilis, and one sample contained Bacillus cereus. Antibiotic sensitivity testing and the antibiotic resistance pattern of the isolates were determined using the disc diffusion method (Kirby–Bauer) as a standard procedure according to the (CLSI) protocol. After 18 hours, the zone of inhibition diameter was measured and compared with available tables for interpretation and recording. All 4 samples were sensitive to the listed antibiotics, with one sample resistant to cefixime and another sample resistant to cefazolin (Fig. 1).

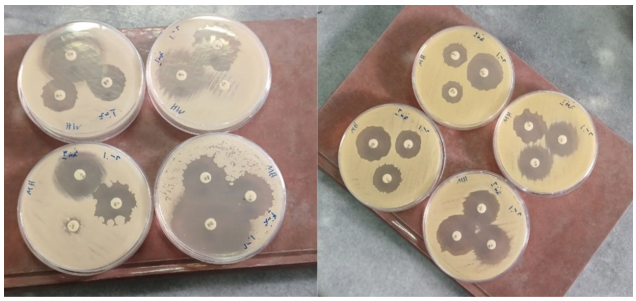

Fig. 1. Antibiotic sensitivity determination and the antibiotic resistance pattern of isolates obtained from handmade sausage samples

Table 2
Primers, target genes, and PCR conditions used in this study

Primers	Primer used (5′→3′)	Number of amplified base pairs (*bp*)
Cow	F: 5'-GCCATATACTCTCCTTGGTGACA-3′ R: 5'-GTAGGCTTGGGAATAGTACGA-3′	68
Sheep	F: 5'-ATGCTGTGGCTATTGC-3′ R: 5'-CCTAGGCATTTGCTTAATTTTA-3′	119
Chicken	F: 5'-CCTCCCAGCTCCATCAAACATCTCATCTTGATGAAA-3′ R: 5'-AAGATACAGATGAAGAAGAATGAGGCG-3′	133
Dog	F: 5'-GAGTTGATCCTTTTAGATTGTT-3′ R: 5'-AAGGGAATGATGAAAGACAT-3′	161
Donkey	F: 5'-CTATCCGACACACCCAGAAGTAAAG-3′ F: 5'-CTATCCGACACACCCAGAAGTAAAG-3′	439

Table 3
Specific primers designed for determining the type of microparasitic cyst species

Primers	Primer used (5'→3')	Number of amplified base pairs (*bp*)
B22	F: 5'- AACGGGCGAGTAGCACCTGAGGAGA -3'	115
B23	R: 5'- TGGGTCTACGTCGATGGCATGACAAC -3'	115
COC1	F: 5'- AAGTATAAGCTTTTATACGGCT -3'	270-350
COC2	R: 5'- CACTGCCACGGTAGTCCAATAC -3'	270-350
SF1	F: 5'- ATGGCGTACAACAATCATAAAGAA -3'	1100
SR5	R: 5'- TAGGTATCATGTAACGCAATATCCAT -3'	1100
Np21	F: 5'- CCCAGTGCGTCCAATCCTGTAAC -3'	337
Np6	R: 5'- CTCGCCAGTCAACCTACGTCTTCT -3'	337

Table 4
The unauthorized tissues observed in handmade sausages samples from provinces of Iran

City	Sample code	Soybean tissue	Cartilage tissue	Bone tissue	Parasitic microcyst	Smooth muscle	Dense connective tissue (percentage)	Skeletal muscle tissue (percentage)	Adipose tissue) (percentage)
Tehran	1			-		-	25.27	45.77	19.56
	2		-		-	-	15.14	36.11	20.14
	3	-	-	-	-	-	19.99	51.59	24.53
	4	-			-		19.21	61.06	17.03
Karaj	5		-			-	26.23	49.25	23.44
	6				-	-	16.01	61.23	18.18
	7	-		-	-	-	14.13	62.05	21.09
Tabriz	8	-		-	-		21.20	49.32	23.14
	9				-	-	12.95	53.89	14.07
	10			-	-	-	21.66	50.74	26.54
Hamedan	11	-	-	-		-	11.75	49.61	25.14
	12	-	-		-		9.58	62.24	17.65
Borujerd	13		-	-	-		19.21	58.32	19.11
Babol	14	-		-	-	-	15.68	52.47	11.57
	15		-		-	-	14.55	60.19	18.66
Mashhad	16		-		-	-	21.07	53.01	16.46
	17	-	-	-	-	-	18.57	55.95	20.88
Shiraz	18						21.44	57.59	14.91
	19		-		-	-	19.64	59.12	8.09
Average (percentage)		f57.89	42.10	52.63	21.05	26.31	18.06	54.18	18.95

3.2. Histology

In the histological study of the collected samples, a total of 57.89% (11 out of 19 samples) of the facial samples contained soybean plant tissue on average. Additionally, 49.10% (8 out of 19 samples) contained cartilage tissue, 52.63% (10 out of 19 samples) contained bone tissue, and 26.31% (5 out of 19 samples) contained smooth muscle tissue. In this study, microcystic sections of parasites were observed in 21.05% (4 out of 19 samples) of the tissue sections. On average, 18.06% of the compositions in these products were dense connective tissue, 54.18% were skeletal muscle tissue, and 18.95% were adipose tissue. The results, broken down by samples from each city, are shown in Table 4 (Fig. 2).Table 4 Results of the histological examination of the studied handmade sausage samples.

3.3. Species Identification

The results from the PCR test to determine the meat origin, using primers for chicken, cow, sheep, donkey, and dog, showed that out of the 19 samples, 4 samples contained the genome of both cow and chicken (simultaneously) (Fig. 3(A)). No dog or odd-toed ungulate genomes were detected in any of the samples (Fig. 3(B)). Finally, only one sample showed the sheep genome (Fig. 3 (C)).

3.4. Identification of Parasitic Cyst Species

In the investigation and species determination of the parasitic microcysts observed in the collected samples using the PCR method for *Toxoplasma gondii* (B22/B23), all samples were negative. Regarding the *Coccidia* primer (COC1/COC2), four samples (21.05%) were positive. When testing with the primer for *Sarcocystis species* (SF1/SR5), all samples were negative. For the *Neospora* primer (Np21/Np6), one sample (5.26%) was positive.

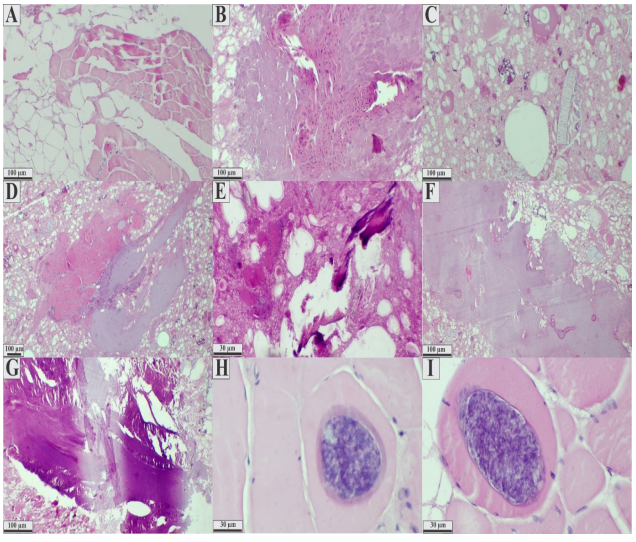

Fig. 2. Authorized and unauthorized tissues observed in the histological sections of the handmade sausages studied. A) Skeletal muscle tissue and fat; B) Smooth muscle tissue; C) Soy plant tissue; D) Cartilage tissue; E) Bone tissue; F and G) Dense connective tissue; H and I) Parasitic microcysts

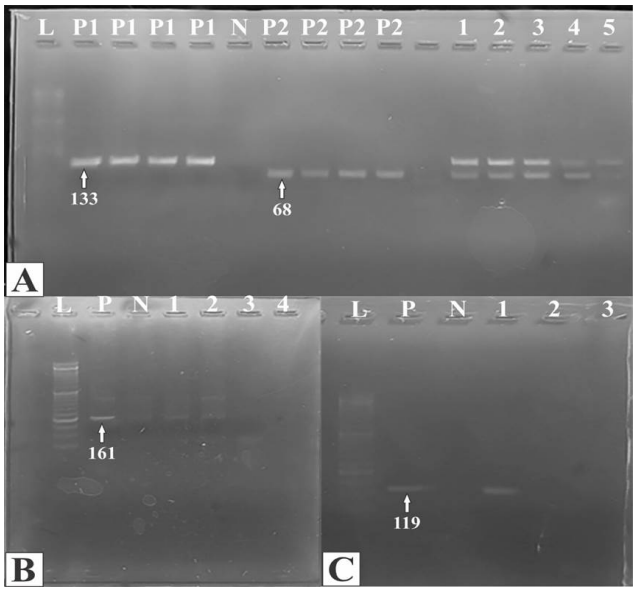

Fig. 3. PCR test images related to the origin of meat used in the production of handmade sausages. A) From left to right, the chicken primer (bp 133) and the cow primer (bp 68) are shown. In the corresponding band, both genomes are expressed in 4 samples, indicating the presence of both cow and chicken meat. B) In the examination with the dog primer (bp 161) and donkey primer (bp 439), no genome was observed in any of the samples. C) In the examination with the sheep primer (bp 119), only one sample was positive

4. Discussion

In the present study, which was conducted on handmade sausage samples collected nationwide, unauthorized tissues such as soybean, cartilage, bone, smooth muscle, and microcystic sections of parasites were observed. Additionally, a high proportion of dense connective tissue and adipose tissue was found in these samples. In the investigation of suspected parasitic cysts with PCR testing, four samples were positive for Coccidia primers, and one sample was positive for Neospora primers. In the bacterial examination of the samples, four samples at a 0.01 dilution contained a single colony, with three samples showing a significant presence of *Bacillus subtilis* and one sample containing *Bacillus cereus*. Among these, all four samples were sensitive to the antibiotic's chloramphenicol, cefixime, penicillin, cefazolin, ampicillin, and vancomycin, while one sample was resistant to cefixime and another was resistant to cefazolin. According to PCR testing, four samples contained both chicken and cow meat simultaneously, and one sample contained sheep meat.

Today, the demand for accurate and transparent information about food products from consumers is greater than ever, and meat products are no exception. The use of PCR technology in meat authenticity testing is an effective method (Cavin *et al.*, 2016). In a study aimed at identifying the animal species used in 91 beef samples through PCR, it was shown that 2.47% of the samples contained chicken meat, and 0.7% contained donkey meat (Mousavi *et al.*, 2015). In another study, the PCR test was used to investigate impurities in meat products in terms of the presence of soy. The results showed that 100% of sausage products, 20% of chicken cutlets, 33% of sausage products, 60% of hamburger products, and 15% of kebab products contained soy (Bazyari *et al.*, 2015). Alikord, *et al.* in 2017 used a composite polymerase chain reaction (PCR) to identify the meat consumed in raw and cooked meat products. The findings showed that, overall, six cases (17%) of fraud were detected, with four cases (11%) involving horse meat and two cases (6%) involving donkey meat. No instances of pork meat were found. The use of beef/sheep meat was confirmed in all samples, indicating that the use of other animal meats was solely to reduce economic costs (Alikord *et al.*, 2017). In a report by Eaqub Ali, *et al.* in 2015, PCR was used to determine the origin of raw materials in the production of meat products. The results indicated the presence of five non-halal species, including cat, pig, dog, monkey, and rat, in products that were sold as halal food (Eaqub Ali *et al.*, 2015).

Studies show that incorrect labeling of meat products occurs in more than 20% of cases (Ballin *et al.*, 2009). The mixing of chicken meat and by-products in meat products labeled as red meat has been reported in recent years (Nešić *et al.*, 2017).

In a study by Hosseni *et al.* in 2014, ten different brands of red meat sausages, made from samples collected in Tehran province, were analyzed using PCR to determine the type of meat used. The specific PCR results revealed that, contrary to the labels on the products, all samples contained poultry by-products (Hosseni *et al.*, 2014). In another study, Lakzadeh and *et al.* in 2016 used Real-Time PCR to identify and measure the amount of chicken tissue in meat products labeled as red meat. The results of the study indicated the presence of 84% and 26% chicken tissue, respectively, in sausage and burger samples (Lakzadeh *et al.*, 2016). Sadeghpour, *et al.*, in 2022 investigated the authenticity of 47 samples of raw meat and red meat products from Tabriz, specifically focusing on the mixing of chicken meat or other poultry parts, using PCR technique. The results showed that poultry by-products were mixed in both processed products with standard certification, such as fully and semi-processed products, as well as in raw meats. Overall, 87.23% of the collected samples were found to be fraudulent (Sadeghpour *et al.*, 2022). Al-Qassab, *et al.*, in 2019 conducted a study on 114 samples of red meat sausages with 40%, 55%, and 70% meat content from 10 different companies in Tehran. After extracting DNA from the sausages, they were amplified using multiplex PCR. The results showed that in 60 sausages (52.6%), only

chicken DNA was detectable. In 48 sausage samples (42.1%), both cow and chicken DNA were present, and in 6 samples (5.3%), only cow DNA was detectable. The use of chicken meat in sausages labeled as red meat may be due to the lower price of chicken compared to red meat, which allows producers to make a higher profit (Al-Qassab *et al.*, 2019). The results of the present study, which focused on handmade sausage samples, showed that 4 samples contained both chicken and cow meat, and 1 sample contained sheep meat.

Julini, *et al.* in 1979 reported the use of unauthorized animal tissues such as nerves, bones, skin, kidneys, and mammary glands in sausages (Julini *et al.*, 1979). Prayson, *et al.* in 2008 conducted histological studies on eight different brands of hamburgers in the United States. They found that all eight brands contained unauthorized tissues including connective tissue, blood vessels, fat, and peripheral nerves. Additionally, skeletal muscle was observed in seven samples, plant tissue in four brands, cartilage tissue in three brands, and bone tissue in two hamburger brands (Prayson *et al.*, 2008). In another study, the presence of unauthorized tissues was investigated in 20 samples, including kebab, sausage, handmade hamburger, and koobideh kebab. The histological test results showed the presence of tissues such as connective tissue in all samples, gizzard in two samples, fat in seven samples, soy in eleven samples, cartilage in five samples, and ovary in one sample (Latorre *et al.*, 2015). In the present study, soy plant tissue, cartilage, bone, smooth muscle, and dense connective tissue were observed, which is consistent with previous studies.

In addition, Jahed Khaniki, *et al.* in 2004 found that the contamination rate of samples with Sarcocystis parasites was 25.6%, and the presence of tissue masses was 5%, with a significant amount of plant tissue also observed (Jahed Khaniki and Rokni, 2004). Furthermore, Jahed Khaniki and Kia in 2006 studied the prevalence of Sarcocystis cysts in Iranian hamburger meat products using histological methods, and the results indicated that the contamination of samples with Sarcocystis parasites was approximately 60% (Jahed Khaniki and Kia, 2006).

In another study, raw hamburgers sold in Tehran were examined for Sarcocystis cysts. The results showed that no parasite cysts were observed in any of the industrially produced samples; however, one handmade sample was found to contain parasite cysts (Hosseini *et al.*, 2008). The results of the present study regarding the observation of parasitic cysts were consistent with previous reports, but they did not align with the species of parasite most commonly reported in studies on Sarcocystis cysts.

Molecular detection of N. caninim herein reported is consistent with previous studies reporting widespread and considerable prevalence of the parasite in muscular tissue of mammalian and avian hosts (refs). In other researches on meat products N. caninum DNA and cysts were reported by PCR (refs) and histology (refs). However, it should be remembers that microscopic cysts of the parasite could be located in one portion of the examined tissue collected from molecular analysis while absent in another portion taken for histological examinations (Karimi *et al.*, 2023).

Unfortunately, the issue of fraud in meat products, which disrupts public health, contradicts religious and ethical principles, and undermines public trust in a system of fair trade, is a very important topic in the food industry. Therefore, it is essential to make adherence to standards and quality control in meat products mandatory for producers, with serious supervision over their practices. Additionally, identifying the type of meat used in meat products is important for religious, health, and economic reasons. In this regard, the present study is the first to examine handmade sausages from across Iran using histological testing and PCR techniques to detect fraud in meat products.

5. Conclusion

Research indicates that the use of unauthorized tissues in meat products is possible. Based on the histological findings in the present study, it can be concluded that some handmade sausages across the country contain unauthorized tissues, Neospora microcysts, and coccidiosis. The primary meat used is beef, although chicken and lamb meat were also observed. Considering that handmade sausages are not subject to health supervision or quality control, there is a possibility of using substandard meat products. Therefore, it is important to emphasize that more rigorous control and supervision should be implemented by regulatory and health organizations.

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