

# Parasitological and Microbiological Investigation of Meat and Offal Consumed in Istanbul

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## Research Article

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## Abstract

In this study, it was aimed to explore the presence of some important foodborne parasitological and microbiological parameters that may seriously risk the consumers' health (total coliform, *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Escherichia coli* EHEC O157:H7, *Salmonella* spp., *Salmonella enteritidis*, *Salmonella typhimurium* and *Toxoplasma gondii*) in meat and offals that are sold in various sales points in different districts of Istanbul. For this purpose, 400 samples (100 mutton, 100 beef, 100 minced meat and 100 sheep brain) were collected and analyzed by using PCR procedures for aforementioned parameters. The data obtained, were interpreted by statistical methods and binary correlations among the parameters will be exposed. Besides, a risk alligment was formed among the sales points and the products. According to the results, *Escherichia coli* EHEC O157:H7 and *Toxoplasma gondii* were determined in any samples. However except the aforementioned parameters all the analyzed microbiological variables were exposed in different samples with different concentrations. The results were verified by real-time PCR procedures. Binary correlation analysis were applied to the positive determined microbiological parameters. The results showed that all the positive determined microbiological parameters were positively correlated with each other. The result of the study showed that applynig good hygien procedures is very important to supply qualified and safe food to the costumers is very improtant in very step of food production chain but especially in the sales points that sell ready to eat foods. Besides, it was concluded that the implementation of effective control / inspections and continiuous education programs which would be held by related government agencies would be very effective for decreasing the incidence of the foodborne pathogens.

## Introduction

The strong connection between food consumption and human diseases was defined by Hippocrates in ancient times and foodborne pathogens were reported as biological agents causing foodborne diseases (Bintsis, 2017). According to the World Health Report, 1/10 of the people worldwide get sick due to food contamination and 1.8 million people die from foodborne diseases every year (Wei and Zhao, 2021). Consumption of animal foods such as meat, milk and eggs is increasing due to globalization, rapid population growth, changing dietary habits and lifestyle. Consequently; Mass production and global movement of products occur, increasing the risk of contamination of foodborne pathogens at any stage of the chain from farm to fork (Abebe et al., 2020). Meats; tools, knives, hands, clothes and air can be easily contaminated during cutting, transportation, processing, packaging and distribution and cause biological, chemical, physical and especially microbial food hazards (Bantawa et al., 2018; Kalogianni et al., 2020). For example; beef was reported to be the vector of 7% of the 1.7 million cases of foodborne illness in England and Wales between 1996 and 2000 (Heredia and García, 2018). *Listeria* spp., *Salmonella* species, *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*) and *Toxoplasma gondii* (*T. gondii*) are the most common and serious pathogens and parasites that can be transmitted through meat (Abay et al., 2017; Assefa and Bihon, 2018; Hinney, 2018; Koutsoumanis et al., 2018). Approximately 1.4 million cases are caused by non-typhoid *Salmonella* serotypes and 270.000 cases are caused by pathogenic *E. coli*, including *E. coli* O157:H7 (Bantawa et al., 2018).

In this study, meat and products (mutton, beef, minced meat and sheep brain) offered to consumers in different types of retail businesses in the Istanbul region were studied. Some important food-borne microbiological and parasitological parameters, which may be serious risk factors for consumer health, were investigated by real time PCR (rt-PCR) method. In our study, in addition to other studies on red meat and offal, sheep brain, which is a type of offal that is frequently consumed by individuals, was also examined.

## Material And Method

### Selection of samples:

Within the scope of the study, 400 sheep meat, beef, minced meat and sheep brain (total 100 samples for each different variety) were collected from different types of sales points in Istanbul that sell directly to the public, and *T. gondii*, *Salmonella* spp., *Salmonella enteritidis* (*S. enteritidis*), *Salmonella typhimurium* (*S. typhimurium*), *Listeria monocytogenes* (*L. monocytogenes*), *S. aureus*, *E. coli* and *E. coli* were analyzed for EHEC O157:H7 parameters.

# Microbiological Analysis

**Total Coliforms:** Firstly, dilution and homogenization procedures were applied to the samples collected using sterile containers and delivered to the laboratory in compliance with the asepsis conditions, and then the standard spreading method was used instead of VRB (Violet Red Bile Agar) agar medium prepared beforehand and poured into petri dishes. After seeding, a second layer of VRB agar was added to the petri dishes. Petri dishes were incubated for 24 hours at 37°C and the typical colonies formed at the end of the incubation period were counted (Manual, 2011).

***S. aureus:*** Dilution and homogenization procedures were applied to the samples, which were collected using sterile containers and delivered to the laboratory in compliance with the asepsis conditions, and then the standard smear method was used instead of the BPA (Baird – Parker Agar) medium prepared beforehand and poured into petri dishes. Petri dishes were incubated for 24 hours at 37°C. At the end of the incubation period, typical colonies were transferred to DNase Agar and DNase plates were again incubated at 37°C for 24 hours. After the typical colonies were subjected to the coagulase test, the identification procedure was completed (Bennett and Lancette, 2001).

***L. monocytogenes:*** 25 g of the sample was transferred into 225 ml of BLEB (Buffered Listeria Enrichment Broth Base), incubated for 4 hours at 30°C, and then selective agents and 25 mg/L natamycin were added to the media for 48 hours at 30°C. time incubated. *Listeria* spp. Cultures were purified by passages from suspected colonies on Trypticase soy agar (TSA) containing/added Yeast Extract. Suspicious isolates were identified. In addition, CAMP test was performed with *S. aureus* and it was determined whether the isolates had CAMP factor (Hitchins et al., 2017).

***E. coli* EHEC O157:H7:** Petri dishes TBX (Tryptone Bile X-glucuronide) were incubated at 44°C for 24 hours and the typical colonies formed at the end of the incubation period were counted. For EHEC O157: H7, after homogenization, the samples were transferred to MTSB (Modified Tryptic Soy Broth) and incubated at 37°C for 24 hours. Afterwards, a loopful sample from each medium was transferred to SMAC Agar (Sorbitol Mac Conkey Agar) and incubated again at 37°C for 24 hours (Manual, 2011).

***Salmonella* spp./*S.typhimurium*/*S.enteritidis:*** Incubation and selective enrichment procedures were performed. For the samples incubated for 24 hours at 37°C, strains showing red color at the top, yellow color and blackening at the bottom on TSI agar (Triple Sugar Iron Agar), strains that grew as a purplish color at the bottom and bottom on LI agar (Lysine Iron Agar), and strains that gave a negative reaction on urea broth *Salmonella* spp. was accepted as positive. The motility of the suspicious colonies on the agar in question at 37°C for 24 hours showed that *Salmonella* spp. evaluated positively. *Salmonella* spp. black “rabbit eye”-like colonies with a metallic bright zone around it were confirmed as *S. enteritidis* on Bismuth Sulphite Agar for samples that were positive, and bluish-gray/dark gray colonies were confirmed as *S. typhimurium* on Hektoen Enteric Agar (FDA, 2014).

## Dna Extraction

***T. gondii:*** Homogenization of 50 grams of meat followed by DNA extraction was performed according to the kit protocol (Macherey-Nagel, Nucleospin®). In the study, two separate DNA preparation procedures were applied for microbiological parameters. One of them is bacterial lysis by heat treatment. The other method is lysis resulting from the treatment of a mixture of lysozyme, proteinase K and SDS with phenol/chloroform extraction (Gutierrez et al., 2010).

## PCR

The presence of *Salmonella* spp., *S.typhimurium*, *S. enteritidis*, *L. monocytogenes*, *S. aureus*, *E. coli* and *E. coli* EHEC O157:H7 was sought in all samples collected as planned by the PCR procedure. For this purpose, the PCR procedure defined for each microbiological parameter was applied to the isolates that were evaluated as suspicious/positive as a result of the cultivation procedure performed with conventional methods for microbiological parameters (Hookey et al., 1999; Holland et al., 2000; Malorny et al., 2001; Malkawi and Gharaibeh 2004; Lim et al., 2005; Aires-De-Sousa et al., 2006; Obi et al., 2007; Onyango et al., 2009; Sudagidan and Aydin, 2009). Meat products were examined by PCR method in order to detect *T. gondii* for parasitological diagnosis. For the detection of *T. gondii* DNA, Gutierrez et al. The primer sequences and PCR protocol reported by (Gutierrez et al., 2010) were applied (Table 1).

Table 1  
Specific primer sets that were used in study

Primer No	Sequence (5' – 3')	Target gene/Amp (bp)	Target microorganism
1	GTGAAATTATCGCCACGTTCCGGCAA	<i>invA</i> / 284	<i>Salmonella</i> spp.
2	TCATCGCACCGTCAAAGGAACC	<i>invA</i> / 284	<i>Salmonella</i> spp.
3	CGGTGTTGCCAGGTTGGTAAT	<i>fliC</i> / 620	<i>S. typhimurium</i>
4	ACTGGTAAAGATGGCT	<i>fliC</i> / 620	<i>S. typhimurium</i>
5	AGCCAACCATTGCTAAATTGG	<i>invA</i> / 488	<i>S. enteritidis</i>
6	GCGTAAATCAGCATCTGCAGTAGC	<i>sefA</i> / 488	<i>S. enteritidis</i>
7	GCTGATTTAAGAGATAGAGGAACA	<i>actA</i> 827	<i>L. monocytogenes</i>
8	TTTATGTGGTTATTTGCTGTC	<i>actA</i> 827	<i>L. monocytogenes</i>
9	GGCAATTGTTTCAATATTAC	<i>nuc</i> / 416	<i>S. aureus</i>
10	TTTTATTTGCATTTTCTACC	<i>nuc</i> / 416	<i>S. aureus</i>
11	ATAGAGATGCTGGTACAGG	<i>coa</i> / 500 - 650	<i>S. aureus</i>
12	GCTTCCGATTGTTTCGATGC	<i>coa</i> / 500 - 650	<i>S. aureus</i>
13	ACA CTG GAT GAT CTC AGT GG	VT1 (SLT1) / 614	<i>E. coli</i>
14	CTG AAT CCC CCT CCA TTA TG	VT1 (SLT1) / 614	<i>E. coli</i>
15	CCA TGA CAA CGG ACA GCA GTT	VT2 (SLT2) / 779	<i>E. coli</i>
16	CCT GTC AAC TGA GCA CTT TG	VT2 (SLT2) / 779	<i>E. coli</i>
17	AAG CGA CTG AGG TCA CT	<i>eaeA</i> / 450	<i>E. coli</i> EHEC O157:H7
18	ACG CTG CTC ACT AGA TGT	<i>eaeA</i> / 450	<i>E. coli</i> EHEC O157:H7
19	CACAGAAGGGACAGAAGT	B1 / 94	<i>T.gondii</i>
20	TCGCCTTCATCTACAGTC	B1 / 94	<i>T.gondii</i>
21	FAM-CTCTCCTCCAAGACGGCTGG-TAMRA	<i>probe</i>	<i>T.gondii</i>

## Statistical Analysis

All data analysis was performed using Statistical Package for Social Sciences (SPSS) version 22 (SPSS Inc., Chicago, IL, USA). The chi square test was used to analyze if there was any significance in the pathogens isolated from the different outlets (butcher, restaurant, peddler, market). P values less than or equal to 0.05 were considered significant.

## Results

Applied microbiological sows showed the presence of *Salmonella* spp. and *S. aureus* in meat and meat products, and this finding was confirmed by PCR. Fig. 1 shows PCR products of the *invA* gene confirming the presence of *Salmonella* spp., while Fig. 2 shows PCR products of *nuc*, *coa* and *spa* genes that indicate the presence of *S. aureus* (Fig. 1, Fig. 2).

Of the mutton obtained from butcher's 22% was positive for coliform, 17% for *S. aureus*, 11% for *E. coli*, 7% for *L. monocytogenes*, 3% for *Salmonella* spp, and of the beef 25% was positive for coliform, 21% for *S. aureus*, 16% for *E. coli*, 5% for *L. monocytogenes*, 4% for *Salmonella* spp. No sample was positive for *E. coli* EHEC O157:H7, *S. typhimurium*, *S. enteritidis* and *T. gondii* (0%). Of the sheep's brain obtained from the butcher's 12% was positive for *S. aureus*, 8% for coliform, 2% for *E. coli* and *Salmonella* spp. No sample was positive for *E. coli* EHEC O157:H7, *S. typhimurium*, *S. enteritidis*, *L. monocytogenes* and *T. gondii* (0%). Of the minced meat obtained from restaurant/kebab house/mobile vendor 37% was positive for coliform, 28% for *S. aureus*, 23% for *E. coli* EHEC

O157:H7, 17% for *Salmonella spp.*, 12% for *L. monocytogenes*, and 4% for *S. typhimurium* and *S. enteritidis*. No sample was positive for *E. coli* EHEC O157:H7 and *T. gondii* (0%). The product with the highest risk in terms of all parameters was minced meat, while the product with the lowest risk was sheep's brain (Fig. 3).

## Discussion

Meat consumption increases worldwide in parallel with increasing challenges in meat hygiene and safety (Iyer et al., 2013). Infections of foodborne *Salmonella spp.* are important worldwide and are recognized as the second most common foodborne pathogen in the European Union (Bonardi, 2017). Types of *Salmonella spp.* isolated from meat products are resistant to many drugs and can cause very serious problems (Soltan Dallal et al., 2014). As a result of *Salmonella spp.* infection in the United States, 26.500 hospitalizations, 1.35 million cases and 420 deaths occur each year (Bjelland et al., 2020). Most of which seen in children aged up to 4, 155.000 deaths occur per year due to Salmonellosis caused by the most common serotypes *S. enteritidis* and *S. typhimurium* (Evangelopoulou et al., 2015). In our study, *Salmonella spp.* and *S. aureus*, ranked first among foodborne pathogens that threaten public health reportedly by World Health Organization (WHO) and the European Union, have shown to have positive correlation and to stimulate their development (Table 2).

Table 2  
Bilateral correlation relations of samples in terms of analyzed parameters

Parameter	Correlation coefficient / Significance	Coliform	<i>E. coli</i>	<i>S. aureus</i>	<i>Salmonella spp.</i>	<i>S. typhimurium</i>	<i>S. enteritidis</i>	<i>L. monocytogenes</i>
Coliform	r	1.000	.276	.421	.665	.534	.221	.900
	p	—	<b>.000</b>	<b>.000</b>	<b>.003</b>	<b>.005</b>	<b>.000</b>	<b>.000</b>
<i>E. coli</i>	r	.220	1.000	.444	.556	.788	.712	.840
	p	<b>.000</b>	—	<b>.000</b>	<b>.004</b>	<b>.000</b>	<b>.000</b>	<b>.000</b>
<i>S. aureus</i>	r	.754	.49*	1.000	.543	.800	.887	.291
	p	<b>.003</b>	<b>.004</b>	—	<b>.000</b>	<b>.000</b>	<b>.000</b>	<b>.000</b>
<i>Salmonella spp.</i>	r	.702	.675	.231	1.000	.398	.506	.312
	p	<b>.005</b>	<b>.000</b>	<b>.000</b>	—	<b>.000</b>	<b>.000</b>	<b>.000</b>
<i>S. typhimurium</i>	r	.342	.754	.300	.802	1.000	.876	.521
	p	<b>.005</b>	.010	<b>.000</b>	.020	—	<b>.000</b>	<b>.000</b>
<i>S. enteritidis</i>	r	.901	.860	.521	.106	.286	1.000	.547
	p	<b>.000</b>	.021	.012	<b>.003</b>	<b>.001</b>	—	<b>.004</b>
<i>L. monocytogenes</i>	r	.723	.055	.467	.602	.866	.377	1.000
	p	<b>.003</b>	<b>.000</b>	.019	.032	.040	<b>.005</b>	—
Bilateral correlation relations are demonstrated by Kendall's tau-b relationship analysis method.								
Since <i>E. coli</i> EHEC O157:H7 and <i>T. gondii</i> were not detected in any example, these parameters are excluded from evaluation.								
Numerals written with bold characters are statistically significant (p<0.005).								
The significance is that it stimulates the reproduction of the other for each binary parameter. That is, the presence of one of both parameters, which is significant, positively affects the reproduction of the other.								

In a study conducted by Zarei et al. (2013); in a total of 210 samples, each 500 g collected from retail outlets and popular supermarkets, the prevalence of *Salmonella spp.* was 7.1% in lamb (n=70), 4.3% in beef (n=70) and 2.8% in buffalo meat (n=70) (Zarei et al., 2013). In our study, prevalence of *Salmonella spp.* was 12% in minced meat obtained from restaurants/kebab

houses/vendors, 4% in beef, 3% in mutton, and 2% in sheep brain obtained from butcher's. Another study examined 60 meat samples obtained from hypermarkets (n=20), groceries (n=20) and butchers (n=20); the prevalence of *Salmonella spp.* was determined to be 45% in butchers, 25% in grocery stores and 5% in hypermarkets (Iyer et al., 2013). In a study conducted on 189 beef and 190 chickens, 45% of chicken samples and 20.2% of beef samples were found to be positive for *Salmonella spp.*, and one of the most isolated serotypes in unpackaged products was *S. enteritidis* (Soltan Dallal et al., 2014). In another study conducted by Ristori et al. (2017) on 552 chilled meat products (138 hot dogs, 138 raw pork sausage, 138 raw minced meat, 138 raw chicken legs); in terms of *Salmonella spp.*, 62.5% of the positive specimens were pork sausage and 37.5% of chicken legs. In addition, while in terms of *Salmonella spp.*, 12.5% of the positive samples belonged to *S. enteritidis* and 28.1% belonged to *S. typhimurium* serotypes, in our study 4% of minced meat was positive for *S. typhimurium* and *S. enteritidis*, but mutton, beef and sheep brains were not positive in terms of mentioned serotypes (Ristori et al., 2017). In another study, *Salmonella spp.* was isolated in 38.06% of 155 poultry offal (Sanda Abdelkader et al., 2019). In a study of pig offal products, 21.8% of 370 samples were positive for *Salmonella spp.*; intestinal (20%), brain (21%), liver, heart (73%) and kidney (87%) samples were determined to be suitable for human consumption (Erickson et al., 2019). This shows that, with the appropriate use of adequate heat treatment of meat products, cooling, freezing processes, application of correct cooking and packaging techniques, outbreaks of these pathogens can be prevented as a result of the absence of cross-contamination of meat products with other products ready for consumption.

In our study, it was determined that the highest risk food in terms of all microbiological parameters was minced meat. The foods that are risky after minced meat for Coliform, *E. coli* EHEC 0157:H7, *S. aureus* and *Salmonella spp.* were beef, mutton and sheep brain, respectively. *Salmonella spp.* and *E. coli* are the main factors that can lead to infections with food poisoning. In a study conducted by Zafar et al. (2016); 30 pieces of minced beef, mutton and chicken meat were examined and the highest microbiological load in terms of *Salmonella spp.* was seen in minced beef (6.57-7.39), followed by chicken and mutton, respectively (Zafar et al., 2016). In another study, 8.34% of the meat collected from the market, 11.86% of mutton, 12.59% of pork and 13.53% of chicken meat were contaminated with *Salmonella spp.*, in which the greatest risk was found to be in chicken meat (Tadesse and Gebremedhin, 2015). In the study conducted by Martínez-Chávez et al. (2015); risk factor for *Salmonella spp.* pathogen was 71% ( $\pm 11$ ) for minced meat, 39% ( $\pm 10$ ) for beef pieces and 18% ( $\pm 6$ ) for beef carcasses, whereas for *E. coli* it was found to be 100% for minced meat, 97% ( $\pm 27$ ) for beef carcasses and 84% ( $\pm 8$ ) for beef pieces. This is due to the fact that during the processing of raw meat in butchers, the microbial load on meat is transferred to minced meat and to beef pieces, and also caused by the sources of cross-contamination such as chopping board-knives, other equipment and staff (Martínez-Chávez et al., 2015). In the study conducted by Zerabruk et al. (2019), it was reported that there were risks in terms of *E. coli* (43.75%), *S. aureus* (37.5%) and *Salmonella spp.*; and also, meat was not separated from offal, sold openly for 5 hours, and butchers were not hygienic (Zerabruk et al., 2019). In another study, the riskiest nutrients for *Salmonella spp.* were lungs (lights) (7.5%) and mombar (2.5%); for *S. enteritidis* (5%) and *S. typhimurium* (2.5%) was lungs (Abd-El-Malek and El-Khateib, 2017). In the study conducted by Ras et al. (2019), the risk ranking for *S. aureus* was found for cattle-sheep kidney (30%), buffalo-camel kidney (20%); buffalo liver (30%), cattle-camel liver (20%) and sheep liver (0%). For *E. coli*, the buffalo and the camel kidney and liver had the same risk (20%). Risk was not present in beef liver-kidney and sheep liver (0%) (Ras, 2019). Both our study and other studies show that in order to prevent the high-risk level observed in terms of mentioned parameters, it is necessary to follow the necessary rules, good production practices, sanitation standard operating procedures and food safety systems during cutting and processing of animals due to contamination of offal.

Although there is not enough data, undercooked and raw meat is considered to be the most important cause of *T. gondii* infections that are effective in muscle and nerve tissues in humans (Sroka et al., 2019). This parasite leads to clinical manifestations such as encephalitis, hepatitis, pneumonia, myalgia and myocarditis in immunosuppressed individuals (Ducrocq et al., 2021). In the study of raw and smoked sausage, ham, dried bacon and minced meat, 5.4% of the samples were positive for *T. gondii*. 45.1% of the positive samples were sausages, 27.4% were smoked meat products, 19.4% were minced meat and 8% were ham (Sroka et al., 2019). In a study conducted in Turkey, *T. gondii* was present in 20% of the ovine muscle, 19% of fermented sausage, 6% of the bovine muscle, 4.17% of the ovine brain and 2% of the bovine brain. With regard to this, it can be said that the risk of toxoplasmosis is high in uncooked/commercial meat and products (Ergin et al., 2009). In our study, on the contrary, no nutrient was positive for *T. gondii*. Based on these findings, it can be said that further study is needed to determine risk factors in *T. gondii* infections (Hussain et al., 2017).

In a study on the offal of the heart, liver, lungs, abdomen, small intestine and large intestine, the most common pathogens found in pork were *Salmonella spp* (23.8%), *S. aureus* (12.7%) and *Clostridium perfringens* (*C. perfringens*) (11.1%); and in cattle were *Salmonella spp.* and *C. perfringens* (7.1%) (Im et al., 2016). In our study, the risk ranking for sheep's brain was *S. aureus* (12%), coliform (8%), *E. coli-Salmonella spp.* (2%). Both studies were similar to the aspect of *S. aureus* risk. In another study, the risk factors for chicken liver were *Salmonella spp.* (24%), *E. coli* (20%), *S. typhimurium* (8%) and *S. enteritidis* (4%); and for gizzard were *Salmonella spp.* (36%), *E. coli* (28%), *S. enteritidis* (12%) and *S. typhimurium* (8%) (Hassanin et al., 2017). According to current studies, it can be said that *Salmonella spp.*, in particular, is an important risk factor for offal. In the study conducted by Wai et al. (2020), chicken gizzard, liver and heart had similar risk for *L. monocytogenes* and *Listeria spp.* (Wai et al., 2020).

*L. monocytogenes* is a major pathogen known worldwide as the causative agent of listeriosis and contaminates with food. This pathogen is a bacterium that causes meningoencephalitis, cerebral abscesses, cerebritis, bacteremia, meningitis and sepsis, especially in immunosuppressed individuals and pregnant women, and has a high mortality rate (20 -30%) (Montero et al., 2015). In a study conducted by Bouymajane et al. (2021) in Morocco, 520 food samples were examined. It was found that 15 (2.9%) of the analyzed samples were contaminated with *L. monocytogenes*. It was observed in 5.7% of raw minced meat and raw sausage, while was found in 1.9% of raw beef, poultry and raw fish samples. Additionally, it was observed that all strains detected carried the *actA* gene (Bouymajane et al., 2021). In another study, the prevalence of *L. monocytogenes* in consumption-ready products was examined and 783 delicatessen products were analyzed. The positive ratio of the products sold with vacuum packaging was 2.7%, while it was reported to be 8.5% in delicatessen meat products packaged in the store (Garrido et al., 2009). Braga et al. (2017) in their study examined 3175 food samples from food factories and retail outlets and 11.2% of them were observed to have been contaminated with *L. monocytogenes*. In our study, 13% of the analyzed samples (sheep: 7%, beef: 5%) were contaminated with *L. monocytogenes*. The results of similar studies conducted in China (22%) (Chen et al., 2017) and Chile (25%) (Montero et al., 2015) were significantly higher than the results observed in our study. It has been reported that one of the causes of this may be the type of food analyzed (Braga et al., 2017).

*S. aureus* causes diseases from mild skin infections and food poisoning to more complicated cases of necrotizing pneumonia, endocarditis, osteomyelitis and toxic shock syndrome. However, it can gain resistance to many antibiotics, including methicillin and vancomycin, and therefore is listed as one of the 'priority pathogens' that threaten public health by the WHO (Shrivastava et al., 2018). In a study conducted in Czech Republic, 23 (35.4%) of 65 raw meat samples (poultry, beef, pork and rabbit) collected from retail outlets were found positive for *S. aureus* (MRSA) (Tegegne et al., 2021). In the study by Hanson et al. (2011), the overall prevalence of *S. aureus* was 16.4% (27/165) in commercially available meat samples. *S. aureus* was most commonly found in turkey (7/36, 19.4%), pork (10/55 samples, 18.2%) and chicken (8/45 samples, 17.8%). Beef meat has been reported to have a much lower prevalence (2/29 samples, 6.9%). In another study, a total of 145 meat samples from Danish supermarkets were examined. *S. aureus* was detected in 69% of meat samples. MRSA was detected in 19 meat samples (13%) and MRSA prevalence was reported as 4% in chicken, 52% in turkey and 15% in pork (Tang et al., 2017). In our study, 50% of the samples analyzed (mutton: 17%, beef: 21%, sheep brain: 12%) were contaminated with *S. aureus*. In this regard, it can be stated that results obtained in our study are lower than the ones in the study conducted in Denmark (Hanson et al., 2011), whereas higher than the ones in the Czech Republic (Tegegne et al., 2021).

In a study, 375 chicken and offal samples were examined and it was determined that 5 samples were contaminated with *E. coli*. *E. coli* O157:H7 was not found in the analyzed samples (Guran et al., 2017). In a study conducted in Morocco, samples of beef (n=52) and lamb (n=52) and veal offal (n=52) were randomly collected from butchers, supermarkets and slaughterhouses. It was reported in the study that half of the samples (n=26) were collected during the cold season and the other half (n=26) in the warm season. Coliform bacteria were determined as  $2.5 \pm 1.2$  cfu/g<sup>-1</sup>,  $2.8 \pm 1.0$  cfu/g<sup>-1</sup> and  $2.3 \pm 1.1$  cfu/g<sup>-1</sup> for beef, lamb and veal offal, respectively. Of all samples analyzed, 59 (37.8%) were positive for *E. coli*, of which 13 (25%) were beef, 23 (44.2%) lamb and 23 (44.2%) were reported as veal offal. However, it was reported that the average number of coliforms in meat samples collected from butchers and supermarkets is significantly higher than the samples taken from slaughterhouses. This is associated with the possibility that hygienic conditions in which the product is processed, ambient temperature and storage after butchering may be inappropriate or insufficient (Cohen et al., 2006). A study by Jaja et al. (2018) collected 400 samples from 2 official slaughterhouses and 112 swab samples from 5 unregistered animal slaughter points. Swabs were made before and after washing the carcasses. The total number of coliform bacteria in slaughterhouses varies between 5.0-6.3 kob/cm<sup>2</sup> before washing and 4.6-

6.3 kob/cm<sup>2</sup> after washing. To ensure that coliform bacteria, and especially *E. coli*, the most prominent hygiene indicator, is not a threat to public health and access to reliable food, it is clear that it is necessary to provide the hygiene conditions of production, equipment and personnel.

Our study found that 22% of mutton meat, 25% of beef and 8% of sheep brain samples were contaminated with coliform bacteria. In *E. coli* analysis, the results obtained were as follows: 11% of mutton, 16% of beef and 2% of sheep's brain were contaminated with *E. coli*. As in the study of Guran et al. (2017), there was no *E. coli* O157:H7 in any sample. As highlighted in other studies, these findings obtained in our study suggest that personnel, equipment, and operational hygiene and critical control points within the enterprise should be provided.

## Conclusions

As a result, in our study, as well as other studies on red meat and offal, the sheep's brain, which is often consumed by individuals, has been studied. When the necessary conditions are not met, it seems that the sheep's brain is potentially at risk of microbiological and parasitological contamination and can therefore threaten public health. Even though significant progress has been made in recent years on food safety and public health, more steps must be taken. A multidisciplinary team, including public health experts, veterinarians and nutritionists, should focus on food hygiene to reduce the number of cases and deaths caused by nutritional pathogens in humans worldwide and this multidisciplinary team should ground on disease surveillance, consumer education, processing and marketing practices.

## Abbreviations

*E. coli*

*Esherichia Coli*

*S. aureus*

*Staphylococcus aureus*

*T. gondii*

*Toxoplasma gondii*

*E. coli* EHEC O157

H7:*Esherichia Coli* O157:H7

rt-PCR

real time PCR

*S. enteritidis*

*Salmonella enteritidis*

*S. typhimurium*

*Salmonella typhimurium*

*L. monocytogenes*

*Listeria monocytogenes*

VRB

Violet Red Bile Agar

BPA

Baird-Parker Agar

BLEB

Buffered Listeria Enrichment Broth Base

TSA

Trypticase soy agar

TBX

Tryptone Bile X-glucuronide

MTSB

Modified Triptic Soy Broth

SMAC Agar  
Sorbitol Mac Conkey Agar  
TSI Agar  
Triple Sugar Iron Agar  
LI Agar  
Lysine Iron Agar  
PCR  
Polymerase Chain Reaction  
SPSS  
Statistical Package for Social Sciences  
WHO  
World Health Organization  
*C. perfringes*  
*Clostridium perfringes*.

## Declarations

### Availability of data and materials

Not applicable. All data sets from which conclusions of the manuscript have been drawn are presented in the paper.

### Competing interests

The authors declare no competing financial interest.

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### Authors' contributions

The author(s) read and approved the final manuscript.

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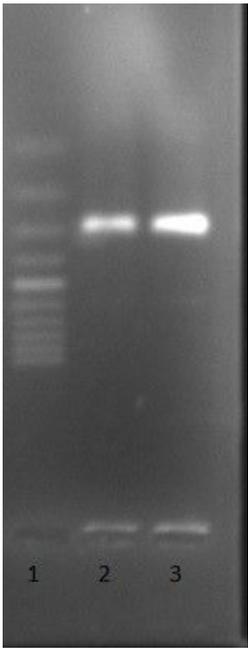
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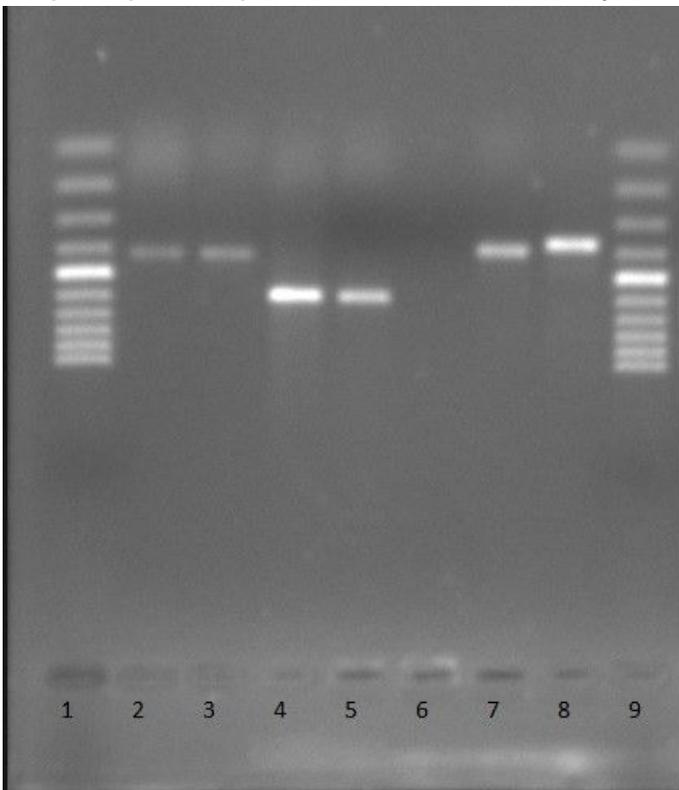
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## Figures



**Figure 1**

Images of gel electrophoresis as a result of PCR analysis of salmonella spp. positive samples. 1:sm 100 bp, 2- 3: invA gene 284 bp



**Figure 2**

Images of gel electrophoresis as a result of PCR analysis of staphylococcus aureus positive samples. 1:100bp sm, 2-3: Staphylococcus aureus nuc gene (responsible for the activity of thermonucleosis ) 416 bp, 4-5: Staphylococcus aureus coa gene (responsible for coagulase activity) 500 -650 bp, 6: NK (-), 7-8: Staphylococcus aureus spa (protein A) gene 100 -450 bp, 9:100 bp sm

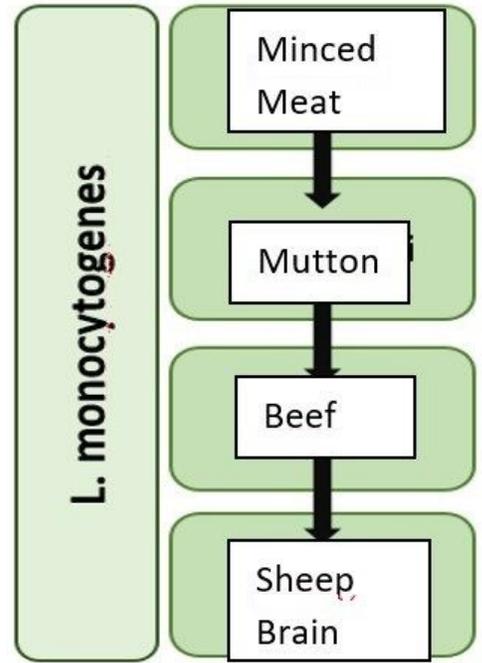
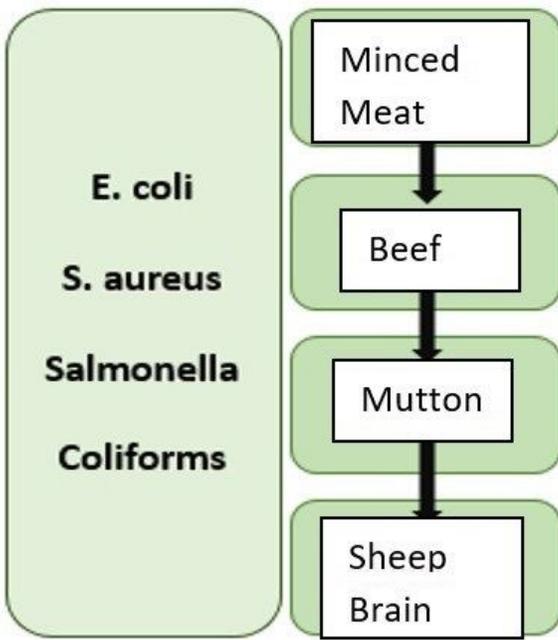


Figure 3

Ranking of meat and products from high risk to low risk in terms of microbiological parameters