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## **BACTERIOLOGICAL EVALUATION OF FROZEN QUAILS SOLD IN MARKETS** (With 3 Tables)

By

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**التقييم البكتريولوجي للسمان المجمد المباع في الأسواق**

**زينب إبراهيم سليمان ، عزة على حسين التابعى**

انتشرت في الآونة الأخيرة مزارع السمان وزاد الإقبال على استهلاك لحومها. ويتميز السمان بطعمه المحبب وطراوته ورخص ثمنه واحتوائه على نسبة قليلة من الكولسترول مما يجعله مصدر صحي ومهم للبروتين يفوق كثيرا من الطيور الأخرى. قد تتعرض لحوم السمان أثناء الذبح والتجهيز للتلوث البكتيري لذلك أجريت هذه الدراسة لتقييم الحالة البكتيرية للسمان المجمد المطروح في الأسواق في محافظة بورسعيد. تم تجميع مائة وخمسون عينة من السمان المجمد وتم تقييمها من حيث العد الكلى البكتيري (Aerobic Colony Count (ACC) والعدد الكلى لكل من الميكروبات المعوية والذهبي العنقودى. كذلك تم فحص العينات لوجود ميكروبات سيدوموناس إيرجينوزا وجنس سيدوموناس، كامبيلوباكتري، الذهبي العنقودى، السالمونيلة، الإشريشيا القولونية نوع O157:H7 والإشريشيا القولونية. أسفرت النتائج على أن معظم العينات (93,3%) تحتوي على عدد كلى بكتيري أقل من  $10^5$  لكل جرام. كذلك كان متوسط العدد الكلى للميكروبات المعوية والذهبي العنقودى فى الحدود المسموح به (أقل من  $10^2$  لكل جرام). كما وجد أن نسبة 61,3% من العينات ملوثة بميكروب سيدوموناس وتم عزل سيدوموناس إيرجينوزا من 20% من العينات. كذلك أظهرت النتائج عن وجود كلا من ميكروبات الإشريشيا القولونية، السالمونيلة والذهبي العنقودى بنسبة 25,3% ، 18% ، 20% على التوالي ولم يتم عزل أي من ميكروبات كامبيلوباكتري والإشريشيا القولونية نوع O157:H7 من العينات التي تم فحصها. كذلك تم توضيح الأهمية الصحية ومدى خطورة الميكروبات المعزولة على صحة المستهلك وكذلك بعض الاشتراطات الصحية الواجب توافرها.

### **SUMMARY**

One hundred and fifty samples of frozen quails were collected from markets in Port Said Governorate for microbiological analysis to assure their quality and safety. The samples were screened for aerobic colony count (ACC), *Enterobacteriaceae* and *Staphylococcus aureus* counts and

pathogen including *Pseudomonas* spp., *Pseudomonas aeruginosa*, *E.coli*, *E. coli* O157:H7, *Salmonella*, *S. aureus* and *Campylobacter*. Bacteriological analysis revealed that in 93.3% of the investigated frozen quails samples, the ACC was  $< 10^5$  cfu/g. The mean values of aerobic plate count, *Enterobacteriaceae* and *S. aureus* counts were  $5.1 \times 10^3 \pm 1.1 \times 10^2$ ,  $1.2 \times 10^3 \pm 3 \times 10^2$  and  $0.3 \times 10^2$  cfu/g, respectively. *Pseudomonas* spp. were detected in 61.3% and *Pseudomonas aeruginosa* in 20% of samples. *E. coli* was detected in 25.3% of samples, but none was serotype O157:H7. *Salmonella* was detected in 27 (18%). *S. aureus* was isolated from 30 (20%). *Campylobacter* was not isolated from any of the investigated samples. The public health aspects for the estimated and isolated criteria were outlined as well as suggested hygienic measures were discussed.

**Key words:** *Quail carcasses, Enterobacteriaceae, Campylobacter, S. aureus*

## INTRODUCTION

In recent years quail meat has been gaining in popularity among consumers. Many quail farms have been established in Egypt both for egg and meat production. Distinct characteristics include rapid growth, marketed for consumption at 5- 6 weeks of age and high disease resistance, enabling quail industries to be accepted in Egypt.

Microbial food safety is an increasing public health concern worldwide. The major cause of foodborne disease is microbiological (Wolf, 1992). It has been estimated that the risk of foodborne disease and malnutrition is a thousand times greater than illness caused by pesticides or additives (Grose, 1988; Wheelock, 1989). Contaminated raw or undercooked poultry and red meats are particularly important in transmitting foodborne pathogens. Poultry meat can be contaminated with a variety of microorganisms, including those capable of spoiling the product during chill storage like *Pseudomonas* spp. (Arnaut-Rollier *et al.*, 1999). Moreover, poultry are perceived to be responsible for significant amount of human illness because of the relatively high frequency of contamination of poultry with *Salmonella* spp. (Geornaras *et al.*, 1995, Geilhausen *et al.*, 1996; Uyttendaele *et al.*, 1999 and Kessel *et al.*, 2001), *Campylobacter* (Rosenquist, *et al.*, 2003), *S. aureus* (Geornaras *et al.*, 1995; Khalifa and Nassar, 2001) and *E.coli* (Zhao *et al.*, 2001). As in case of chicken, quail meat can be contaminated with foodborne pathogens during slaughtering, defeathering, evisceration and other preparation processes.

Freezing is an excellent way to preserve animal products such as meat, poultry, fish and shellfish. Freezing does not sterilize food, the extreme cold simply retards the growth of microorganisms and slows down the changes that affect quality or cause spoilage in food (Banwart, 1979). The quality and safety of the final product depends on how the product is handled before, during and after freezing. To ensure the continued growth and competitiveness of this industry, it is essential that quails meat quality and safety are maintained during production and processing. Assessment of the quality and safety of foods requires microbiological analysis. The objectives of this investigation were (1) to determine the microbiological quality of frozen quail and (2) to document the presence or absence of some pathogens in frozen quail meat.

## **MATERIALS and METHODS**

### **Samples**

One hundred and fifty samples of frozen quails collected from local retail supermarkets in Port Said Governorate were transferred in an icebox. Each carcass was left to defrost at 4°C for 6 hours.

### **Microbiological quality analysis:**

Twenty-five gm muscle meat with skin sampled randomly from different parts of each carcass were blended with 225 ml buffered peptone water (1%). Tenfold serial dilution in 0.1% sterile peptone water was prepared. Aerobic colony count (ACC), *Enterobacteriaceae* and *S. aureus* counts were carried out according to APHA (1992). Total plate count was determined using plate count agar, *Enterobacteriaceae* counts by Violet Red Bile Agar + 1% glucose and *S. aureus* counts on Baird Parker tellurite egg yolk agar (BPA). The characteristic black colonies of *S. aureus* with peripheral clearance zone on BPA were counted and typical isolates were tested for coagulase activity.

### **Determination of *Pseudomonas* organisms:**

Presence of *Pseudomonas* organisms was determined according the technique recommended by Anon (1986). Isolation and identification of *Pseudomonas aeruginosa* were carried out according to Quinn *et al.*, (1994).

### **Microbiological safety analysis:**

Isolation and identification of *E. coli*, *Salmonella*, *S. aureus* and *Campylobacter* organisms were carried out according to the methods obtained by Food and Drug Administration (FDA, 1998).

**Identification of *E. coli* O157:H7:**

Typical *E. coli* were identified serologically by slide agglutination test using diagnostic *E. coli* O antisera and H 7 antisera (*Escherichia coli* antisera, Denka Seiken Co., Ltd, Tokyo, Japan), following the manufacturer's specification.

**RESULTS**

**Table 1:** Statistical analytical results of bacterial counts, *Enterobacteriaceae* and *S. aureus* in frozen quails samples (n= 150).

	Minimum	Maximum	Mean	± SE
Aerobic colony count	<10 <sup>2</sup>	2.3×10 <sup>6</sup>	5.1×10 <sup>3</sup>	1.1×10 <sup>2</sup>
<i>Enterobacteriaceae</i>	<10 <sup>2</sup>	8×10 <sup>3</sup>	1.2×10 <sup>3</sup>	3×10 <sup>2</sup>
<i>S. aureus</i>	<10 <sup>2</sup>	1.5×10 <sup>3</sup>	0.3×10 <sup>2</sup>	1.2

<10<sup>2</sup> = Non detectable level.

**Table 2:** Frequency distribution of bacterial counts, *Enterobacteriaceae* and *S. aureus* in frozen quails samples(n= 150).

Frequency range (cfu/g)	ACC		<i>Enterobacteriaceae</i>		<i>S. aureus</i>	
	No.	%	No.	%	No.	%
<10 <sup>2</sup>	80	53.3	98	65.3	120	80
10 <sup>2</sup> -<10 <sup>3</sup>	30	20	18	12	10	6.7
10 <sup>3</sup> -<10 <sup>4</sup>	18	12	34	22.6	20	13.3
10 <sup>4</sup> -<10 <sup>5</sup>	12	8	0	0	0	0
>10 <sup>5</sup>	10	6.7	0	0	0	0

ACC : Aerobic colony count

<10<sup>2</sup> = Non detectable level.

**Table 3:** Incidence of the isolated bacteria from the examined frozen quails samples (n= 150).

Organism	No. of positive samples	% of positive samples
<i>Pseudomonas</i> spp.	92	61.3
<i>P. aeruginosa</i>	30	20
<i>E. coli</i>	38	25.3
<i>E. coli</i> O157:H7	0	0
<i>Salmonella</i> spp.	27	18
<i>S. aureus</i>	30	20
<i>Campylobacter</i> spp.	0	0

## DISCUSSION

Quail meat have many advantages and superiority on the other species of poultry due to its low content of cholesterol and are considered as a valuable source of protein and minerals. The microbiological safety and quality of poultry meat are equally important to producers, retailers and consumers.

The total aerobic colony count, the *Enterobacteriaceae* and *Staphylococcus aureus* counting results obtained from 150 frozen quails samples collected from markets in Port Said were given in Tables (1 and 2). The achieved results revealed that, minimum, maximum and mean values of aerobic colony counts of frozen quails samples were  $<10^2$ ,  $2.3 \times 10^6$  and  $5.1 \times 10^3$  cfu/gm, respectively. Moreover, Table (2) indicated that 93.3% of the examined samples were within the permissible limit ( $10^5$  cfu/gm) which obtained by the Egyptian Organization for Standardization and Quality Control (EOSQC, 1995). However, only 6.7 % of the examined frozen quails samples had countable numbers of total bacteria, ranged from  $2 \times 10^5$  to  $1.2 \times 10^6$  cfu/gm. This may attributed to temperature abuse of the product prior to freezing or from poor sanitation in handling or processing and indicates that if it is subjected to temperature abuse, spoilage may occur in short time. The mean *Enterobacteriaceae* count of the examined samples was  $1.2 \times 10^3 \pm 3 \times 10^2$  cfu/g with a minimum value  $<10^2$  cfu/g and a maximum value  $8 \times 10^3$ . *Enterobacteriaceae* counts in food samples have been used as indicators of hygiene and contamination after processing, satisfactory  $<100$ , acceptable  $100-10^4$  and unsatisfactory  $>10^4$  (Gilbert, *et al.*, 2000). Accordingly, all examined samples are satisfactory and/or acceptable. With respect to *S. aureus*; minimum, maximum and mean values were  $<10^2$ ,  $1.5 \times 10^3$  and  $0.3 \times 10^2$  the maximum count of *S. aureus* recorded in investigated quails samples was  $1.5 \times 10^3$  cfu /gm, respectively (Table 1). The data obtained in Table (2) proved that 80% of the examined samples were contain *S. aureus* below the permissible limit ( $10^2$  cfu/gm) which suggested by EOSQC, (1995), while only 20% of frozen quails samples were contained *S. aureus* exceeded the permissible limit.

Poultry meat can be contaminated with a variety of microorganisms, including those capable of spoiling the product during chill storage, and certain foodborne pathogens. When poultry meat is stored aerobically under chill conditions, the organisms that predominate are invariably *Pseudomonas* spp. (Arnaut- Rollier *et al.*, 1999).

The results of the present study revealed that *Pseudomonas* spp. were detected in 92 (61.3%) of the investigated frozen quails samples. The incidence of *Pseudomonas aeruginosa* was found to be 20% (Table 3). The genus *Pseudomonas* represents the most psychrotrophic bacteria and can be taken as indicator for keeping quality and a measure for spoilage of meat. These organisms are highly proteolytic and/ or strong lipolytic and lead to biological changes in composition of meat particularly at low temperature (Gill and Newton, 1982). *Pseudomonas aeruginosa* has the ability to cause spoilage of meat and leads to several outbreaks of food poisoning (Pererra *et al.*, 1977). In this concern, Lukasova and Marz (1986) found that freezing at -18 °C reduced *Pseudomonas aeruginosa* count but did not results in complete reduction of the organism.

The presence of pathogenic organisms on foods (*Salmonella* spp, *Campylobacter*, *Staphylococcus aureus* and *E. coli* O157:H7) poses a food poisoning threat and following a number of recent high publicity food-related health scare (Mead, 2004).

Data in Table (3) showed that *E. coli*, *Salmonella* , and *S. aureus* were recovered at rate of 25.3 %, 18 % and 20 % from the examined samples, respectively. Neither *Campylobacter* nor *E. coli* O157:H7 were isolated in this study. Several other researchers have investigated the prevalence of many microorganisms that have public health significant in quails (Bottarelli *et al.*, 1994, Mostafa, 1997, Abd- El-Wahab *et al.*, 1998, Mossad *et al.*, 2000 and Medani *et al.*, 2002).

The rate of microbial contamination of retail frozen quails with *E. coli* in this study was 25.3%. The presence of *E. coli* and other *Enterobacteriaceae* in food samples most likely indicates fecal contamination (Aslam *et al.*, 2003). The importance of *E. coli* as a cause of diarrhoeic disease has been increasingly recognized (Desmarchelier and Grau, 1997). All *E. coli* isolates identified in this study were negative for the presence of *E. coli* O157:H7. Several studies have shown that *E. coli* O157:H7 is present in retail meat products; mostly beef products (Samadpour *et al.*, 1994). Despite the rarity of *E. coli* O157 in poultry, an outbreak in the UK that associated with eating turkey roll was reported by Salmon *et al.* (1989).

In terms of bacteria contamination, *Salmonella* spp. remains the most important infective agent of foodborne disease. Isolation of *Salmonella* from 18 % of the examined samples was of concern. Most *Salmonellas* found on poultry meat are non-host-specific and are considered capable of causing human food poisoning. *Salmonella*

positive birds at the time of slaughter have high numbers of organisms in their intestines as well as on the external surfaces (fecal contamination or feathers). Cross contamination during processing may also lead to increased prevalence of *Salmonella* in the finished products. Salmonellosis is a leading cause of enteric illness, with symptoms ranging from mild gastroenteritis to systemic illness such as septicemia and other longer-term conditions (Bryan and Doyle, 1995). A wide range of foods has been implicated in food-borne salmonellosis. However, as the disease is primarily zoonotic, foods of animal origin (in particular poultry) have been consistently implicated as the main sources of human salmonellosis (FAO/WHO, 2002).

*Staphylococcus aureus* is a ubiquitous organism in warm-blooded animals and its presence in a low number in raw foods of animal origin is to be expected. The presence of *S. aureus* may be due to contamination of food, human being normally harbour *S. aureus*, the main reservoir is the nasal cavity. The organisms find their way to skin, air and dust, which may contaminate meat and may cause staphylococcal food poisoning (Forbes *et al.*, 1998). Illness resulting from consumption of cooked poultry meat contaminated by *S. aureus* presents a risk due to the inactivation of competing microorganisms during cooking. Time and temperature abuse could allow growth of *S. aureus* that subsequently produce enterotoxin. (Ray, 2001). The production of enterotoxin (heat stable toxins) by *S. aureus* in food cause nausea, vomiting, retching, abdominal cramping and diarrhea in human (Gracey *et al.*, 1999).

*Campylobacter* spp. are recognized as an important cause of foodborne diarrhea in humans. (Brieseman, 1990). Concern has been expressed about the potential for infection of this bacterium via raw poultry (Kramer *et al.*, 2000). However, *Campylobacter* was not isolated in this study. The failure to isolate *Campylobacter* spp. may relate to lack of contamination with this organism. In addition *Campylobacter* spp. do not survive well at freezer temperatures (Moorhead and Dykes, 2002).

For food to be entirely safe from the microbiological viewpoint, it would need to be free from all pathogenic organisms. However, that this is not a realistic goal. There is still no economically viable means of eliminating foodborne pathogens in poultry-meat production because of the technological limitations in the process that can lead to cross-contamination of the carcasses being processed. In the processing microbial hazards can be introduced into poultry meat or grow to potentially hazardous levels, through: direct contamination by food

handlers, contaminated utensils, processing operations and the processing environment. Human illness may follow from handling of raw meat, undercooking or mishandling of the cooked product. Bacterial growth during thawing may be particularly relevant for pathogens such as *S. aureus* that are likely of minimal concern at low levels but can cause serious disease if allowed to grow and produce toxins. To diminish contamination rates in retail quails, it is critical that risk reduction strategies are used throughout the food chain. These strategies include on-farm practices that reduce pathogen carriage; Controlled slaughter and handling processes may be helpful. Additionally, consumption of undercooked food and cross-contamination during food handling and preparation must be avoided to ensure food safety at home and in the food service industry.

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