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Microbiological Evaluation of Some Edible Bovine By-products

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A B S T R A C T

Microbiological evaluation of edible bovine by-products (intestine, lung, rumen meat, head flesh, heart, tongue, kidney and liver) commonly consumed in Assiut city, Egypt were determined by enumerating total viable bacterial count (TVBC), total enterobacteriacae count, yeast and mold contaminants and determine the presence of *Salmonella* spp. and *E. coli* O157:H7 organisms. The obtained results showed that the mean TVBC of intestine, lung, rumen meat, head flesh, heart, tongue, kidney and liver were 9×10^6, 14×10^6, 6×10^7, 8×10^7, 7×10^6, 9×10^6, 7×10^6 and 5×10^6 cfu/g, respectively. While, the mean enterobacteriacae count of intestine, lung, rumen meat, head flesh, heart, tongue, kidney and liver were 3×10^6, 3×10^6, 3×10^7, 4×10^6, 7×10^5, 3×10^6, 3×10^6 and 3×10^6 cfu/g, respectively. Furthermore, the mean total fungal count of intestine, lung, rumen meat, head flesh, heart, tongue, kidney and liver were 3×10^4, 8×10^4, 2×10^4, 6×10^4, 1×10^4, 7×10^4, 9×10^5 and 8×10^4 cfu/g, respectively. Two *S. enteritidis* could be isolated from intestine and lung samples. One isolate of *S. typhimurium* was detected in intestinal sample. *E. coli* 157:H7 contamination was found in intestine, lung, rumen meat and head flesh, respectively. The results of this study show that edible bovine by-products are cross-contaminated by *E. coli* 0157:H7, *S. enteritidis* and *S. typhimurium* and thus 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.

Introduction

Edible meat by-products are a very economical source of high quality protein. They are rich in mineral and vitamin contents (Oztan, 2005; Seong *et al.*, 2014). In Egypt, the continuous increase in meat price lead the consumer to search for another suitable cheaper source of protein such as edible bovine by-products (Ockerman and Hansen, 2000). Edible bovine by-products are edible parts of internal organs also called variety meat or offal such as liver, kidney, heart, tripe and lung are eaten in large quantities by the population and most popular in Egypt especially in poor places. Also, they are consumed in many countries all over the world like Turkey (Nazlı *et al.*, 2005). Rumen meat, also, known as tripe is one of the important edible bovine by-products obtained from the first two chambers of a cattle's stomach: the rumen and the reticulum (Anna

Anandh *et al.,* 2012). Tripe is one of the high proteinnacious by product obtainable from slaughter house and is inexpensive with a distinguished taste (Ndeddy and Babalola, 2011). The intestine is a very long organ and is ideal for a casing of the sausage. Mumbar means stuffed intestines made by stuffing the large intestine which are short and tubby, while those made from the small intestine are long and slender. Due to readily available nutrients and poor hygienic conditions during handling, collection and processing, edible bovine by-products generally possess poor microbial quality (Selvan *et al.,* 2007). As well as they have high loads of microorganisms (Oztan, 2005). They can be contaminated more frequently than animal carcasses by Salmonella (Little *et al.,* 2008). Edible beef by-products have recently received significant attention worldwide (Im *et al.,* 2016).

However, studies evaluating the microbial safety of diverse edible beef by-products and specifically investigating contamination by pathogens that cause foodborne illnesses are rare. The scarcity of the published information about the microbiological evaluation of edible bovine by-products obtained from different butcher's shops and street vendors. Therefore, this study was carried out to enumerate total viable bacterial count, total Enterobacteriacae count and total fungal contaminants and to determine the presence of *Salmonella* spp. and *Escherichia coli* O157:H7 in some edible bovine by-products commonly consumed in Assiut city, Egypt.

Materials and Methods

Samples

A total of 132 edible bovine by-products (included 36 intestine, 27 lung, 26 rumen meat, 11 head flesh, 6 heart, 6 tongue, ten liver and ten kidney) samples were collected randomly from different butchers open shops and street vendors in Assiut city, Egypt. Samples were collected within 3 hours post-slaughter and during early morning, in order to minimize the microbial changes due to environmental temperatures and post-slaughter timings.

Each sample was aseptically placed into a sterile plastic bag, labelled and transferred immediately to the laboratory for bacteriological analysis. The selected internal organs were washed under running tap water to remove adhering blood, food remnants, feces, impurities, trimmed off of visible fats and connective tissues. Rumen meat was cut into small chunks of about 2.5 cm (Anna Anandh *et al.,* 2004).

Microbial analysis (PHLS, 1998)

The microbiological examinations of bovine by-products were assessed on the basis of Total Viable Bacterial Count (TVBC), total Enterobacteriacae count and total fungal count. TVBC determined on standard plate count agar (OXOID, CM0463), Enterobacteriacae cultured on MacConkey agar (Biolife, CB 5502) and total fungal detected on Malt extract agar Base (HIMEDIA, M137). The standard procedure recommended by (ISI, 1980) was followed for microbial analysis with above respective media.

All plates were incubated under aerobic conditions at 36±1°C for 24- 72 hrs. The mean number of colonies counted was expressed as colony forming units (cfu)/ per gram. Detection and serological identification of *Salmonella* spp. according to Kauffman (1974) and ISO (2002). Isolation and serology of E. *coli* 0157:H7 as recommended by De Boer and Heuvelink (2000) and Kok *et al.,* (1996). Data analysis was performed using SPSS v.16 statistical software package.

Results and Discussion

Total Viable Bacterial Count (TVBC)

The obtained results demonstrated in Table 1 showed that the mean TVBC of intestine, lung, rumen, head muscles, trachea, heart, tongue, diaphragm, kidney and liver were 9×10^6, 14×10^6, 6×10^7, 8×10^7, 9×10^6, 7×10^6, 9×10^6, 1×10^7, 7×10^6 and 5×10^6 cfu/g, respectively. The achieved results revealed that the TVBC of the edible bovine offal samples were higher than the permissible limits recommended by Egyptian Standard (E S, 2005). Similarly, in a related study conducted in Benha, Egypt, the mean value of TVBC of lung, liver, kidneys and heart were 4.01×10^7, 1.28×10^7, 3.90×10^6 and 2.15×10^6 cfu/g, respectively (Gafer-Rasha, 2013). During slaughter and processing, all edible bovine tissues are subjected to contamination from a variety of sources within and outside animal. The contaminating organisms are derived mainly from the hide of the animal and also comprise organisms that originate from feces (Datta *et al.*, 2012).

Enterobacteriaceae count

According to the data recorded in Table 2, the mean enterobacteriacae count of intestine, lung, rumen, head muscles, trachea, heart, tongue, diaphragm, kidney and liver were 3×10^6, 3×10^6, 3×10^7, 4×10^6, 4×10^6, 7×10^5, 3×10^6, 2×10^7, 3×10^6 and 3×10^6 cfu/g, respectively. These results revealed that the total enterobacteriacae count of the edible bovine offal samples were higher than the permissible limits sets by Egyptian Standard (E S, 2005). The achieved results in the current study revealed a higher counts of enterobacteriacae in different edible bovine offals compared with other authors such as Faten *et al.*, (2013) who recorded that the mean values of total Enterobacteriaceae count/g of lung, liver and heart samples were 8.53×10^4, 3.96×10^4 and 9.17×10^3, respectively and Hafez *et al.*, (1994) who found that Enterobacteriacae count of heart and liver was 2×10^3 and 4×10^4, respectively. Also, lower findings were reported by Ishak (1992) who found that Enterobacteriacae count 3.4×10^3 cfu/g in abattoir samples; El-Seiiedy (1997) who found that Enterobacteriacae count of cattle liver was $4.28\text{x}10^4$ and Ammar *et al.*, (2012) who recorded that Enterobacteriacae count in examined beef liver was 2.2×10^3. The Enterobacteriaceae family is one of the main bacterial groups implicated in the contamination of bovine tripe. Their presence in a food is an indication of improper hygienic measures (Gill and Landers, 2004).

Total fungal count (TFC)

Our results in Table 3 found that the mean total fungal count of intestine, lung, rumen, head muscles, trachea, heart, tongue, diaphragm, kidney and liver were 3×10^4, 8×10^4, 2×10^4, 6×10^4, 12×10^4, 1×10^4, 7×10^4, 8×10^4, 9×10^5 and 8×10^4 cfu/g, respectively. As E S (2005) does not establish safety limits for yeast and mould, it cannot be stated whether the values obtained here (1×10^4-8×10^4 cfu g) imply a risk to human health. On the other hand, there are studies with higher percentages such as that carried out by Gafer-Rasha (2013), who reported that the mean value of total mycotic counts/g of lung, liver, kidneys and heart were $1.55\text{x}10^5$, $2.97\text{x}10^5$, $1.04\text{x}10^6$ and $1.75\text{x}10^6$ cfu/g, respectively. Meanwhile, there is no fungus was found in the sample of meat and meat products except 2 samples (Datta *et al.*, 2012). The presence of yeast /mould in the food sample is due to it's disperse in the form of spores which are abundant in the environment and can be introduce through dust and soil (Apinis, 2003). Their presence in these food samples is a serious public health concern as these fungi may be associated with the production of mycotoxin (Makun *et al.*, 2009).

Salmonelle spp.

Regarding *Salmonella* spp., the results of this study demonstrated that two *S. enteritidis* were isolated from intestine and lung (one sample from each) with percentage 3.7 and 2.7 %, respectively. One strain of *S. typhimurium* was isolated from one intestine sample with incidence 2.7%. Moreover, *S. muenster* (3.7%) was isolated from one lung sample. The prevalence of *Salmonella* spp. in edible bovine by-products was evaluated by many investigators. In Germany Sinell *et al.,* (1984) could isolate Salmonella with percentage of 68.9% and 28.9% in bovine lung and rumen meat samples, respectively. The recovery rates of *Salmonella* spp. were 10% for the liver and rumen meat samples and 20% for the brain samples obtained from local butcheries in another study conducted in Turkey Oflaz (2005). In a study performed by Akkaya *et al.*, (2012), the detection rate of *Salmonella* spp. was 16% in the liver and 4% in the kidney, tripe and brain samples. Overall Salmonella prevalence (7.1%) in cattle offal from slaughterhouses in Korea was reported by Im *et al.*, (2016).

On the other hand, Ulutürk (1993) failed to detect salmonella in liver and rumen meat samples collected from abattoirs in Turkey. Also, Keven and Ay (2003) reported the same findings from Turkey that none of the liver, tripe and brain samples from abattoirs were found positive. The highest level of contamination of *Salmonella* spp. was reported from the lung samples by Sinell *et al.*, (1984) and from the liver samples by Samuel *et al.*, (1980).

Similarly, in our study the highest level of contamination with salmonella from intestine, lung and liver. The presence of *Salmonella* spp. in our samples may be due to multiple sources of contamination that cross-contamiante the offal during handling and processing post-slaughter and also reflects the intestinal bacterial load of slaughtered animals and hygienic standards of the abattoir (Akkaya *et al.,* 2012). In addition, the sources of the Salmonella were probably the contents of the gastrointestinal tract. Furthermore, 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). In our study, we failed to detect salmonella in rumen meat, head flesh, trachea, heart, tongue, diaphragms and kidney. This is in accordance with the results of Selvan *et al.,* (2007) who did not recover Salmonella from samples of retail meat products and Datta *et al.,* (2012) who reported that none of the samples contained Salmonella and *Shigella*. The absence of Salmonella in these offals samples indicate the quality of raw meat and other hygienic processing including the quality of the water used in washing and cleaning. Other researchers could isolate *Salmonella* spp. with different percentages such as Edris *et al.*, (2013) who could isolate *S. typhimurium* (4%) from lung samples and *S. typhimurium* (4%) and *S. entertidis* (4%) from liver samples and *S. entertidis* (4%) from kidney samples, respectively. Also, lower results were reported by Khalafalla *et al.*, (1989) who examined 25 samples of bovine livers and could found *S. typhimurium* (4%).

From the achieved results of the present study, it declared that *S. enteritidis* and *S. typhimurium* are the most prevalent serotypes. This result is compatible with Miller and Pegues (2005) who emphasized that *S. typhimurium* and *S. enteritidis* are the most common serotypes in the United States. Furthermore, historically, *S. typhimurium* is the most common agent of human food-borne disease, although in the last few decades *S. enteritidis* has become more common (Braden, 2006).

Table.1 Total viable bacterial count (TVBC) of examined edible bovine by-products (cfu/g)

Samples	No.	Min.	Max.	X ¯	SE±
Intestine	36	8×10^4	8×10^7	$9 \times 10^{6*}$	3.3×10^6
Lung	27	1×10^5	2×10^9	$14 \times 10^{6*}$	9.6×10^7
Rumen meat	26	9×10^4	3×10^9	$6 \times 10^{7*}$	1.4×10^9
Head flesh	11	1×10^5	2×10^9	$8 \times 10^{7*}$	1×10^4
Heart	6	2×10^5	5×10^6	$7 \times 10^{6*}$	4.8×10^5
Tongue	6	2×10^4	2×10^7	$9 \times 10^{6*}$	2×10^6
Kidney	10	1×10^7	1×10^7	$7 \times 10^{6*}$	7.8×10^6
Liver	10	6×10^5	1×10^7	$5 \times 10^{6*}$	7.4×10^5

*Higher than Egyptian standards contamination load (ES, 2005).
Min. = minimum Max. = maximum X ¯ = mean value

Table.2 Entrobacteriacae count of examined edible bovine by-products (cfu/g)

Samples	No.	Min.	Max.	X ¯	SE±
Intestine	36	5×10^4	3×10^7	$3 \times 10^{6*}$	1.2×10^6
Lung	27	4×10^4	3×10^7	$3 \times 10^{6*}$	1.4×10^6
Rumen meat	26	4×10^4	1×10^9	$3 \times 10^{7*}$	4.9×10^7
Head flesh	11	2×10^4	2×10^7	$4 \times 10^{6*}$	1.5×10^6
Heart	6	1×10^5	5×10^6	$7 \times 10^{5*}$	5×10^5
Tongue	6	9×10^4	7×10^6	$3 \times 10^{6*}$	7×10^5
Kidney	10	9.5×10^4	6×10^6	$3 \times 10^{6*}$	4.6×10^5
Liver	10	1×10^5	5×10^6	$3 \times 10^{6*}$	3.8×10^5

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

Table.3 Total fungal count of examined edible bovine by-products (cfu/g)

Samples	No.	Min.	Max.	X ¯	SE±
Intestine	36	1×10^2	2×10^5	3×10^4	8×10^3
Lung	27	6×10^3	9×10^5	8×10^4	4×10^4
Rumen meat	26	1×10^2	4×10^5	2×10^4	1.9×10^4
Head flesh	11	4×10^3	3×10^5	4×10^6	2.2×10^4
Heart	6	3×10^3	3×10^4	1×10^4	2.7×10^3
Tongue	6	2×10^4	2×10^5	7×10^4	1.8×10^4
Kidney	10	6×10^3	9×10^5	8×10^4	4×10^4
Liver	10	8×10^4	8×10^4	8×10^4	6.2×10^3

Table.4 Incidence of Entrobacteriaceae in the examined samples of edible bovine by-products

Samples	No.	*E. coli* 0157:H7		*E. coli* 0111:H4		*Salmonella* spp.		*Shigella* spp.		*Providencia rettgeri*	
		No.	%	No.	%	No.	%	No.	%	No.	%
Intestine	36	2	5.6	-	-	2	5.6	2	5.6	1	2.8
Lung	27	4	14.8	-	-	2	7.4	4	14.8	-	-
Rumen meat	26	3	11.5	1	3.8	-	-	7	26.9	-	-
Head flesh	11	1	9.09	-	-	-	-	2	18.8	-	-
Heart	6	-	-	-	-	-	-	1	16.6	-	-
Tongue	6	-	-	-	-	-	-	-	-	-	-
Kidney	10	-	-	-	-	-	-	1	10	-	-
Liver	10	-	-	-	-	1	10	-	-	-	-
Total	132	10	7.57	1	0.75	5	3.78	17	12.8	1	0.75

The stomach of a cow is home to millions of microbes and therefore contamination of tripe derived from the rumen and reticulum is difficult to avoid especially during the evisceration process (Bensink *et al.,* 2002; Ndeddy and Babalola, 2011).

E. coli 0157:H7

Concerning *E. coli*, in the current study *E. coli* 0157:H7 could be isolated from intestine (5.6%), lung (14.8%), rumen meat (11.5%) and head flesh (9.09%) samples, respectively. In a related study, Asakura *et al.,* (2012) screened 229 bovine offal products for the presence of Shiga toxin (stx) gene and found that eight (3.5%) were positive for *E. coli* 0157:H7. Also, *E. coli* O157:H7 was isolated from 4 tongues (6.7%), 1 liver (1.7%), 3 omasa (5.0%) (Asakura *et al.,* 2014). On the other hand, lower incidence (4.9%) of *E. coli* O157:H7 in the rumen meat were recorded by Walker *et al.,* (2010). In the present study, rumen meat was found contaminated with *E. coli* O111:H4 serovers (3.8%). Nearly similar results obtained by Braden (2006) who could detect *E. coli* O111:H4 serovers (EHEC) in lung samples (4%) and heart samples (4%), respectively and Bensink *et al.,* (2002) who isolated *E. coli* from 25 samples of cattle livers. The isolated *E. coli* is serotyped as O111, O128 and O26. Higher incidence achieved by Hassan and Osaman (2008) who could determine *E. coli* O111 serovers (EHEC) in lung samples with percentage of 8%.

Shigella

Regarding *Shigella* data outlined in Table 4 showed that *Shigella* can be found in intestine, lung, rumen meat, head flesh, heart and kidney samples with an incidence of 5.6, 14.8, 26.9, 18.18, 16.6 and 10%, respectively. The presence of *Shigella* usually indicates improper sanitary conditions and poor personal hygiene and is principally a disease – shigellosis – of humans, as well as other primates (Asghar *et al.,* 2002).

Providencia rettgeri

Concerning *Providencia rettgeri*, the findings presented in Table 4 revealed that *Providencia rettgeri* were isolated from only one out of 36 examined intestinal samples with an incidence of 2.8%. The genus *Providencia*, belonging to the family Enterobacteriaceae, consists of 9 spp., among these, *P. rettgeri* may be associated with

diarrhea (Yoh *et al.*, 2005). Shima *et al.*, (2016) could isolate *P. rettgeri* in 2 (8%) from retail meats in Thailand indicating that food animals, in particular meats, might be source of *Providencia* infection to human. Control measures as prevention of contamination of abattoir environment by bacteria, application of ante-mortem and post-mortem inspection in abattoirs and implementation of proper hygienic conditions by personnel working in meat industry and cleaning/disinfection of the equipments and machines used for processing are important to minimize infections caused by these pathogenic microorganisms (Akkaya *et al.*, 2015).

The results obtained in the present study showed that the TVBC, total enterobacteriacae count of the edible bovine offal samples were higher than the permissible limits recommended by Egyptian Standards (ES, 2005). The prevalence of *E. coli* 0157:H7 in the edible beef by-products is relatively high and the most common *Salmonella* serotypes 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 edible bovine by-products should be separated from the viscera at evisceration process. Moreover, hygiene improvements are needed in the establishments selling edible beef by-products to protect public health.

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