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# STUDY OF THE EFFECT OF BOILING AND THAWING ON THE BACTERIAL COUNT OF *SHIGELLA* SPP. ISOLATED FROM RAW MEAT OF BEEF AND SHEEP IN BAGHDAD CITY, IRAQ

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**ABSTRACT**

This study was conducted to detect the contamination of beef and sheep meat with *Shigella* species in AL-Karkh and AL-Rasafa district of Baghdad province. A 160 samples of beef and sheep raw meat were collected from butcher shops in Baghdad city and examined for microbial load. The results showed there was 85 samples positive to *Shigella,* 18 and 21 isolates form sheep and beef respectively in AL-Karkh area, while number of the *Shigella* spp. that isolated from tested meat samples in AL-Rasafa district was 20 and 26 isolates form sheep and beef respectively. The total count of the *Shigella* spp. in the raw meat of sheep and beef samples that collected from AL-Karkh district was 5.03±0.11 log cfu/ml and 5.47±0.14 log cfu/ml respectively, while the total count of the *Shigella* spp. in the meat samples that collected from AL-Rasafa district was 5.16±0.12 log cfu/ml for sheep and 5.59±0.13 log cfu/ml for beef. The result of treating meat samples by boiling and pressure cooking showed no bacterial growth in all examined samples after treatment. We concluded that about 50% of the meat samples from butcher shops were contaminated with *Shigella* spp. and the contamination was more in beef meat than sheep meat and the isolated *Shigella* sensitive to heat and pressure.

***Keywords*:** Beef and sheep meat, Contamination, *Shigella* spp.

## Introduction

Safe food is defined as not causing harm or illness to the consumer (Zeeshan *et al*., 2017). Meat are an excellent source of a wide variety of nutrients, high quality proteins, vitamins and certain minerals. It is contain an large quantity of all nutrients required to the growth and multiplication of most microorganisms, so good manufacturing practices and the hygienic conditions of these practices are very important during the procedures of handling, preparation, and storage because the contaminated from different sources may lead to spoilage of these products and/or act as a public health hazard to consumers (El-Sharkaway *et al*., 2016). Foodborne pathogens represent a serious threat to food safety especially in developing countries where operational practices and sanitation facilities in retail shops and abattoirs are substandard. The incidence of foodborne infections have greatly increased worldwide and it is estimated that nearly a quarter of the population is at risk (CDC, 2013). Meat is one of the most perishable foods, and its composition is ideal for the growth of a wide range of spoilage and pathogenic bacteria (Birhanu *et al*., 2017).

The main sources of contamination are the slaughtered animals themselves, the workers and working environment, and to a lesser degree, contamination from air via aerosols and from carcass dressing water (Okonko *et al*., 2010 and Birhanu *et al*., 2017 5). Moreover, the contaminating organisms are derived mainly from the hide of the animals and comprise organisms that originate from stomachs and intestines, which are excreted in their feces (Norrung *et al*., 2009).

Gram negative organisms such as *Salmonella, Shigella,* and *Escherichia coli* form the majority of meat contamination (Zweifel *et al*., 2008). There are four species of *Shigella*, classified according to biochemical and serological characteristics: *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii*, and *Shigella sonnei* (Seidlein *et al*., 2006). *Shigella* transmission occur by the fecal-oral route, including direct person-to-person contact and may be indirect through ingestion of contaminated food or water (Jomezadeh *et al*., 2014 and Makabanyane *et al*., 2015). This study was conducted to investigate the contamination of the beef and sheep raw meat with *Shigella* spp. in AL-Karkh and AL-Rasafa districts of Baghdad province.

## Materials and Methods

### Samples collection

160 meat samples of sheep and beef raw meat were collected from butcher shops from different areas of Baghdad Province. The samples were collected in a sterile plastic containers and transported to the laboratory for microbial analyses.

### Samples preparation

25g of collected meat samples were weighed, minced and moved to sterile flasks containing 225 ml of tryptone soya broth and yeast extract which homogenized and incubated at 37˚C for 24hr.

### Bacterial count in meat samples

A serial dilutions were made using 1ml of the homogenized meat sample with 9 ml of peptone water, then 0.1 ml of proper dilution was plated on specific media and

incubated at 37˚C for 24hr for counting of *Shigella* spp. (ISO/TS, 2009). At the end of the incubation period, colonies were counted manually. The number of the bacteria for each plate were stated as colony forming unit of the dilution (CFU/g).

**Effect of salting, boiling and pressure on *Shigella* count in sheep and beef meat**

Each sample was treated with different concentration of sodium chloride solution 1%, 2%, 3% and cooked by hot plate at 100°C for half hour and by pressure by autoclave at 121°C and 15 psi for 15min. The final viable bacterial count for each sample was made after each treatment.

**Effect of thawing method on the viable count of *Shigella* spp. in meat**

Three samples were used in this experiment. After initial viable count of the *Shigella,* all meat samples were stored inside the freezer at -18°C until starting the bacteriological tests of thawing meat. Three methods for frozen meat thawing were used for each sample, in the first method we used tap water at ≤ 15°C, and in the second method the frozen meat samples were left at room temperature 20 -23.5 °C, while in the third method the frozen meat samples were stored inside the refrigerator 4°C for 20 hr. The final viable bacterial count was made after 1, 2, and 3 days from the beginning of the thawing for each sample.

## Results

The total number of the *Shigella spp*. that isolated from the 160 tested samples was 85 isolates (Fig. 1). The number of the *Shigella* spp. that isolated from tested meat samples were 18 and 21 isolates form sheep and beef respectively in AL-Karkh area (table 1), while number of the *Shigella* spp. that isolated from tested meat samples in AL-Rasafa district was 20 and 26 isolates form sheep and beef respectively (table 2).

**Table 1 :** Number of *Shigella* spp. isolated from sheep and beef meat samples in AL-Karkh district

| No. of samples / Type of sample | No. of tested samples | No. of positive samples | No. of negative samples | percent of positive samples |
|---|---|---|---|---|
| Sheep | 40 | 18 | 22 | 45% |
| Beef | 40 | 21 | 19 | 52.5% |

**Table 2 :** Number of *Shigella* spp. isolated from sheep and beef meat samples in AL-Rasafa district

| No. of samples / Type of sample | No. of tested samples | No. of positive samples | No. of negative samples | percent of positive samples |
|---|---|---|---|---|
| Sheep | 40 | 20 | 20 | 50% |
| Beef | 40 | 26 | 14 | 65% |

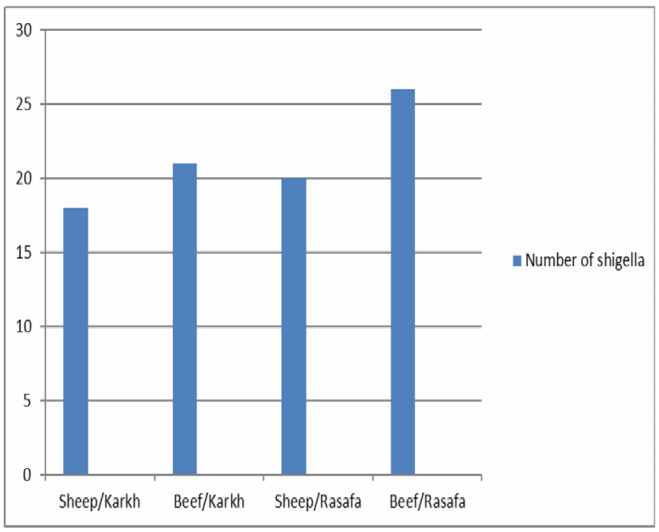


**Fig. 1 :** Prevalence of *Shigella spp* in AL-Karkh and AL-Rasafa district

**Bacterial count in meat samples**

The total count of the *Shigella* spp. in the raw sheep and beef meat samples that collected from AL-Karkh district was 5.03±0.11 log cfu/ml and 5.47±0.14 log cfu/ml respectively, while the total count of the *Shigella* spp. in the meat samples that collected from AL-Rasafa district was 5.16±0.12 log cfu/ml for sheep and 5.59±0.13 log cfu/ml for beef (table 3; figure 2).

**Table 3 :** Mean log cfu/ml of *Shigella* spp. for the examined meat samples of sheep and beef in AL-Karkh and AL-Rasafa district

| District / Type of sample | AL-Karkh | AL-Rasafa |
|---|---|---|
| Sheep | 5.03±0.11 A b | 5.16±0.11 A b |
| Beef | 5.47±0.14 A a | 5.59±0.13 A a |
| LSD | 0.3644 | |

Means with a different capital letter in the same row are significantly different(P≤ 0.05).

Means with a different small letter in the same column are significantly different(P≤ 0.05).

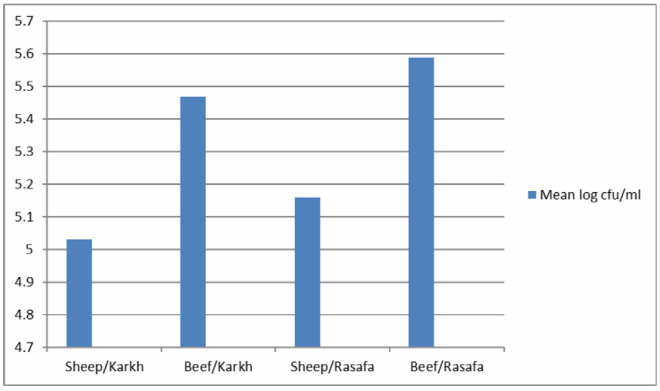


**Fig. 2 :** Mean log cfu/ml of *Shigella* spp. for the examined meat samples of sheep and beef in AL-Karkh and AL-Rasafa district.

**Effect of salting, boiling and pressure on of *Shigella* count in sheep and beef meat**

The result of treating meat samples by boiling and pressure cooking showed no bacterial growth in all examined samples after treatment (table 4).

**Table 4 :** Mean log cfu/ml of *Shigella* spp. for the examined beef meat samples treated with boiling and pressure cooking

| Salt conc. | Mean±SE | |
|---|---|---|
| | Boiling cooking | Pressure cooking |
| Control | 4.91±0.00 | 4.91±0.00 |
| 1% | Ng | Ng |
| 2% | Ng | Ng |
| 3% | Ng | Ng |

Ng: no growth

The results of the effect of the different methods of thawing on the log cfu/ml of *Shigella* spp. in the meat samples showed that the refrigerator thawing was the best method in reducing the increment in the bacterial count as compared with other two methods water thawing and room temperature thawing (table 5). In comparison between three methods of thawing, the data revealed there was important increasing ($P<0.05$) in the bacterial load in samples which thawed in room temperature after 1 day (6.31±0.55 log cfu/ml) and water thawing (6.09±0.62 log cfu/ml) as equated with thawing by refrigerator (5.65±0.65 log cfu/ml). Afterward 2 and 3 days of thawing the increment in bacterial count was the lowest in meat samples that thawed by refrigerator 6.01±0.59; 6.66±0.33 log cfu/ml with significant difference ($P<0.05$) as compared with water thawing 6.80±0.41; 7.59±0.73 and room temperature thawing 7.12±0.24 and 7.85±0.50 log cfu/ml.

The comparison between days within same treatment showed there was statistical increasing ($P<0.05$) in bacterial load in meat samples that thawed by all methods of thawing at day 3 in comparison with other periods (0, 1 and 2 days), also the increment was significant between day 2 and day 0.

**Table 5 :** Mean log cfu/ml of *Shigella* spp. for the examined beef meat samples treated with different methods of thawing

| Days | Mean±SE | | |
|---|---|---|---|
| | Refrigerator thawing | Water thawing | Room temperature thawing |
| 0 | 5.28±0.32<br>A c | 5.28±0.32<br>A d | 5.28±0.32<br>A d |
| 1 | 5.65±0.65<br>B bc | 6.09±0.62<br>AB c | 6.31±0.55<br>A c |
| 2 | 6.01±0.59<br>B b | 6.80±0.41<br>A b | 7.12±0.24<br>A b |
| 3 | 6.66±0.33<br>B a | 7.59±0.73<br>A a | 7.85±0.50<br>A a |
| LSD | 0.4416 | | |

Values with a dissimilar capital letter in the similar row are statistically different ($P<0.05$)
Values with a dissimilar small letter in the similar column are statistically different ($P<0.05$)

## Discussion

The results of this study showed there was approximately 50% percent of the tasted meat samples were contaminated with *Shigella* spp. The presence of bacteria in meat has been widely reported from different parts of the world (Kinsella *et al*., 2008), where the presence of a high microbial load rises the chances of meat spoilage in a short time as described by the Agriculture and Consumer Protection Department (FAO, 2010), therefore it is necessary to protect the meat from farm until consumption. The data about meat contamination with *Shigella* are very little. This study was agreed with results that obtained by Bersisa and colleagues 2019 that isolated *Shigella* from meat samples (Bersisa *et al*., 2019), study conducted by Bantawa *et al*., 2018 where the *Shigella spp.* were detected in 6% of the tested samples (Bantawa *et al*., 2018), results of Garedew *et al*., 2016 where the percent of positive meat samples to *Shigella* was 7.4% (Garedew *et al*., 2016), and with study that showed contamination of meat with *Shigella* spp. at 0.6% (Tassew *et al*., 2010).

Meat contamination may happen due to many reasons that attributed to unclean sites of slaughtering or to workers themselves represented by a lack or low level of information of the workers on how to handle meat because they didn't receive sufficient training. It was recommended that new applicants could be examined clinically and bacteriologically before they are employed and at regular intervals afterwards, examination should include medical history to determine past infections with special reference to dysentery, typhoid, and paratyphoid fevers, venereal and skin diseases, and bacteriological examination of stool and urine (WHO, 1959). All of the right food handling indicators assessed were against safe food handling requirements, which include cleaning the equipment and premises, personal hygiene, temperature control and the prevention of cross contamination (MLA, 2014). Microbial contamination in meat can start from the first skin incision made to remove the blood, especially if the tools and equipment used by the operator are not sterile. Subsequent contamination can occur on the surface of the meat during meat preparation, carcass or meat cutting, manufacturing of processed meat products, packing, storage, and distribution. So, anything that can contact meat directly or indirectly, can be a source of microbial contamination (Soeparno, 2009). According to Rajakumar and colleagues, chopping boards in households are the reason for cross contamination of meat with microbial pathogens of animal origin (Rajakumar *et al*., 2012). Knives, wooden boards, and weighing scales from retail shops are sources of bacterial contamination, like *Shigella* species (Ali *et al*., 2010). A lack of sanitary conditions is the most common cause of contamination of meat from different sources, including money, where found similar organisms both on the meat and the sources of contamination (Ukwuru, and Gabriel, 2012).

Blood removal performed on the floor is very unsanitary and it is may be contribute to meat contamination. The process should take place on a clean stainless-steel table which should be cleaned frequently and the knife should be changed after operation and returned to a sanitizer (FAO, 1991). Bleeding and skinning of neck, cheeks, shoulder, and legs were done on the floor and contamination from the hide of one animal to others was transmissible. The cutting of carcasses, involves the use of utensils, equipment and knives and may allow for the transfer of more microorganism to beef tissues. Furthermore, workers' hands, clothes and their instruments may spread contamination onto the surface of beef carcasses (Gracey, and Collins, 1992). In most developing countries, the absence or poor hygienic practices in slaughtering, dressing and evisceration has been found to be one of the major causes of high surface contamination of beef carcasses by pathogenic and nonpathogenic microorganism (Eugène *et al*., 2013). Water used in slaughterhouse can also contaminate the meat during washing water used for cleaning procedures and meat processing in the slaughterhouses must meet drinking water standards (Adebowale *et al*., 2010), so adequate supply of potable water should be available to meet operational and cleanup needs and it should be analyzed frequently to confirm its quality (CFIA, 2010).

In general, contamination of carcasses is reduced by using automatic hide removal because there is less handling of the carcass and less use of knives. Vertical rail dressing improves hygienic practice by reducing carcass contact with operators, equipment, and other carcasses (Bakhtiary *et al*., 2016). Also workers must be educated about the principle of personnel hygiene as their hands must be washed after using the toilet, also the water used for washing such carcasses and offals must be bacteriologically examined. The result of treating meat samples by boiling and pressure cooking showed no bacterial growth in all examined samples after treatment, this is attributed to the fact that *Shigella* spp. are heat sensitive and unable to survive during pasteurization and cooking temperatures (Osborne, 2013).

The results of our study revealed there were significant differences between different methods of thawing, where the refrigerator thawing was the best method in reducing the increment in the bacterial count as compared with other two methods water thawing and room temperature thawing and this results agreed with other data obtained by authors. *Shigella* spp. presence was significantly higher when using room temperature thawing method (Kinman *et al.*, 2018). However, thawing for longer periods could lead to a faster bacterial growth (Sage and Ingham, 1998; Ingham, 2005). The ground beef which was thawed at room temperature for 8 h at 22ºC led to elevation of the bacterial count (Ingham, 2005). From the results of the other study concluded that refrigerator thawing (4°C) is more suitable in minimizing bacterial growth than running tap water or warm water (40°C) thawing (Rahman *et al*., 2014). Thawing of frozen minced red meat inside the refrigerator for overnight was the best and suitable to get meat with stable microbiological quality (Ismail *et al*., 2016).

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