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PREVALENCE AND SEROTYPING OF *SALMONELLA* IN BEEF CARCASSES SOLD IN MARKETS OF HAMA CITY, SYRIA

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Keywords: *Salmonella, meat, serotyping, cow, public health*

Abstract
Salmonella is one the most important pathogenic bacteria, which causes food poisoning in human consumers worldwide. This study aimed to detect the prevalence and serovars of Salmonella in beef in markets of Hama city, Syria. The study was carried out on 200 beef samples from 20 retail shops in markets of Hama city, Syria. Investigation was conducted using bacterial cultures and serotyping. Bacterial cultures showed that 11 samples out of 200 samples from different retail shops were positive, with an overall prevalence of 5.5% (95% CI: 2.34–8.66). The isolated serotypes were S. Typhimurium (36.36%), S. Enteritidis (27.27%), S. Reading (9.09%), S. Bredeney (9.09%), S. Chester (9.09%), and S. Anatum (9.09%). Significantly higher ($p < 0.05$) prevalence was revealed in the east district (8%, 4/50) compared to other districts, in informal shops (7.5%, 6/80) than in other outlets, in female animals (10%, 4/40) compared to male animals, in slaughtered animals at the age of more than 12 months (7.5%; 6/80), in summer (12%, 6/50) compared to other seasons, in unclean shops (7.38%, 9/122), and in liver (7.5%, 6/80) compared to other meat types. These results are considered an important threat to public health and indicate food contamination.

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Introduction

Beef is one of the animal products that are widely consumed by humans and is one of the foods that are most exposed to bacterial contamination as it contains many nutrients that bacteria need to multiply [1,2]. Cattle carcasses consist of parts that are edible for human consumers and others that are not edible. Among the edible parts are the carcass meat, liver, heart, kidneys, tongue and brain. Despite the high nutritional value of beef, it is a potential nutritional material for the transmission of foodborne pathogens, as it contains a high level of protein and a low percentage of carbohydrates in addition to a moderate acidity with sufficient water, which helps in the growth and survival of pathogenic bacteria [3,4,5]. The storage conditions of beef, which include many factors such as humidity and temperature of meat storage, especially with regard to poor cooling, are conducive to the growth and proliferation of bacteria, including bacteria that cause zoonotic diseases in humans [6]. The most important sources of beef contamination are the skin of animals (soil attached to the skin) as well as the intestines (feces) of animals processed for human consumption. Microbial contamination occurs especially when the entrails of slaughtered animals are removed in slaughterhouses [7,8]. Contaminated tools used in slaughtering animals and tools for cutting and cleaning carcasses such as knives and cleavers are potential sources of bacterial contamination of this meat [9].

Another potential source of beef contamination are vehicles used to transport meat from slaughterhouses to retail outlets due to poor hygiene, not to mention the poor hygiene of the stores where this meat is displayed [8].

Bacteria, including *Salmonella*, *Shigella*, *Escherichia coli*, *Campylobacter*, and *Staphylococcus aureus*, are among the most important foodborne causative agents and are very common in beef, in addition to other pathogens such as viruses and parasites that cause zoonotic diseases in humans who consume this meat [10,11].

Salmonella is a genus of bacteria that is a leading cause of foodborne illness globally, responsible for millions of infections each year [12]. The *Salmonella* genus is currently comprised of two species *Salmonella enterica* and *Salmonella bongori*, with *Salmonella enterica* being the most clinically significant. Within *S. enterica*, over 2,500 serotypes have been identified, distinguished by their unique combi-

nations of surface antigens, specifically O (somatic) and H (flagellar) antigens [13]. This classification system is known as serotyping and is vital for epidemiological surveillance, outbreak investigations, and understanding the transmission dynamics of *Salmonella* infections.

Serotyping plays a crucial role in identifying specific serotypes associated with particular sources of infection. For example, *Salmonella* Typhimurium and *Salmonella* Enteritidis are frequently linked to poultry and egg products, while *Salmonella Typh*i is associated with typhoid fever and human carriers [14]. Understanding the diversity of *Salmonella* serotypes is essential for developing targeted public health interventions and improving food safety practices.

Recent advancements in molecular techniques, such as whole-genome sequencing (WGS), have enhanced our ability to characterize *Salmonella* strains more precisely. However, traditional serotyping methods remain fundamental due to their cost-effectiveness and ease of use in routine diagnostics [15]. The combination of traditional serotyping and modern genomic approaches provides a comprehensive framework for tracking transmission pathways and identifying contamination sources.

There are several serotypes of *Salmonella* detected in contaminated beef sold in local markets in many areas that have been investigated in several previous studies. These studies reported that the most prevalent serotypes of *Salmonella* are *Salmonella* Typhimurium and *Salmonella* Enteritidis in addition to other serotypes identified in meat [16].

Salmonella are facultatively anaerobic, gram-negative, oxidase negative, catalase positive, nonspore forming rods. Almost all *Salmonella* serotypes are motile via peritrichous flagella except *S.* Pullorum and *S.* Gallinarum [17]. The optimal growth temperature of *Salmonella* is 37 °C; however, growth has been recorded between 2 and 4 °C and as high as 45 °C, *Salmonella* can live in a wide pH range from as low as pH 3.8 to as high as pH 9.5 with an optimum pH of 6.5–7.5 [18].

It can ferment glucose, mannitol, arabinose, maltose, dulcitol and sorbitol, forming acid and gas except for *S.* Typhi, *S.* Gallinarum and rare aerogenic variants in other subtypes form only acid and no gas. Generally, *Salmonella* does not ferment lactose, sucrose, salicin or adonitol. It is indole negative, Methyl Red positive (MR), Voges Proskauer negative (VP), and citrate positive (IMViC – + – +) except for *S.* Typhi and *S.* Paratyphi *A*, which are citrate negative as they need tryptophan as the growth factor. Hydrogen sulfide is produced except for *S.* Paratyphi *A*, *S.* Choleraesuis, *S.* Typhisuis and *S.* Sendai. Urease is not hydrolyzed by *Salmonella* [19].

Salmonella is a significant foodborne pathogen that poses a serious public health risk worldwide, particularly in relation to the consumption of contaminated beef. According to the Centers for Disease Control and Prevention (CDC), *Salmonella* is one of the leading causes of bacterial food poisoning in the United States, contributing to approximately 1.35 million infections annually [20]. Beef, as a widely consumed protein source, has been identified as a common vehicle for *Salmonella* transmission, with outbreaks often linked to undercooked or improperly handled meat [21].

The contamination of beef with *Salmonella* can occur at various stages of the production chain, including slaughter, processing, and distribution [22]. Factors such as inadequate hygiene practices, cross-contamination, and improper cooking temperatures contribute to the persistence of *Salmonella* in beef products [15].

In addition to direct contamination from livestock, environmental factors and feed sources also play crucial roles in the prevalence of *Salmonella* in beef [23]. The emergence of antibiotic-resistant strains of *Salmonella* further complicates the issue, as these strains pose challenges for treatment and control measures [24].

Given the public health implications associated with beef contaminated with *Salmonella*, it is essential to implement rigorous food safety practices throughout the meat production and supply chain. This includes monitoring and controlling *Salmonella* levels in cattle herds, enhancing hygiene practices during processing, and educating consumers on safe cooking methods [12].

The control of foodborne pathogens such as *Salmonella enterica* is difficult because of their ability to survive during food production, processing, storage and improper cooking. Therefore, it is important to understand the ecology of *Salmonella enterica* and the genetic variation of different strains in order to design specific management practices to reduce risks associated with this pathogen. Several molecular typing methods are used to differentiate *Salmonella enterica* isolates, including multilocus variable-number tandem-repeat analysis, multilocus sequence typing or multiplex-PCR-based methods and whole genome sequencing [25].

Meat production is central to livelihoods in many countries, with meat from livestock and poultry being a key protein source in subsistence communities [26]. In many low-resource settings, industrialization, urbanization, and the shift from planned to market economies are leading to rapid changes in the way that food is produced, distributed, sold, and consumed [27]. Such market-driven changes within agricultural production towards wider distribution networks, centralized processing, larger-scale and more intensive systems, have been linked to the emergence of zoonotic diseases [28].

Therefore, it was necessary to investigate *Salmonella* and its serotypes in beef carcasses sold in markets of Hama city, Syria.

Materials and methods

Samples

Beef samples were taken from four different districts for bacteriological examination. A total of 200 beef samples were randomly taken from 20 retail shops, during a period from October 2023 to September 2024. Samples were used to detect *Salmonella*.

Sample collection

Samples of beef were collected in sterile plastic bags and kept in them at a temperature of 4–8 °C for the period of transference to the research laboratory for microbiological analysis.

Epidemiological data collection

Epidemiological data on the studied beef and retail shops were collected using special questionnaires based on previous studies, which involved information about retail shops such as shop name, district, age of animals, sex of animals, outlet, and season as well as cleanness of retail shops. Questionnaires were filled out during visits to the studied retail shops.

Microbiological analysis of Salmonella

Twenty five grams of the examined samples were weighed aseptically into sterile blender container and thoroughly homogenized with 225 ml of sterile lactose broth. The homogenate was incubated at 37 °C for 24 hours. In order to detect *Salmonella* using the traditional method, the following procedure was used [29].

Enrichment in a selective liquid medium: In this stage, Tetrathionate broth manufactured by HiMedia® was used, with potassium iodide solution added to it. Amounts of 0.1 ml and 1 ml of the incubated pre-enrichment homogenate were transferred to 10 ml Tetrathionate broth as selective enrichment. Tetrathionate broth was incubated at 42 °C for 24–48 hours.

Isolation and growth in selective solid medium: XLD (Xylose Lysine Deoxycholate) agar (HiMedia®) was used, which was prepared and poured into petri dishes according to the manufacturer's instructions. Following primary enrichment, 20 µl from the TTB culture was streaked onto XLD medium. The dishes were incubated in the incubator at 37 °C for 24–48 hours. After incubation, the cultural properties of the growing colonies were studied. Small, round colonies with a smooth surface and a black center with a metallic sheen or brown, green or gray colonies appeared were considered to be *Salmonella* colonies.

Several biochemical tests were performed to identify *Salmonella*. The biochemical tests performed were: catalase — oxidase — indole — methyl red — Voges-Proskauer — citrate — urease.

Serotyping of Salmonella

Biochemically confirmed *Salmonella* sp. isolates were further serologically identified using a series of slide agglutination specific for O and H antigens (White — Kauffmann — Le Minor scheme) [30]. These tests were performed at the Animal Health Directorate of the Ministry of Agriculture and Agrarian Reform of Syria, Damascus, Syria.

Statistical analysis

Field and laboratory recorded data were entered into a Microsoft Excel 2010 spreadsheet (Los Angeles, CA, USA). Then the data was checked to detect errors and the data was coded in preparation for statistical analysis, which was done using the statistical program SPSS version 22 (IBM Inc., Chicago, IL, USA), after exporting the data to it.

Descriptive statistics for recorded data

Absolute frequency and relative frequency were calculated for the studied variables with a categorical pattern. The value of the prevalence of *Salmonella* in beef was also calculated based on laboratory results using bacterial culture for each of the categorical variables studied. For the recorded prevalence, 95% confidence intervals (CI) were calculated.

Analytical statistics of recorded data

Analytical statistics were conducted for each of the categorical variables studied and included in the questionnaires, which are: districts (4), outlets (3), sex of animals (2), type of meat (3), age of animals (4), seasons (4), and cleanness of retail shops (2).

The association between the prevalence of *Salmonella* and the variables (risk factors) under consideration was studied using chi-square method. $P < 0.05$ was statistically considered significant.

Ethical Approval

Those responsible for taking meat samples from retail shops adhered to ethical principles and general rules. The shop owners agreed with collection of samples from their shops.

Results

Distribution of the studied samples

As can be seen from Table 1, the number of the samples taken from four districts and during four seasons was equal and made up 25% for each category of these variables. As regards other variables and categories, most samples were taken from male animals (80%), animals at the age of 7–12 months (40%), liver (40%), supermarkets and butchery shops (80%), and unclean retail shops (61%).

Prevalence

The study recorded an overall prevalence of *Salmonella* of 5.5% (11/200; 95% CI: 2.34–8.66%) in retail shops according to bacterial cultures. The highest prevalence was revealed in the east district (8%; 95% CI: 4.24–11.76%); in informal shops (7.5%; 95% CI: 3.85–11.15%); in liver (7.5%; 95% CI: 3.85–11.15%); in summer (12%, 95% CI: 7.5–16.5%); in unclean shops (7.38%; 95% CI: 3.75–11%); in female animals (10%; 95% CI: 5.84–14.16%); and in animals at the age of 13–24 months (7.5%; 95% CI: 3.85–11.15%) as shown in Table 1.

Distribution of Salmonella serovars in beef in retail shops

Only six *Salmonella* serovars were identified in beef samples: *S.* Typhimurium (36.36%), *S.* Enteritidis (27.27%), *S.* Reading (9.09%), *S.* Bredeney (9.09%), *S.* Chester (9.09%), and *S.* Anatum (9.09%) (Table 2).

Table 1. Prevalence of *Salmonella* in beef meat in markets of Hama city, Syria and its association with categories of studied variables

Variable	Category	N	positive	%	95% CI		*p*-value
					lower	upper	
Districts	East	50	4	8.00	4.24	11.76	0.00
	West	50	3	6.00	2.71	9.29	
	North	50	1	2.00	0.06	3.94	
	South	50	3	6.00	2.71	9.29	
Outlets	Informal shops	80	6	7.50	3.85	11.15	0.00
	Butchery shops	80	4	5.00	1.98	8.02	
	Supermarkets	40	1	2.50	0.34	4.66	
Type of meat	Thigh	60	3	5.00	1.98	8.02	0.00
	Shoulder	60	2	3.33	0.85	5.82	
	Liver	80	6	7.50	3.85	11.15	
Seasons	Winter	50	1	2.00	0.06	3.94	0.00
	Spring	50	2	4.00	1.28	6.72	
	Summer	50	6	12.00	7.50	16.50	
	Autumn	50	2	4.00	1.28	6.72	
Cleanness of shops	Clean	78	2	2.56	0.37	4.75	0.00
	Unclean	122	9	7.38	3.75	11.00	
Sex of animals	Male	160	7	4.38	1.54	7.21	0.00
	Female	40	4	10.00	5.84	14.16	
Age of animals	0–6 months	40	1	2.50	0.34	4.66	0.00
	7–12 months	80	4	5.00	1.98	8.02	
	13–24 months	40	3	7.50	3.85	11.15	
	Above 24 months	40	3	7.50	3.85	11.15	

Table 2. Distribution of *Salmonella* serovars in beef in retail shops in markets of Hama city, Syria

Salmonella serovars	n	%
S. Typhimurium	4	36.36
S. Enteritidis	3	27.27
S. Reading	1	9.09
S. Bredeney	1	9.09
S. Chester	1	9.09
S. Anatum	1	9.09
TOTAL	11	100%

Study of variables associated with the prevalence of Salmonella

The study showed a relationship between the prevalence of *Salmonella* in beef and several studied variables that were considered risk factors for this prevalence in the studied retail shops, as shown in Table 1. A statistically significant relationship was observed for each of the following variables: district ($P < 0.001$), outlet ($P < 0.001$), type of meat ($P < 0.001$), season ($P < 0.001$) cleanness of shop ($P < 0.001$), sex of animals ($P < 0.001$), and age of animals ($P < 0.001$).

Discussion

The study is one of the quantitative epidemiological studies carried out for the first time in Syria on the prevalence of *Salmonella* in markets of Hama city, which included different retail shops.

Twenty different retail shops were studied in markets of Hama city, Syria, where beef is sold, and 200 beef samples were collected from markets to study the prevalence of *Salmonella* in beef. The study showed that prevalence of *Salmonella* was 5.5% of the total beef samples examined according to the scientific methodology.

Salmonella contamination in beef remains a critical public health concern, particularly given its association with foodborne illnesses. The present research indicates that approximately 5% of beef samples were positive for *Salmonella*, highlighting the need for effective monitoring and control measures throughout the beef supply chain.

The presence of *Salmonella* in beef can be attributed to several factors, including animal husbandry practices, processing conditions, and environmental factors. Cattle can harbor *Salmonella* in their gastrointestinal tracts without showing clinical signs of illness, making it challenging to detect and manage [31]. During slaughter and processing, improper handling and cross-contamination can facilitate the transfer of the pathogen to beef products.

Consumer handling also plays a crucial role in the risk of *Salmonella* infection. According to [22], improper cooking and cross-contamination in home kitchens contribute significantly to foodborne illness outbreaks associated with beef. The recommended cooking temperature for ground beef is 71 °C, which is effective in killing *Salmonella*; however, many consumers do not adhere to these guidelines [32]. This gap in consumer knowledge and practice can exacerbate the risks associated with even low levels of contamination.

Moreover, the emergence of antibiotic-resistant strains of *Salmonella* poses additional challenges for public health. Studies have shown that certain strains found in beef have developed resistance to commonly used antibiotics,

complicating treatment options for infected individuals [24]. This highlights the importance of implementing robust surveillance systems and improving biosecurity measures on farms to reduce the prevalence of *Salmonella* in cattle.

The results of our study are consistent with the studies on the prevalence and risk factors for contamination by *Salmonella* that were conducted in Namibia, where the prevalence of *Salmonella* in beef carcass in markets was 2.67% [33], and in Istanbul, Turkey, where the prevalence of *Salmonella* in ground beef was 0.98% [34].

On the other hand, the prevalence of *Salmonella* in beef meat in markets of Hama city was less than what was stated by Hassanein et al. [23] in Egypt, where the prevalence of *Salmonella* in beef retail supermarkets was 20%. This percent is also similar to previous epidemiological studies conducted by researchers [35,36] in separate areas of the western Asian continent such as Tehran, Iran (20.2%) and Malaysia (15.4%). The study conducted in Vietnam by Van et al. [37] revealed the presence of *Salmonella* in retail beef samples at a level of 62%, which was much higher than the level of *Salmonella* positive beef samples (48.6%) recorded by Phan et al. [38] in the same country.

This varying prevalence of *Salmonella* in retail shops may be attributed to many reasons, including the differences in the show conditions of beef in retail shops, differences in breeding systems, differences in methods of diagnosing the bacteria.

The present study recorded that the highest prevalence (8%) of the contamination was in the east district in the Hama city, Syria, compared to the other districts in the city ($P < 0.00$). This may be attributed to the fact that the east district contains a higher number of retail shops than the other regions, and is an open area for other districts, which helps in the entry of illegal meat into it.

The study showed that the prevalence of *Salmonella* in informal shops is higher compared to other outlets ($P < 0.00$), which is consistent with the findings of Shafini et al. [36]. This is due to the lack of proper sanitary conditions for selling beef in informal shops.

Contamination by *Salmonella* was more common in carcasses of female animals compared to males ($P < 0.00$) in this study, which is consistent with [39,40,41]. Apparently, this result was obtained because females are more exposed to pathogens than males.

The study recorded a higher prevalence of *Salmonella* in beef from animals more than 12 months old compared to other age groups, with significant differences ($P < 0.05$) This can be attributed to previous infections in older animals [42].

The current study showed that liver had higher prevalence of *Salmonella* compared to other types of meat ($P < 0.00$). This result confirms that the liver is more contaminated by *Salmonella* due to its closeness to intestines and is consistent with the results of [43]. Intestinal perforation may occur during opening the abdominal cavity of the carcass.

The current study also confirmed that the prevalence of *Salmonella* is more in the summer compared to other seasons ($P < 0.00$). This contradicts the findings of Brichta–Harhay et al. [44].

The study recorded a higher prevalence of *Salmonella* in beef in unclean shops compared to others, with significant differences ($P < 0.05$), which may be due to cross contamination with existing pathogens in the shop [45].

In our study, *Salmonella* Typhimurium and *Salmonella* Enteritidis were found to be among the most common serotypes in contaminated beef, which is consistent with several previous studies [46,47,48].

Conclusions

Contamination of beef by *Salmonella* in retail shops in Hama city, Syria, is considered an important health problem as it may be a cause of food poisoning in human consumers. There are several predisposing factors to contamination by *Salmonella*, such as the district of retail shops, sex and age of slaughtered animals and type of outlets, in addition to the season and cleanness of shops. We propose improving health practices in places where beef is sold, and adhering to the high hygienic conditions of selling and trading beef in markets of Hama city, Syria.

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