Bacteriological criteria of chicken giblets

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A B S T R A C T

A total of 50 random samples of liver and gizzard from freshly slaughtered chicken carcasses (slaughtered, plucked and eviscerated) (25 of each) were collected from local commercial retail shops in Menofia government. It is evident from the results that the mean value of aerobic plate count *(APC)* (cuf/g) of examined samples of chicken giblets was $4.86\times10^4\pm0.92\times10^4$ in liver and $7.73\times10^4\pm1.68\times10^5$ in gizzard and the incidence of *E.coli* in the examined chicken liver and gizzard were 20% and 28% , respectively. Also, the serologically identified *E.coli* isolates in the examined samples were $O_{26}: H_{11}$ (4%) , $O_{55}: H_7$ (4%), $O_{91}: H_{21}$ (4%)and $O_{128}: H_2$(8%) in liver and $O_{26}: H_{11}$ (8%) , O_{78}(4%), $O_{111}: H_2$ (4%), $O_{119}: H_6$(4%), O_{124}(4%)and $O_{126}: H_{21}$(4%) in gizzard. *Salmonella* was isolated from 24% and 36% of liver and gizzard and incidence of the isolated serotypes were S.Entritidis (4% and 12%), S.Infantis (4% and 4%), S.Kentucky (4% and 4%), S.Typhimurium (8% and 8%), S.Labadi 4% & S.Virchow 4% in gizzard and S.Larochelle 4% in liver only.

Key words: Liver, Gizzard, *APC*, *E. coli*, *Salmonella*.

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1. INTRODUCTION

Chicken giblets, edible viscera or edible offal (liver, gizzard and heart), the neck is usually part of giblets but is collected later on after evisceration (Alen, 2001). Chicken giblets namely are popular for the Egyptian people because of its palatability, fast preparation and its highly nutritive value (Hashim, 2005). The majority of Egyptian people prefer to eat fresh chicken, chicken parts and chicken giblets. This matter leads to dealing with small scale manual poultry shops. These shops didn´t implement effective hygienic measures or food safety instruction, as most of the recommended hygienic measures in the processing chain in the modern poultry processing plant are not applicable (Mira and Eskandar, 2007). Foodborne infection and intoxication outbreaks are increasing day by day in industrial and developing countries, the majority of cases of foodborne diseases were due to bacterial agents (Stevenson and Bernard, 1995). Aerobic plate count (*APC*) is the most reliable index of meat quality, sanitary processing and storage life of meat products (ICMSF, 1980), high APC of

mesophilic bacteria, for example, when applied to raw products, often consists of the normal microflora, or perhaps indicate incipient spoilage, rather than any potential health hazard (ICMSF, 1978). The presence of *Escherichia coli (E. coli)* in food of animal origin is considered as indicator of faults during preparation, handling, storage or service (Tebbut, 1999). The most commonly isolated bacteria from livers of apparently healthy chicken were*Esherichia coli* (Shah-Majid and Jah, 1987). *Escherichia coli* is considered as one of the most common causes of food poisoning outbreaks all over the world (Mead et al., 1999). *Salmonella* is responsible for most cases of food poisoning in the developing countries. Food borne Salmonellosis is still the most important food borne infection in human (Bhaduri and Cottrell, 2001). Therefore, this work was planned out to study the bacteriological contamination in chicken giblets from local commercial retail shops in Menofia government.

2. Materials and methods

2.1. Collection of samples:

A grand total of 50 random samples of liver and gizzard from freshly slaughtered chicken carcasses (slaughtered, plucked and eviscerated) (25 of each) were collected from local commercial retail shops in Menofia government,each sample weighting about 10gm. The collected samples were kept in separate plastic bags, transferred directly to the laboratory in an insulated ice box under complete aseptic conditions without any delay to evaluate their bacteriological quality.

2.2. *Preparation of samples* (USDA, 2011) under complete aseptic conditions, the examined samples were prepared. Twenty five grams of the examined samples were taken by sterile scissors and forceps after surface sterilization by hot spatula, transferred to a sterile polyethylene bag, and 225 ml of 0.1 % sterile buffered peptone water were aseptically added to the content of the bag. Each sample was then homogenized in a homogenizer. One ml from the original dilution was transferred with sterile pipette to another sterile test tube containing 9 ml of sterile buffered peptone water 0.1 % and mixed well to make the next dilution, from which further decimal serial dilutions were prepared. The prepared dilutions were subjected to the following examinations.

2.3. *Determination of aerobic plate count:* it carried according to (USDA, 2011).

2.4. Isolation and identification of E. coli:

The method described by ISO, (2004)Typical colonies of *E.coli* appeared on Eosin Methylene Blue agar plates greenish metallic with dark purple center, suspected colonies were purified and subcultured onto nutrient agar slopes and incubated at 37ºC for 24 hrs. The purified colonies were subjected for further morphological, biochemical and serological examination.

2.5.Isolation and identification of Salmonellae:

The method described by (FDA, 2011)Plates were examined for suspected salmonellae colonies which appear as red with or without black centers on Xylose Lysine Desoxycholate (XLD) agar media The purified colonies were subjected for further morphological, biochemical and serological examination .

2.6. Statistical analysis: the data was statistically treated by one-way ANOVA using SPSS program for windows (Version 16) (SPSS Inc. Chicago, IL and USA) and Duncan's post hoc test with $p < 0.05$ considered to be statistically significant.

2. RESULTS

It is evident from the results recorded in table (1) that the mean value of *APC* (cuf/g) of examined samples of chicken giblets were $4.86\times10^4\pm0.92\times10^4$ $7.73\times10^4\pm1.68\times10^5$ in liver and gizzard.

According to EOS (2005), only 8% of the examined samples of liver and 20% of gizzard were exceeded the permissible limits (10^5).

Result achieved in table (3) indicated that the incidence of *E.coli* in the examined chicken giblets were 20% and 28% in liver and gizzard and the serologically identified *E.coli* isolates in the examined samples of liver were $O_{26}: H_{11}$ (4%) , $O_{55}: H_7$ (4%), $O_{91}: H_{21}$ (4%)and $O_{128}: H_2$(8%) and $O_{26}: H_{11}$ (8%) , O_{78}(4%), $O_{111}: H_2$ (4%), $O_{119}: H_6$(4%), O_{124}(4%) and $O_{126}: H_{21}$(4%) in gizzard samples.

According to EOS (2005) 20% of liver and 28% of gizzard samples were unaccepted based on their contamination with *E.coli* (free) according to table (4).

As shown in (table 5), *Salmonella* was isolated from 24% and 36% of liver and gizzard samples, respectively. Incidence of *Salmonella* serotypes were S.Entritidis (4% & 12%), S.Infantis 4% , S.Kentucky4% & S.Typhimurium 8% of both, S.Labadi 4% & S.Virchow 4% in gizzard only, S.Larochelle 4% in liver only.

S.Typhimurium 8% of both, S.Labadi 4% & S.Virchow 4% in gizzard only, S.Larochelle 4% in liver only.

Seventy six percent and sixty four percent of the examined samples of liver and gizzard were acceptable for salmonella (count within the permissible limits 10^2/g) according to EOS (2005) (table 6).

3. DISCUSSION

Microbial contamination of poultry carcasses is a natural result of different procedures necessary to produce retailed products from living birds. Most of bacterial contaminants are nonpathogenic; however, poultry are known to harbor a large number of bacteria that are pathogenic to human being (Zhang et al., 2001).

Several indicators can be useful to evaluate hygiene levels during meat slaughtering process. Aerobic plate count (*APC*) is commonly used to evaluate the hygiene of the entire meat production process.

According to table (1) Nearly similar results were reported by Hashim (2005) (8.83×10^4 and 4.09×10^4 cuf/g) Moawad (2008) ($5\times10^4\pm1.3\times10^5$ and $3.1\times10^4\pm1.1\times10^5$) in liver and gizzard.

On the other hand, higher counts were reported by Cox et al.(1983) and Hassan (1996) 1.5×10^8 and 1.2×10^8 cuf/g, El-Kewaiey(1997) $4.2\times10^6\pm1.2\times10^8$ and $1.9\times10^6\pm8.9\times10^7$ cuf/g and Osman(2001) $5.8\times10^5\pm4\times10^6$ and $7.7\times10^6\pm1.83\times10^7$ cuf/g in liver and gizzard.

Lower counts were reported by Mira and Eskandar(2007) $0.1\times10^1\pm2.5\times10^6$ in liver and $0.15\times10^1\pm2.5\times10^6$ (cuf/g) in gizzard.

Results of incidence of E.coli in table (3) is nearly similar to Srinivasan et al.(2003) 20% and Abd-El-Moneim(1998) 20% in liver, Moawad (2008) 25% in gizzard , but higher than Samaha et al.(1993) 4.76%in liver and Samaha et al.(1993) 11.9% in gizzard .Moawad (2008) failed to detect *E.coli* in liver.

While the current results for the examined samples were lower than those recorded by Saha et al.(2003) (54.28% in liver) and, Abd-El-Moneim(1998) (32% in gizzard) .

Table (1): Aerobic plate counts/g (APC)(cuf/g) in the examined samples of chicken giblets (n=25).

Chicken tissues	Min	Max	Mean ± S.E*
Liver	$10^3 \times 8.0$	$10^5 \times 1.1$	$10^4 \times 10^4 \pm 0.92 \times 4.86$
Gizzard	$10^4 \times 1.5$	$10^5 \times 1.7$	$10^5 \times 10^4 \pm 1.68 \times 7.73$

S.E* = Standard error of mean

Table (2): Acceptability of the examined samples of chicken giblets based on their APC/g (n=25).

Products	APC /g*	Unaccepted samples	
		No.	%
Liver	$>10^5$	2	8
Gizzard	$>10^5$	5	20

* Egyptian Organization of Standardization "EOS" (2005)

Table (3): Incidence of *E. coli* isolated from the examined samples of chicken giblets (n=25).

Chicken tissues / *E.coli*strains	Liver		Gizzard		Strain Characteristics
	No.	%	No.	%	
O26 : H11	1	4	2	8	EHEC
O55 : H7	1	4	0	0	EPEC
O78	0	0	1	4	EPEC
O91 : H21	1	4	0	0	EHEC
O111 : H2	0	0	1	4	EHEC
O119 : H6	0	0	1	4	EPEC
O124	0	0	1	4	EIEC
O126 : H21	0	0	1	4	ETEC
O128 :H2	2	8	0	0	ETEC
Total	5	20	7	28	

EPEC=Enteropathogenic *E. coli*

EIEC =Enteroinvasive *E. coli*

ETEC=Enterotoxigenic *E. coli*

EHEC =Enterohaemorrhagic *E. coli*

Table (4): Acceptability of the examined samples of chicken giblets based on their contamination with *E. coli* (n=25).

Chicken tissues	*E. coli* /g*	Unaccepted samples	
		No.	%
Liver	Free	5	20
Gizzard	Free	7	28

* Egyptian Organization of Standardization "EOS" (2005)

Table (5): Incidence of Salmonella organisms isolated from the examined samples of chicken giblets (n=25).

Products / Salmonella Strains	Liver		Gizzard		Group	Antigenic structure	
	No.	%	No.	%		O	H
S. Enteritidis	1	4	3	12	D1	1,9,12	g,m : --
S. Infantis	1	4	1	4	C1	6,7	r : 1,5
S. Kentucky	1	4	1	4	C3	8,20	i : Z6
S. Labadi	0	0	1	4	C3	8,20	d : Z6
S. Larochelle	1	4	0	0	C1	6,7	e,h : 1,2
S. Typhimurium	2	8	2	8	B	1,4,5,12	i : 1,2
S. Virchow	0	0	1	4	C2	6,7,14	r : 1,2
Total	6	24	9	36			

Table (6): Acceptability of the examined samples of chicken giblets based on their contamination with *Salmonellae* (n=25).

Chicken tissues	cuf /g*	Unaccepted samples	
		No.	%
Liver	Free	6	24
Gizzard	Free	9	36

* Egyptian Organization of Standardization "EOS" (2005)

Moawad (2008) indicated that the serology of isolated *E.coli* serovars in fresh chicken liver were O_{111} K_{58} (b_4), $O_{127}K63$ *(B8)* and *O119K69 (B14),* at a percentage of 5.88%for each serotype and the number of the isolated strains were (2) serovers for each serotype. Also, in fresh gizzard was recorded as *O111K58 (B4); O127K63 (B8)* and *O119K69 (B4),* at a percentage of 5.88% for each serotype and it´s isolates number were (2) isolates for each serotype

Abd-el-moneim (1998) recorded that the isolated *E.coli* serovers were *O111K58(B4)* and *O126K71(B16)* at a percentage of 40% and 20%and its isolates were 2 and 1 in fresh liver, *O26K60(B6), O111K58(B4), O119K69(B14), O125K70(B15)* and *O128K67(B12)* in gizzard at a percentage of 12.5%, 25%, 12.5% and 12.5%, in isolates number of 1,1,2,1 & 1 and 1,1,2,1 & 1, respectively.

The pathogenic strains of *E. coli* associated with food borne illness were classified into 4 categories, Enteropathogenic *E.coli* (EPEC), Enteroinvasive *E.coli* (EIEC), Enterotoxigenic *E.coli* (ETEC) and Enterohaemorrhagic *E. coli* (EHEC) (Doyle, 1990).

Enterotoxigenic *E.coli* (ETEC) strains (*O78 and O128*) are considered the common cause of traveller's diarrhea and / or children diarrhea. It can produce either heat labile (LT) and/or heat stable (ST) toxins which are mainly attributed to the colonization factors that are specific for the host animal species and enable the organism to adhere to the epithelium of the small intestine (David *etal.*, 1990).

Although *E. coli* O_{157} is mostly found in ruminant animal and it is occasionally associated with other livestock and various foods of animal origin. Experience suggests that it is rare in poultry, whether in the live birds or on processed products (Mbata, 2005).

Result of salmonella in table (5) agrees with those reported by D´Aoust(1985) (21%), Ibrahim et al.(1989) (18%), Tibaijuka et al.. (2003) (28%) and Plummer et al.(1995) (24.5%)in liver but for gizzard Plummer et al.(1995) (37.1%) and Molaa and Mesfin (2003) (41.1 %).

Higher percentage of *Salmonella* were reported by Jerngklinchan et al.(1994) (86%), Arumugaswamy et al. (1995) (44%) and Molaa and Mesfin (2003) (34.5 %) in liver and Arumugaswamy et al. (1995) (44%) and Tibaijuka et al.. (2003) (53.1) in gizzard.

Moreover, Mira and Eskandar (2007) investigated that the percentage of Salmonella in fresh giblets were 30%.

On the other hand lower results obtained by Hassan(1996) (15%&20%), Abd-El-Moneim(1998) (12%&12%), Al-Mater et al. (2005) (16.67% and 6.67%) in liver &gizzard, Raguz et al.(1987) (6.9%) to (10.7%), Hashim(2005)(10%) and Moawad(2008) (5%) in liver, Ibrahim et al.(1989) (20%), El-Kewaiey (1997) (12%) and Tibaijuka et al.. (2003) (17.5%) in gizzard. Moawad-Shimaa(2008) failed to isolate *salmonella* from all gizzard samples.

The leading source of contamination of carcasses by salmonella is the evisceration step at the slaughterhouse (Bouchrif et al., 2009).

Moawad (2008) found that the serological identification of the isolated *Salmonellae* in liver was S.Typhimurium, S.Newport, S.Entritidis and S.Infanntis with its counts were 2, 0, 0 and 2 with a percentage 15.38%, 0%,0% and 15.38%, respectively.

Tha isolated serotypes as Salmonella Infantis and S.Typhimurium were recorded by Mossel et al., (1983), Guthrie (1991) and D´Aoust et al., (1985). Who found that the most frequent serotypes isolated from chicken liver were S.Infantis.

In liver Nine Salmonella serotypes were identified by Krabisch and Dorn (1986) who isolated S. Bovimorbificans (16.7%), S. Typhimurium (12.4 %), S. Infantis (11.1 %) and S.Saintpaul, S. Agona, S.Munchen, S. Entritidis and Typhimuriumvar Copenhagen.

Ibrahim et al.,(1989) said that the incidence of S.Infantis was 6% in gizzard and liver, while S.Typhimurium was 8% in liver and 6% in gizzard.

While mean, Vural et al. (2006) isolated Salmonella from giblets at a percentage of 8%.

Arroyo (1995) found that only 3 different serotypes were identified in chicken livers as S.Virchow and S.Entritidis.

Plummer et al. (1995) isolated S.Typhimurium from 23.1% of giblet samples.

Mira and Eskandar (2007) recorded that the isolated *Salmonella* serotypes in chicken giblets were *S.Infantis* and *S.Typhimurium*.

The source of Salmonella infection in poultry were feedstuffs, water, breeding eggs, hatcheries, flock house environment and transport cages (Bryan, 1979 and Barrow, 1993) Salmonella species is an important food-borne pathogen responsible for disease in animals and humans. It has been the leading cause of many outbreaks and infections around the world and is considered as one of the major causes of human gastroenteritis worldwide (Rasschaert et al., 2005).

Raw poultry products are perceived to be responsible for significant amount of human illness because of the relatively high frequency of contamination of poultry with *Salmonella spp*. (Kessel et al., 2001).

4. CONCLUSION

The examined chicken giblets (liver and gizzard) from local commercial retail shops in Menofia government harbor a high microbial loads especially *APC*, *E.coli* and *salmonella*. This is due to incorrect handling and processing as well as negligence of hygienic aspects at the production level. Chicken giblets characterized by wide public consumers without regarding to their social positions and ages carried and contaminated by varied types of microorganisms which harbor a dangerous effect on the consumer's health so it is of a great importance to safe guard consumer from being infected with these pathogens.

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