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A study on bacterial contamination of table eggs sold for consumption in Sana'a city.

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Summary

Bacterial pathogen contamination and their penetrability to table chicken egg shell were studied. A total of 60 table eggs,30 cracked (Group A) and 30 uncracked; apparent normal; eggs (Group B) were randomly purchased from whole-sale chicken egg distributors located at different market places in Sana'a city (Capital of Yemen). Egg shells as well as albumin and yolks were subjected to microbiological analyses. A total of 8 bacterial species were isolated from uncracked egg 8/30 (26.66%) six of them were Gram negative. These bacterial species including 22 isolates in rate of 63.33%. A total of 9 bacterial species were isolated from cracked egg shells 9/30 (30%); 7 out of them were Gram negative. These bacterial species including 28 isolates in rate of 93.33%. Generally, in this study 50 isolates were obtained from 60 eggs. Most of gram negative isolates were *E. coli* (19/50) in a percentage of (38%) out of total examined samples. One *S. Enteritidis* isolate was obtained from cracked eggs. Other Gram-negative bacterial species *spp., Klebsiella spp., Proteus spp., Campylobacter spp, and Pseudomonas spp.,* had also been found in eggs with intact or damaged shells with low proportion. Egg albumin of intact eggs was negative, while that of cracked shell had one *E. coli* isolate. *Staphylococcus aureus* and *Streptococcus* were isolated from both cracked and un-cracked egg shells. While *Staphylococcus epidermidis* was recovered only from uncracked egg shell.

The presence of cracks on egg shells was found to increase the load of bacterial groups, therefore we recommended the application of table egg sanitation to minimize the risk of bacterial contamination and the possible related food infection or bacterial poisoning.

Key words: Table eggs , Bacteria , Bacterial contamination ,
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Introduction

The poultry sector investment in Yemen is estimated to be more than 2 milliards US$. Big companies produce 30 percent of table eggs, while middle size producers or farms produce about 50 % of the table eggs. The small producer's/ farmers contribute with producing about 20 % of table eggs ***(FAO, 2008)*** (Table1). Because of the risk of spreading diseases, this aspect of food quality and hygiene of animal products for human consumption is an important aspect of egg production. Moreover, Hygiene is an important link, not only in term of health and production performance but also in terms of food safety ***(Vucemilo, et al. 2010).***

Campylobacteriosis and Salmonellosis are two zoonotic infections that can be transmitted to humans by contact with either the poultry itself or their eggs *(Willey et al., 2009)*. Eggs can be contaminated by coming in contact with contaminants like dust or droppings in the nest or on the litter floor *(Perry, 2004)* but in fact, much of Salmonellosis originates from a feeding gradient and can cause gastrointestinal illness in humans. *E. coli* are found naturally in the gastrointestinal tract of all warm blooded animals. Most strains of *E. coli* are not considered as pathogenic. These bacteria are commonly used as an indicator of fecal contamination of food Is products originates from a feed ingredient *(Willey et al, 2009)*. Egg shell quality is of primary importance to the egg industry worldwide. Egg shells need to be strong enough to remain intact throughout the chain from the time that the egg is laid until it is used by the consumer *(Roberts, 2010)*. Cracks in the egg shells are another quality concern. Eggs with cracks spoil faster than intact eggs *(Gletema, 2005)*. On the other hand, pathogenic bacteria can more easily invade those *(Rose, 1997)* If not infected during formation; an egg with intact shell is almost completely free of bacteria inside. The finest" hairline cracks "can only be detected when shining a bright light through it, so called candling *(Solomon, 1991)*.

The objective of this study was to evaluate the hygienic conditions of Yemeni egg production, and establishing the contamination and species variety of microorganisms in poultry table eggs, taken after about 7 days in storage.

Material and Methods

Egg samples:

Sixty eggs were used as including 30 cracked shell (Group A) and 30 uncracked (normal) shell eggs (Group B) were randomly purchased from whole-sale egg distributors located at different market places in Sana'a city (Capital of Yemen). This category of egg sellers usually sell cracked eggs along with un-cracked eggs. All egg samples were aseptically collected using sterile disposable hand gloves to prevent additional contamination. The collected eggs were put separately into sterile glass beakers, covered with sterile aluminum foils, transferred into ice blocks and quickly taken to the laboratory for microbiological evaluation and analyses.

Glass wares:

All laboratory glass wares were sterilized by autoclaving at 121°C for 15 min.

The Culture media:

Fluid media (nutrient broth and selenite-F-broth media) and sold agar media including MacConkey agar for Enterobacteriaceae, Nutrient and Blood agar for Gram- positive bacteria as well as Skirrow's, Butzler and Skirrow's media for Campylobacter and nutrient agar for *Pseudomonas aeruginosa (P. aeruginosa)* were prepared and used according to *Collee et al (1996)*.

Isolation of organisms:

From the collected samples an egg

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was picked up from (Group A), and put into a sterile glass and then washed with distilled water. After washing, 0.1ml of the

water was inoculated on the agar plates to isolate the organisms from the egg surface. At the same time, this egg was disinfected by 70% alcohol and broken with sterile blade, and 0.1 yolk and 0,1 albumen was inoculated separately on the previous media using a sterile Pasteur pipette . This procedure was also carried out for (Group B) un- cracked eggs. All culture media plates were labeled A and B incubated at the recommended temperature, time and precautions and then examined for bacterial growth according to ***Quinn et al. (1994) and Collee et al. (1996).***

Identification of Isolates:

The obtained isolates were identified and characterized on the basis of the results obtained from biochemical characterization complemented with the API identification kits (API System, France) and analyzed using Bergey's manual of systematic bacteriology ***(Sneath, et al. 1986).***

Results and Discussion

The collected official data (Table 1-3) (***MAI (2006)***about yearly egg production and the market share of each commercial and rural sectors pointed out that there was an continuous increase in all rectors from 1,105 X 10^6 in 2003 to reach1.291 X 10^6 in 2007 and 1,195 in 2011. Table eggs were represent 83% from the produced egg at 2003 and 82% at 2007 .The production of table eggs covers all the local needs (Table, 1) and the total local consumption of table eggs is shown in the table (2).

Hen's eggs can be contaminated or infected horizontally (through the shell) or vertically (transovarian) that makes them a potential source of pathogen participating in the etiology of diseases in poultry or food borne diseases in humans ***(Saif et al., 2003 and Stepien-Pysniak, 2010).*** Although it has been assumed that avian eggs in general are germ free at oviposition. Three routes of egg infection have been considered including the transovarian which resulted in yolk infection, oviducal resulted in vetelline membrane and\or albumin infection and trans-shell which resulted in translocation of bacteria from the outer to inner surface of the shell (***Duguid and North, 1991; Bruce and Drysdale, 1994 and Saif et al., 2003).***

A strong intact shell helps to keep the contents of the egg safe from microbial contamination. Egg shell quality is also very important in breeder flocks as one of the factors affecting hatchability. Eggs possess range of antimicrobial properties; the cuticle on the outside of the egg provides a mechanical barrier to bacterial ingress ***(Berrang et al., 1991)*** and its removal can increase the penetration of the eggshell by bacteria ***(Board et al., 1979).*** The egg shell itself provides a significant mechanical barrier to bacterial entry into the egg contents. The organic matrix of the egg shell and the shell membranes posses antimicrobial properties. Egg albumin has antimicrobial properties and the perivit-elline membrane provides a mechanical barrier to the entry of bacteria into the egg yolk.

It is clear that 22 isolates were isolated and identified, from egg shell (19 isolates) and egg yolk (3 isolates) of uncracked eggs (Tables 4). In the other hand 28 isolates including; 20 from egg shell, one from albumin and 7 from yolk of cracked eggs (Table 5). It is clear that bacterial isolates depends on quality of eggshell as the number of isolates was more in cracked eggs than those of un-cracked eggs. This could be explained that cracks in egg shell could increase the chances of the organism, as eggs without cracks in shell have many natural and built-in chemical and physical properties that prevent bacteria from entering and growing ***(Edema and Atayese, 2006).*** A total of 10 bacterial genera mostly of Gram negative were isolated from all egg samples with different percentage (Table 4 and 5). This comes in accordance of ***Moats (1980).*** It was clear that most isolates were *E.coli* (19 out of 50) and a percentage of (38%) out of total examined samples, Other Gram-negative bacteria, such as *Citrobacter spp., Klebsiella spp., Proteus spp., Campylobacter spp, and Pseudomonas spp.* have also been found in eggs with intact or damaged shells with low proportion which seem to be in agreement with those reported by ***Stepien-Pysniak (2010) and Board et al. (1964)*** who found that *Escherichia* was present on most eggs examined but in small numbers; while, *Pseudomonas, Proteus, and Serratia* were occasionally recovered. Moreover, ***(Florain and Trussell , 1956)*** correlated

the presence of E. *coli, Proteus, Pseudomonas and Aerobacter* with different rot in eggs examined. ***Musgrove et al. (2004)*** isolated *Citrobacter , Escherichia, Klebsiella and Salmonella* from the shells of eggs examined.

Table (1): Annual table and hatching eggs Production in Yemeni ($X10^6$)*.

Type	Year				
	2003	2004	2005	2006	2007
Commercial table eggs	890	908	930	967	1,022
Local table eggs	27	28	29	30	31
Hatching eggs for commercial broiler	124	131	138	151	165
Hatching eggs for commercial layer	9	9	9	10	11
Hatching eggs for local broiler and layer	55	57	85	60	62
Total	1,105	1,132	1,191	1,227	1,291

* Source: Agricultural Statistical Yearbook 2006 and DGAR information for 2007.

Table (2): Local total consumption of table eggs ($X10^6$) *.

Type	Year				
	2003	2004	2005	2006	2007
Table eggs eggs	913	936	959	1,006	1,053

* Source: Agricultural Statistical Yearbook 2006 and DGAR information for 2007 .

Table (3): Eggs production(MT) 2007- 2011 ($X10^6$) *.

Type	Year				
	2007	2008	2009	2010	2011
Eggs	1,031	1,085	1,128	1,166	1,195

* Source: Agricultural Statistical Yearbook 2011.

Table (4): Bacterial isolates obtained from egg shell, albumin and egg yolk of uncracked table eggs (n= 30).

Bacterial isolates		Egg shell		Egg Albumin	Egg Yolk		Total Isolates	
		No	%	-	No	%	No	%
E.coli		7	23.3	-	2	6.66	9	30
P.vulgaris		1	3.33	-			1	3.33
C.frundii		3	10	-			3	10
K. pneumonia		1	3.33	-			1	3.33
P. aeruginosa		2	6.66	-	1	3.33	3	10
C.jejuni		1	0.03	-			1	3.33
Staph.	*aureus*	2	6.66	-			2	6.66
	epidermidis	1	3.33	-			1	3.33
Streptococcus		1	3.33	-			1	3.33
Total number		19	63.33	-	3	10	22	73.3

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Table(5): Bacterial isolates obtained from egg shell, Albumin and egg yolk Bacteria of cracked table eggs (n=30).

Bacterial isolates		Egg shell		Egg Albumin		Egg Yolk		Total isolates	
		No	%	No	%	No	%	No	%
E.coli		6	20	1	3.33	3	10	10	33.3
S.Enteritidis		1	3.33					1	3.33
P.	vulgaris	1	3.33			1	3.33	2	6.66
	mirabilis	1	3.33					1	3.33
C.freundii		4	13.33					4	13.33
K.pneumonia		1	3.33					1	3.33
P.aeruginosa		2	6.66			1	3.33	3	10
C.jejuni		1	3.33			1	3.33	2	6.66
Staph. aureus		2	6.66			1	3.33	3	10
Streptococcus		1	3.33					1	3.33
Total number		20	66.66	1	3.33	7	23.33	28	93.3

Only one *Salmonella* was isolated from egg shell of cracked egg and two isolates of *Campylobacter jejuni* from table uncracked eggs, as epidemiological studies show that poultry meat and eggs are important sources for consumers' exposure to zoonotic pathogens such as Salmonella and Campylobacter ***(Anonymous, 2007 , 2008a and b and De Jong and Ekdahl, 2006).*** Salmonella infections are the most common food borne illnesses transmitted from animals to humans, and epidemics of Salmonellosis occur as a result of eating improperly cooked, contaminated foods, primarily eggs and egg products ***(Patrick et al., 2004) and USDA FSIS , 2005).***

P.aeruginosa is a known contaminant; it was isolated from egg shells and egg yolk of both uncracked and cracked eggs. *P.seudomonas* has the ability to dissolve cuticle when humidity is available that leading to higher frequency of penetration ***(Board et al., 1979).*** Furthermore; there is evidence available of chemotactic effect directing *P.aeruginosa* toward yolk ***(Lock et al., 1968).*** The low contamination of albumen and yolk in all egg groups can be referred to the storage conditions may lead to low penetrability of bacteria, The egg albumen has some prevention potential against bacteria and antimicrobial defense mechanisms, such as its organization in the albumenous sac and the viscosity of its protein. Chemical antimicrobial defense by lysozyme C, ovomucin, alkaline state (pH 9.5) and potential chelating of ovotransferrin. Other toxic components such as certain cations and vitamins made unavailable to organism by some proteins. The reason of low contamination of bacteria in yolks is that the perivitelline membrane provides a mechanical barrier to the entry of bacteria into yolk ***(Robert, 2010)*** beside egg collection and grading were performed immediately after laying ***(Kalidari et al., 2009).***

In conclusion, our study showed that market table eggs were contaminated by different microorganisms. A lot of bacterial types and isolates were detected on egg shell and some were recovered from inner egg parts of the eggs that indicate primary heavy contamination and prevailing poor storage conditions.

Therefore; it is strongly recommended to adopt quality control standards in egg collection, transportation and storage conditions. As management strategies that can be practiced to reduce egg contamination includes: eggs collection frequently to minimize the time that they are exposed to a contaminated environment, keeping eggs laying areas as clean as possible (including the nest litter or pads) avoid abrasive cleaning of the egg which can the effect of the integrity of the shell, use authorized egg shell sanitation or fumigation programs properly and removing eggs to the cooler as soon as possible after lay.

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دراسة عن التلوث البكتيري في بيض المائدة المعد للبيع للاستهلاك في مدينة صنعاء.

الملخص العربى

تمت دراسة التلوث البكتيرى الممرض ونفاذيتة لقشرة بيض الدجاج المائدة . العدد الكلى للبيض الذى خضع للفحص هو ٦٠ ، منها ٣٠ بيضة متصدعة (المجموعة الأولى) و ٣٠ بيضة سليمة (المجموعة B) تم شراؤها بشكل عشوائي من الموزعين من أماكن مختلفة في سوق مدينة صنعاء (عاصمة اليمن). تعرض قشرة البيض وكذلك الزلال والصفار كل على حده إلى الفحص الميكروبيولوجية. ثمانية الأنواع البكتيرية تم عزلها من البيض طبيعى القشرة (٣٠/٨) بمعدل ٢٦.٦٦% ٦ منها سلبية الغرام. شملت هذه الانواع البكتيرية ٢٢ معزولة بمعدل ٦٣.٣٣%. مجموع الانواع البعتيرية التى عزلت من البيض المتصدع القشرة كانت ٩ انواع من ٣٠ بيضه بنسبة ٣٠% منها ٧ سالبة الجرام وشملت تلك الانواع ٢٨ معزولة بمعدل ٩٣.٣٣%.

بصفة عامة اوضحت الدراسة التوصل الى ٥٠ معزولة من ٦٠ بيضة. وان معظم المعزولات سلبية الغرام تنتمى الى الميكروب القولونى (*E. coli*) ٥٠/١٩ بمعدل ٣٨%. كما تم الحصول على معزولة من السلمونيلا المعوية (*S. enteritidis*) من البيض متصدع. الأنواع البكتيرية الأخرى سلبية الغرام من البيض السليم والمتصدع كانت تنتمى الى الكليبسيلا (*Klebsiella*) ، و البروتيوس (*Proteus*) ، الكمبيلوباكتر (*Campylobacter*) ، والسيدوموناس (*Pseudomonas*) . كان زلال البيض السليم سلبي، في حين أن متصدع كان به عترة من الميكروب الفولونى . اما العنقودية الذهبية (*Staphylococcus aureus*) والعقدية السبحية (*Streptococcus*) فقد تم عزلها من كل من قشر البيض متصدع وغير متصدع. في حين تم عزل المكورات العنقودية البشروية (*Staphylococcus epidermidis*) من كلا النوعين المتصدع والطبيعى القشرة.

وجود الشقوق على قشر البيض يؤدى الى زيادة الحمولة من المجموعات البكتيرية، ولذلك أوصينا بتطبيق الشروط الصحية على بيض المائدة للحد من مخاطر التلوث الجرثومي واحتمال العدوى الغذائية ذات الصلة أو التسمم البكتيري.