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BACTERIAL CONTAMINATION AND PREVALENCE OF SOME FOODBORNE PATHOGENS IN EDIBLE BOVINE OFFAL IN ASSIUT CITY

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ABSTRACT

This study was carried out to evaluate the bacterial contamination and prevalence of *Salmonella* spp. and *Bacillus cereus* in 150 samples of edible bovine by-products which were collected from different retail butcher's shops in Assiut city. The obtained results revealed that the mean values of total viable bacterial count (T.V.B.C.) of Tripe, Mombar (Intestine), Lung and Cerebrum (Brain) were $2x10^5\pm0.89$, $1x10^5\pm0.89$, $1x10^5\pm0.79$ and $8x10^4\pm1.06$ CFU/g, respectively. Wheras, it was indicated that 90 of the examined samples of edible offal (32 Tripe, 29 Mombar, 16 Lung and 13 Cerebrum) were more than 10^3 (MPN/g) for total colliform count. Concerning *Salmonella* spp., 4 samples (2.7%) only were positive for *Salmonella* spp. Isolated serovars were identified as *S*. Enteritidis, *S*. Typhimurium and *S*. Anatum. Regarding *B. cereus*, 12 samples (8%) were positive, which included 5 samples of Tripe, 4 samples of Mombar and 3 samples of Cerebrum, while Lung samples were free. The average count of *B. cereus* in positive samples was $1.6x10^3$ CFU/g, where average number in Tripe, Mombar and Cerebrum were $3x10^3$, $1.4x10^3$ and $5x10^2$ CFU/g, respectively. The achieved results indicated that consumption of edible bovine by-products such as Tripe, Mombar, Lung and Cerebrum constitute a public health hazard as they may be associated with food poisoning microorganisms such as *S*. Typhimurium and *S*. Enteritidis.

Key Words: Edible bovine by-products, bacteriological contamination, S. Typhimurium, S. Enteritidis, B. cereus.

INTRODUCTION

Edible bovine by-products (offal) have recently received significant attention worldwide (Im et al., 2016). In Egypt, edible bovine by-products such as Tripe, Mombar (Intestine), Lung and Cerebrum (Brain) are more popular, less expensive and have high nutritive value for consumers. They have a good nutritional value due to the high protein and low fat levels as well as good content in vitamins and minerals (Anderson, 1988). Nowadays, Salmonella is one of the most pathogenic genera implicated in food-borne bacterial outbreaks and diseases and that constitute an important public health problem. There are numerous transmission routes for Salmonellosis, but the majority of the human infections are derived from consumption of contaminated foods especially those of animal origin (Saha et al., 2016). Also, B. cereus was identified as a causative agent in 19 % of foodborne outbreaks. The majority of these outbreaks were traced back to rice (50 %) and meat (24 %) (Bennet et al., 2013).

Numerous studies have described the numbers and types of bacteria on fresh meats. Whereas, studies

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evaluating the microbial contamination of diverse offal specifically investigating edible and contamination by pathogens that cause foodborne illnesses are rare. Moreover, there is scarce of the published information about the prevalence of Salmonella spp. and B. cereus in edible bovine byproducts obtained from retail butcher's shops in Assiut city. Such microbial contamination of edible bovine by-products may lead to public health problems. Therefore, the main objectives of this study were to enumerate total viable bacterial count, total coliform count and total Bacillus cereus count and determine the presence of Salmonella spp. and B. cereus on some edible bovine by-products commonly consumed in Assiut city, Egypt.

MATERIALS AND METHODS

1. Samples collection:

A total of 150 samples of edible bovine by-products including 40 samples of Tripe, 40 samples of Mombar (Intestine), 40 samples of Lung and 30 samples of Cerebrum (Brain) were collected from different butcher's shops in Assiut city. Each sample was obtained in separate sterile plastic bag. The collected samples were transferred directly to the laboratory where they were prepared for bacteriological examination.

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2. Samples preparation:

At the laboratory, each sample was aseptically and carefully freed from it's plastic bag and cut into fine particles by sterile scissor, then homogenized and thoroughly mixed. The fat and adhering extraneous materials on the surface of tripe were removed by knife and it was cut into small chunks of about 2.5 cm (Anna Anandh, 2004).

3. Bacteriological analysis:

RESULTS

3.1- Total viable bacterial count: (ISO protocol, 2003).

3.2- Total coliform count: (ISO protocol, 2006).

3.3- Count and isolation of *B. cereus*: (BAM, 2012). **3.4- Isolation of** *Salmonella* **spp**: Identification of presumptive colonies by biochemical reactions (ISO protocol, 2002). Serological identification of *Salmonellae* was carried out according to Kauffman – White scheme (Kauffman, 1974) for the determination of Somatic (O) and Flagellar (H) antigens using Salmonella antisera (Denka Seiken Co., Japan).

 Table 1: Statistical values of total viable bacterial count (CFU/g) of examined samples of edible bovine by-products.

| | | Total viable bacterial count (cfu/g) | | | |
|----------|----------------|--------------------------------------|--------------|--------------------|---------------|
| Samples | No. of samples | Min. | Max. | Mean | <u>+</u> SD |
| Tripe | 40 | $1 x 10^4$ | $2x10^{7}$ | $2x10^{5*}$ | <u>+</u> 0.89 |
| Mombar | 40 | $6x10^{3}$ | $3x10^{7}$ | $1 \times 10^{5*}$ | <u>+</u> 0.89 |
| Lung | 40 | $3x10^{3}$ | $9x10^{6}$ | $1 x 10^{5*}$ | <u>+</u> 0.79 |
| Cerebrum | 30 | 1×10^{3} | $1 x 10^{7}$ | $8 \times 10^{4*}$ | <u>+</u> 1.06 |

*Lower than Egyptian standards contamination load (ES, 2005).

Table 2: Statistical values of total coliform count (MPN/g) of examined samples of edible bovine by-products.

| Samples | Coliform count (MPN/g) | | | | | |
|----------|------------------------|----|-------|---------------|-------------|--|
| | No. | <3 | 3 ≤10 | $10 \le 10^2$ | $\geq 10^3$ | |
| Tripe | 40 | 0 | 2 | 6 | 32* | |
| Mombar | 40 | 2 | 1 | 8 | 29* | |
| Lung | 40 | 0 | 8 | 16 | 16* | |
| Cerebrum | 30 | 1 | 4 | 12 | 13* | |
| Total | 150 | 3 | 15 | 42 | 90 | |

*Higher than Egyptian standards contamination load (ES, 2005).

| Table 3: Incidence of Salmonella spr | . in examined samples | of edible bovine by-products. |
|---|-----------------------|-------------------------------|
|---|-----------------------|-------------------------------|

| Samples | No. of examined | Salmonella spp. S. Enteritidis | | eritidis | S. Typhimurium | | S. Anatum | | |
|----------|-----------------|--------------------------------|-----|----------|----------------|-----|-----------|-----|---|
| | samples | No. | % | No. | % | No. | % | No. | No. % 0 0 1 2.5 |
| Tripe | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mombar | 40 | 1 | 2.5 | 0 | 0 | 0 | 0 | 1 | 2.5 |
| Lung | 40 | 3 | 7.5 | 2 | 5 | 1 | 2.5 | 0 | 0 |
| Cerebrum | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 150 | 4 | 2.7 | 2 | 1.33 | 1 | 0.67 | 1 | 0.67 |

Table 4: Incidence and mean values of positive B. cereus from examined samples of edible bovine by-products.

| Samples | No. of samples | +ve B. cereus | % of +ve B. cereus | Mean |
|----------|----------------|---------------|--------------------|---------------------|
| | | | | (CFU/g) |
| Tripe | 40 | 5 | 12.5 | $3x10^3$ |
| Mombar | 40 | 4 | 10 | $1.4 \text{x} 10^3$ |
| Lung | 40 | 0 | 0 | 0 |
| Cerebrum | 30 | 3 | 10 | 5x10 ² |
| Total | 150 | 12 | 8 | 1.6x10 ³ |

DISCUSSION

The obtained results in Table (1) indicated that the mean values of T.V.B.C. of total 150 examined samples of edible bovine by-products was ranged from $2x10^5$ to $8x10^4$ CFU/g which were lower than standards contamination load set by Egyptian Standards (ES, 2005). Meanwhile, other investigators such as Isabel-Legarreta (2011) recorded lower results. In addition, the finding in Table (1) indicated that the mean number of T.V.B.C. of examined samples of Mombar was 1×10^5 CFU/g, lower results were reported by Mboto et al. (2012) who found that the bacterial count of Mombar samples was 1×10^{3} CFU/g. The results also showed that mean values of T.B.C. of examined samples of Mombar was 1x10⁵, of lung was 10⁵ and of Cerebrum was 8x10⁴ log CFU/g, Ahmed and Sarangi (2013) obtained nearly similar results as our findings.

According to the safe permissible limit stipulated by Egyptian Standards limits (ES, 2005) for total coliform count in edible offal (not exceed 10^2 CFU/gm), it was indicated that 90 of the examined samples of edible offal (32 Tripe, 29 Mombar, 16 Lung and 13 Cerebrum) were accordingly unaccepted (Table 2). These obtained results were in agreement with other studies as Selvan *et al.* (2007) who recorded higher results of coliform count (1x10⁵ CFU/g).

From the achieved results illustrated in Table (3), it could be concluded that 4 samples were positive for *Salmonella* spp. with overall percentage of 2.7%. Three samples of lung contaminated with *Salmonella* (two samples were classified as *S*. Entertitidis and one sample was identified as *S*. Typhimurium). Moreover, one sample was categorized as *S*. Anatum in Mombar. These results might be occurred due to faulty rupture of gastrointestinal tract during slaughtering process which regarded as one of the most important sources of carcass and organ contamination with *Salmonella* at abattoirs (Stopforth *et al.*, 2006).

The results demonstrated in Table (3) revealed that the *Salmonella* spp. failed to be isolated from Tripe and Cerebrum samples, this finding was disagree with the results recorded by Akkaya *et al.* (2012), who found *Salmonella* in these organs. On the other hand, higher overall Salmonella prevalence (7.1%) in cattle offal were reported by Im *et al.* (2016).

The obtained data outlined in Table (4) showed that there were 12 samples were positive for *B. cereus* (5 tripe, 4 Mombar and 3 Cerebrum samples) and these numbers represent 8% of total number of samples. The mean number of *B. cereus* in Tripe samples was $3x10^3$ CFU/g and Mombar samples was $1.4x10^3$ CFU/g while in Cerebrum samples was $5x10^2$ CFU/g. From Table (4) it was obvious that the mean number of overall count of *B. cereus* in this study was 1.6×10^3 CFU/g. This value were below the pathogenic number of *B. cereus* which estimated as 10^4 CFU/g (Lee *et al.*, 2006). This obtained result was disagree with a related study conducted by Iroha et al. (2011), who found lower incidence of *B. cereus* (2%) from 100 samples of beef meat included lung and Cerebrum samples and other internal organs.

CONCLUSION AND RECOMMENDATIONS

The achieved results in the present study indicated that total coliform count of the edible bovine offal samples were higher than the permissible limits and the most common *Salmonella* serotypes isolated were *S*. Enteritidis and *S*. Typhimurium so the presence of such food poisoning microorganisms may pose potential risk for public health. It is recommended that hygiene improvements are needed in the establishments selling edible bovine by-products to protect public health.

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التلوث البكتيرى وانتشار بعض البكتيريا الممرضة في أحشاء الأبقار الصالحة للأكل في مدينة أسيوط

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أجريت هذه الدراسة على عدد ١٥٠ عينة من أحشاء الأبقار الصالحة للأكل، حيث اشتملت على ٤٠ عينة من كلا من المعدة (الكرشة)، الممبار والرئة (الفشة) و ٣٠ عينة من المخ لتقييمها ميكروبيولوجيا عن طريق إجراء العد الكلى للبكتريا وعد بكتريا القولون الموجودة فى هذه الأحشاء. وكذلك الكشف عن وجود بكتريا السالمونيلا والباسيليس سيريس. وقد أوضحت نتائج هذه الدراسة أن متوسط قيم العدد الكلى للبكتريا للعينات التى تم فحصها يتراوح ما بين ٨ ×١٠ – ٢ × ١٠ مستعمرة بكتيرية/جم. فى حين أشارت النتائج إلى أن العدد الكلى للبكتريا القولون كان أكبر من ١٠ مستعمرة بكتيرية/جم فى ٩٠ عينة (٢٢ من المعدة، ٢٩ أشارت النتائج إلى أن العدد الكلى للبكتريا القولون كان أكبر من ١٠ مستعمرة بكتيرية/جم فى ٩٠ عينة (٢٣ من المعدة، ٢ الممبار، ٢٦ من الرئة و ٢٣عينة من المخ) وقد تم عزل بكتريا السالمونيلا فى ٤ عينات بما يمثل ٢٢ % من العدد الكلى للعينات وكانت عترات السالمونيلا المعزولة عتره واحدة سالمونيلا تيفيميوريام وعتر اتان سالمونيلا انتيريتيدس وعترة واحدة سالمونيلا اناتيم. أما بكتريا الباسيليس سيريس فقد وجدت فى ١٢ عينة بما يمثل ٨% من اجمالى عدد العينات، وكان متوسط عدد الكلى للعينات العينات ٢٦, ١٠ مستعمرة بكتيرية/جم. واحدة سالمونيلا تيفيميوريام وعتر اتان سالمونيلا انتيريتيدس وعترة واحدة سالمونيلا اناتيم. أما بكتريا الباسيليس سيريس فقد وجدت فى ١٢ عينة بما يمثل ٨% من اجمالى عدد العينات، وكان متوسط عدد البكتريا فى هذه العينات ٢٦, ١٠٦ مستعمرة بكتيرية/جم. ولقد خلصت نتائج هذه الدراسة إلى أن أحشاء الأبقار الصالحة للاستخدام الأدمى مثل المعدة (الكرشة) ، الممبار، الرئة (الفشة) والمخ قد تمثل خطورة كبيرة على صحة المستهلكين نظرا لزيادة العدد الكلى لبكتريا القولون بها وأيضا لوجود البكتريا المسببة للتسمم الغذائي مثل السالمونيلا تيفيميوريام والمالمونيلا الرائية الأدلمة