Yersinia enterocolitica and *Pseudomonas aeruginosa* in marketed eggs in Sohag Governorate.

F. Abd El-Latif Wassel, N. Makram Awad and Azza M. Moslah
Veterinary Sohag Lab., Animal Health Research Institute

Abstract

Commercial hens and duck eggs (300 eggs) representing 100 groups (3 eggs each as sample) were randomly collected from different Sohag city markets and different groceries. Hen eggs represent farm hens and native breeds. The collected samples were examined for incidence of *Yersinia enterocolitica* and *Pseudomonas aeruginosa* on egg shells and contents. *Yersinia enterocolitica* could not be isolated from shell and contents of farm hen eggs (shell and content), and the organism recovered from three samples (7.5%) of shell and 6 samples (15%) of the samples of native breeds eggs. Ducks' eggs were contaminated by the ratio of 10% from shell and 20% from contents. *Pseudomonas aeruginosa* could not recover from any native breeds eggs (shells and contents) but could be isolated 6.7% of shells and contents of farm eggs (2 sample each). Ducks' eggs proved to contain the organism in the contents (4 samples) by ratio 13.3% and on shell (2 sample) by ratio 6.7%. Total leucocytic count and differential were done to hens and ducks. Also sodium, potassium and chloride were assayed to sicked birds . The public health hazard of isolated organisms and suggestive measures were discuss.

Key words:
Yersinia enterocolitica, *Pseudomonas aeruginosa*, hens egg, duck egg, shell

Introduction

It has been recognized that eggs are important and popular food in all countries almost for all eges . Eggs provide a unique well balanced source of nutrients, including high quality protein, vitamins, useful amount of minerals and easily digested lipids, However, the nutrients that make eggs a high quality food for humans are also a good growth medium for bacteria. In the rare event that egg contains bacteria, as the inside of an egg was once considered almost sterile **(Brooks and Taylor, 1955** and **Frazier and Westhoff, 1978)**. But, over the recent years, different types of microorganisms have been found inside eggs. As, if the ovary is infected with bacterial pathogens, the egg may become infected before it is laid, Besides, other types of microorganisms could be deposited along with dirt on the outside of an egg and from fecal matters, from hens, by lining of the nest, by wash if the eggs are to be washed, by handling and perhaps by materials in which eggs are packed (**Boardand Fuller, 1994** and **Cox *et al*., 2000)**.

Some food Poisoning outbreaks due to consumption of eggs had been reported **(Adler, 1965; Bowmer, 1965** and **Godoy *et al*., 2000)**. Different types of bacteria including Psudomonas have been isolated from eggs **(Longree, 1980)**. Also, it has been proved that egg shells are pervious to microorganisms such as *Salmonella typhi, E. coli* **(Lifshitz *et al*., 1964** and **Maha *et al*., 2013)**.

Yersinia enterocolitica is a food borne pathogen that is widely distributed in nature, animal and aquatic reservoir. They are able to

grow at low temperature **(Stern *et al*., 1980)** and can produce toxins in foods **(Boyce *et al* ., 1979** and **Francis *et al.,* 1980).** These enterotoxins may be able to resist the temperature used in food processing and storage **(Boyce *et al.,* 1979)**. *Y. enterocolitica* could lead to enterocolitis, mesenteric lymphadenitis and terminal ileitis. Also it has been reported that *Y. enterocolitica* invades the epithelium cells of gastrointestinal tract to produce intestinal diseases in animals and humans . There has been interest in the recovery of *Y. enterocolitica* from foods since the implication of *Yersinia enterocolitia* in food poisoning occurred in New York due to consumption of chocolate milk **(Black *et al*., 1978)**. The reports on *Y. enterocolitica* prevalence in eggs and egg productsare sketchy, however, it has been shown that *Y.enterocolitica* can be found on the surface of egg shells **(Favir *et al*., 2000)**.

Pseudomonas aesuginosa, has been implicated in various human infections, including enteritis, various respiratory diseases , urinary tract infection, infections of bones, joints, nails skin and wounds ***(*Winso, 1957;Burlina, 1962; Chernosky and Duckes, 1963** and **Baron *et al*., 2013*)*** As reported by **Gantois *et al.,* 2009*)***, *Pseudomonas aeruginosa* can grow and then penetrate through intact shell to the egg contents. This phenomenon was previously assured by **Garibaldi and Stokes (1958)** that egg shells were pervious to Pseudomonas aeruginosa. **Ahmed *et al.* (1985)** could isolate the organism from the shells of examined hens' eggs. In a study conducted by **Das *et al.* (1996)**, *Pseudomonas aeruginosa* was one of the causative organisms of nosocomail diarrhea occurred in Calcutta, the eggs were among the sources of infection. Therefore, this work aims to investigate the incidence of *Yersinia enterocolitica* and *Pseudomonas aeruginosa* on shells and in contents of commercial hens and ducks' eggs.

Materials and Methods

Collection of samples :

A total of 300 eggs representing 100 groups of commercial hens and ducks' eggs, were collected randomly from Sohag city markets and different groceries. Hens' eggs included farms and native breed hens. Each group (3 eggs) represent one sample was placed in a sterile plastic bag and dispatched to the laboratory with a minimum of delay. The eggs were prepared and examined for the presence of *Yersinia enterocolitica* and *Pseudomonas aeruginosa* .

3ml of blood obtained from the wing vein using 3 ml syringe then immediately transferred in 2 tubes (1ml into the first tube containing EDTA for hematological determination and 2 ml into the second tube without anticoagulant for sodium, potassium, and chloride determination.

Preparation of sample :

(A) Egg shells were tested by surface method as described by **Moats (1979).**

(B) Egg contents . The egg was prepared for evacuation of its content according to **Speck (1976)**.

Experimental techniques :

The rinse solution of egg shells, as well as, the homogenous egg contents was subjected to the following examination:

1- Isolation and identification of *Yersinia entrocolitica*

(a) Enrichment procedure : 1 ml of rinse solution of egg shells, as well as, from the homogenous egg contents was placed aseptically into enrichment broth (Trypticase soya broth) and incubated at 37C^{o}for 24 hours (**Greenwood and Hooper, 1989)**.

(b) Isolation technique : A loopful of the incubated broth was streaked directly onto Cefsuldin Irgasan Novobiocin (CIN) as described by **Schiemann (1979**). And then incubated at 37^{o}C for 24th. The presumptive colonies were identified according to **Schiemann and Devenish (1982)** .

2- Isolation and identification of Pseudomonas aeruginosa.

(a) Enrichment procedure :1ml of shell rinse

solution, as well as , from homogenous egg contents was inoculated into Citrimide broth and incubated at 42°C for 48 hours (**Grover and Srinivasan, 1988)**.

(b) Isolation technique : Loopfuls from incubated broth tubes were streaked on to citrimide agar plates and incubated at 42°C for 24-48 hours (**Shrininwas, 1975)** Loopfuls from suspected colonies were picked up into agar slants and incubated at 42°C for 24 hours for further identification according to **Finegold Martin (1982)**.

3-Hematology :

-Differential leucocytic count were made on monolayer blood films, fixed and stained with Giemsa stain , total red blood cell and total leucocytic were determined manually using hemocytometer (**Schalm, 1986)**.

-Sodium , potassium , and chloride were determined by atomic spectrophotometry.

Results

The obtained results were recorded in Tables :

Table (1). Incidence of *Y. enterocolitica* in the examined samples Hens and ducks' eggs**.**

Examined samples	No . of samples examined	Positive samples			
		Egg shell		Egg content	
A. Hens' eggs		No	%	No	%
Farms	30		-	-	-
Native breeds	40	3	7.5	6	15
B. Ducks' eggs	30	3	10	6	20

Tables (2). Incidence of *Ps. aeruginosa* in the examined samples of Hens and ducks' eggs .

Examined samples	No . of samples examined	Positive samples			
		Egg shells		Egg contents	
A. Hens' eggs		No	%	No	%
Farms	30	2	6.7	2	6.7
Native breeds	40	-	-	-	-
B. Ducks' eggs	30	2	6.7	4	13.3

Table (3). Total and differential leucocytic count.

	Hens						Ducks		
	Native hens			Farm hens			Diseases ducks		
	Control	Yersinia	Ps.	Control	Yersinia	Ps.	Control	Yersinia	Ps.
TLC	9.83±0.92*	11.56±057*	10.09±0.75	8.97±1.14	11.76±079	11.07±1.0	10.33±0.78	11.76±0.79*	11.07±1.09
Heterophil	28.17±3.0	35.0±4.8*	32.0±3.1*	26.58±3.7	38.67±1.3*	31.33±1.3*	26.75±4.2	37.3±1.3*	31.6±2.5
Lympho-cyte	60.75±3.2	57.67±5.3*	58.6±2.78*	63.58±3.4	55.0±1.73*	60.67±0.5*	63.2+4.8	55.0±0.47*	60.0±3.1*
Monocyte	6.58±1.0	3.67±0.5*	4.33±0.5	6.67±0.89	3.33±0.5*	4.67±0.5*	6.58±0.79	4.33±1.3*	4.33±0.5*
Eosinophil	2.92±0.67	3.3±0.5	4.0±0.78	3.33±0.78	3.0±0.0	3.3±0.5	3.42±1.16	3.33±0.5	4.3±0.5*

*= significant increase. Total leucocytic count showed significant increase in both *Y.enterocolitica* and *P.aeroginosa* infection in hens and ducks compared with control ones. (Ps.=*Pseudomonas aeroginosa*)

Heterophil percent showed significant increase in both *Y.enterocolitica* and *P.aeroginosa*infection in hens and ducks compared with control one. Lymphocyte percent showed significant decrease in both *Y.enterocolitica*and *P.aeroginosa* infection in hens and ducks compared with control one. Monocyte percent showed significant decrease in both *Y.enterocolitica* and *P.aeroginosa*in fection in hens and ducks compared with control one. Eosinophil percent showed significant increase in Pseudomonas infection in native hens and ducks compared with control one.

Table (4). Mean &SD of sodium, potassium and chloride in serum of infected hens and duck with *Y. entralitica*

	hens			ducks	
	Control	Native hens	Farm hens	Control ducks	Diseased duck
Sodium (mmol/l)	133.53±4.01	141.87±4.45	139.73±1.6	136.8±4.45	145.57±3.7
Potassium (mmol/l)	4.27±0.22	4.91±0.45	4.25±048	5.21±0.09	5.07±0.35
Chloride (mmol/l)	101.5±4.3	100.0±3.12	95.67±1.8	103.0±3.1	102.3±0.5

Significant decrease in serum chloride level in farm hens compared with control ones.

Table (5). Means & SD of sodium, potassium and in serum of infected hens and ducks with *P.aeroginosa*.

	Hens			Ducks	
Mean±SD	Control	Native hens	Farm hens	Control ducks	Diseased ducks
Sodium (mmol/l)	139.07±4 34	141.33±2.0	140.47±2.82	141.93±5.43	143.47±4.09
Potassium (mmol/l)	5.05±0.19	4.85±0.93**	5.01±0.16	5.22±0.20	5.06±0.21
Choride (mmol/l)	107.0±4.86	100.67±2.18**	99.0±0.87**	106.53±3.58	100.67±1.32**

**=Significant decrease in serum potassium level in native hens compared with control ones also significant decrease in serum chloride levels in native and farm hens as well as diseased ducks.

Table (6). Means &SD of sodium, potassium, sodium and chloride in mg/100gm egg content in diseased samples.

			Hens		Ducks	
	Y.enterocolitica		*P.aeroginosa*			
	Farm	Native	Farm	Native	Yersinia	Ps.
Sodium (mg/100gm egg content)	149*	153	148	150	155	158
Chloride (mg/100gm egg content)	170	177	168	163	174	171
potassium(mg/100gm egg content)	138	143	131	133	148	142

* 100 gm

FAO (2010) whole chicken egg potassium value was 126 mg/100gm egg content (Food and agriculture article).

Discussion

As recorded in (**Table 1),** *Y. enterocolitica* could not be detected on egg shells or in contents samples of the examined hens' eggs collected from poultry farms. The data proved that 3/40 (7.5%) of egg shells and 6/40 (15%) of egg contents samples of native breed hens were positive for the organism. Duck's eggs samples were found to be contaminated by *Y. enterocolitica* on 3/30 (10%) of their shells and in 6/30 (20 %) of their contents. No data available to compare with except the research newsletter published by **Favir *et al.* (2000)** that *Y. enterocolitica* could not be detected among the natural flora on any of the egg tested.

Presence of *Y. enterocolitica* on egg shells and in egg contents samples could be attributed to the fecal matter soiled the egg shells of native breed hens and ducks' eggs Furthermore , Previous isolation of Coilforms, *E.coli*, Salmonella, Enterococci, Alkaligenes and pseudomonas organisms from egg shells and contents by **(Alford *et al*., 1950; Ahmed *et al*., 1985 & 1987; El-Prince, 1988 and Bastawrows *et al*., 1997)**, assures the possibility of contamination by *Y. enterocolitica*. **(Melina, 2010)** stated that egg shells are frequently contaminated by infected fecal matter and under favorable conditions, the organisms penetrate the shell into the egg contents. Also, **(Matthes, 1984)** proved that storage temperature and types of packing materials for transportation influence the penetration through egg shells into the contents.

The summarized data in (**Table, 2)** revealed that hens' eggs of poultry farms were contaminated by *Pseudomonas aeruginosa* on 2/30 (6.7 %) of their shells samples and by the same ratio in their contents . In case of egg samples of native bread hens, *P. aeruginosa* failed to recover from any of their shells or contents. While, 4/30(13.3%) of ducks' eggs content were positive for *P. aeruginosa*, and 2/30 (6.7%) of their shells carried the organism. *P. aeruginosa* was previously isolated from egg shells of commercial hens' eggs by **(Garibaldi and Stokes, 1985 and Ahmed *et al.,* 1985)** in different percentages.

The investigation carried by **(Moursy *et al*., 1982)** pointed out that ***P. aeruginosa*** could be detected in 18.18% of contents of unsold aged hens' eggs . However, in a previous study conducted by **(Miller and Crawford, 1953)**, ***P. aeruginosa*** could be isolated from 9% of examined egg content samples of commercial hens' eggs . It was noticed that ***P. aeruginosa*** can grow and then penetrate through the intact shell to the egg content and the rate of penetration depends on the temperature of storage and humidity **(Haines and Moran, 1940)** furthermore, **(Garibaldi and Stokes, 1985)** had proved that eggshells are pervious to ***P. aeruginosa*** .

Total leucocytic count in our study in healthy laying hens showed (10.33$\pm$ 0.78), marked decrease TLC was noticed compared with (20.64$\pm$ 0.45), (14.60$\pm$ 2.5) **(Simaraka *et al*., 2004 and Hassan *et al*., 2011)**. Also slight increase in heterophil% (26.75 $\pm$ 0.4) compared with **(Simaraka *et al*., 2004)** and the same result with **(Hassan *et al*., 2011)**.

Lymphocyte % nearly same result of our study (63.2 $\pm$ 4.0) with **Simaraka *et al*., 2004** (63.68 ± 9.36) and highly increase when compared with **(Hassan *et al*., 2011)** (55.0±7.2).

Our study showed the measurement of sodium, chloride and potassium were showed in table (6) potassium showed marked increase in both *Y. enterocolitica* and *Ps.aeruginosa*. infected hen eggs compared with **(Matt *et al*., 2009)** that said potassium in chick egg content in mg/100gm was 122$\pm$ 25 in native hens and 131 $\pm$ 27 in farm hens and sodium level was 134 $\pm$ 25 in native hens and 131$\pm$ 20 in farm hens and nearly the same result recorded by **(Nys and Sauveur, 2004)** sodium, chloride and potassium in mg/100gm egg contents without shell were 155, 175 and 140, respectively.

And slight decrease compared with **FAO (2010)** that recorded whole chicken egg potassium value was 126 mg/100gm egg contents.

No record was found for hematological changes or electrolyte levels in case of *Y. enterocolitica* or *Pseudomonas aeruginosa* in laying hens or for duck eggs electrolyte.

The public health hazard of *P. aeruginosa* has

been stated, since its implication in nosocomial diarrhea occurred in Calcutta, where the eggs were among the source of infection **(Das *et al*., 1996)**.

From the mentioned results, it is clearly evident that *Y.enterocolitica* and *P. aeruginosa* recovered from the examined egg samples should be considered a public health hazard concern. These pathogens may thrive from shells or contents into other egg products, grow and multiply sufficiently to the risk level of food poisoning .

Recommendation for prevention of their risk include, proper hygienic measure should be adopted in poultry farms side by side with educational program for the employee , and for the public to know how to handle and store egg or egg products . Likewise, the practice of cleaning eggs by removal of dirt and faecal matter, washing by sanitizing solution is recommended and is common in egg industry. Moreover, pasteurization of egg products must be adopted as a statutory requirement.

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اليرسينيا انتيروكوليتيكا والسيدوموناس ايروجينوزا فى البيض المسوق فى محافظة سوهاج

فيصل عبد اللطيف واصل , نهال مكرم عوض، عزه محمود مصلح

معهد بحوث صحة الحيوان - معمل بيطرى سوهاج

الملخص العربى

يعتبر البيض ذو قيمته غذائيه عالية ألا انه قد يصبح مصدرا لا يستهان به لنقل العديد من الامراض التى تهدد صحه الانسان و ذلك لانه عرضه للتلوث بانواع مختلفه من الميكروبات الممرضه و المفسده و التى تصل اليه من مصادر متعدده منذ انتاجه و اثناء توزيعه حتى يصل الى المستهلك . لذلك قمنا بجمع 300 عينه من بيض الفراخ البلدى و بيض فراخ المزارع ممثله فى 100مجموعه (لكل نوع) جمعت عشوائيا من اسواق مدينه سوهاج و محلات البقاله و المزارع . و قد تم فحص البيض بكتريولوجيا لمعرفه مدى تلوث قشر البيض من الخارج وكذلك محتوياته بميكروبى اليريسينا انتيروليتكا و السيدوموناس ايروجينوزا . و لقد تبين من الفحص ان ميكروب اليريسينا انتيروليتكا تواجد فى عدد 6عينات من محتويات بيض الفراخ البلدى بنسبه 15% و امكن عزله من 3عينات من على قشر ذلك البيض بنسبه 7.5% بينما لم يتم عزل الميكروب من بيض فراخ المزارع سواء من المحتويات او من على القشر وتم عزل ميكروب اليريسينيا من قشر بيض البط (عدد3) بنسبه 10% ومن محتويات البيض (عدد 6) بنسبه 20% . اما بالنسبه لميكروب السيدوموناس ايروجينوزا فقد تم عزل الميكروب من عدد 2عينه من قشر بيض فراخ المزارع بنسبه 6.7%و كذلك من عدد2 عينه من المحتويات بنفس النسبه , بينما لم يتم عزل الميكروب من محتويات او قشر بيض الفراخ البلدي وتم عزل الميكروب بالبط بنفس النسبة من علي قشرة البيض وعدد 4 من محتويات البيض بنسبة 13.3 %. وتم عمل عد الدم الكلى و النوعى للطيور مصابه البيض و اجريت تحاليل قياس الصوديوم و البوتاسيوم و الكلوريد من هذه الطيور وسجلت النتائج . و لقد ناقش البحث اسباب التلوث بهذين الميكروبين و خطوره تلوث منتجات البيض اثناء التصنيع و الخطر الذى قد يشكله هذا على صحه الانسان , كذلك الطرق الواجب اتباعها لمنع تلوث البيض بهما.