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Prevalence of some enteric pathogens in table eggs with special reference to *E. coli* O157: H7

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The present investigation aims to study the incidence of some enteric pathogens in table eggs with special references to *E. coli* O157: H7. A total of 250 table egg samples (75 Baladi hen's, 75 white farm hen's, 75 brown farm hen's, and 25 duck eggs) were collected randomly from poultry farms, groceries, supermarkets, and street vendors in El Fayoum city, Egypt. Each Baladi hen's egg sample is represented by five eggs, while each farm hen's and duck egg are represented by three eggs. The samples were analysed for the presence of coliforms, faecal coliforms, *E. coli*, *E. coli* O157: H7, Shiga like toxin genes 1&2, *Salmonella typhimurium*, and *Yersinia enterocolitica*. The isolates were identified by biochemical, serological & molecular (PCR) methods. The obtained results in the present study showed that the examined samples of shells and contents of Baladi hens', poultry farms' (white and brown) and ducks' eggs were contaminated with coliforms with incidences of 25.33, 5.33, 1.33, 4.00, 5.33, 0.00, 0.00 and 2.66%, respectively, while faecal coliforms were in 8.0, 2.7, 0.0, 20.0, 0.0, 0.0, 0.0 and 0.0 %, respectively, *E. coli* was present in 2.7, 1.3, 0.0, 8.0, 0.0, 0.0, 0.0 and 0.0 %, respectively. Despite Shiga like toxin genes 1 &2 being found in the shells of Baladi hens' eggs and ducks' eggs, respectively, *E. coli* O157: H7 failed to be detected. Moreover, *Salmonella typhimurium* was isolated only in 4% of ducks' eggshells, while *Yersinia enterocolitica* failed to be isolated in this study. The highest rates of contamination were observed in ducks' and Baladi hens' eggs, while poultry farms' (white and brown) eggs were the best types and advised to be consumed. The potential health hazards and the proposed control measures for the isolated strains were discussed.

1. Introduction

Table eggs are consumed worldwide and are a reasonable choice as part of a healthy, balanced diet. Eggs are one of the most balanced and economical sources of protein. In addition, eggs contain all the vitamins and minerals needed for human beings except vitamin C. Fully mixed eggs contain 65% water, 12% protein and 11% fat (Jay et al., 2005).

Eggs own a natural defence system against contaminating microorganisms, such as cuticles, calcium hard shells, and shell membranes (Jerzy & Dagmara, 2009). The albumen proteins have antimicrobial properties, especially the lysozyme. Another proteinase is ovomucoid, which prevents bacteria from using the protein in albumen. Also, the pH in albumen is about

9–10, and the viscosity of the egg white is not suitable for microbial growth (Froning, 1998).

The egg can be contaminated with a variety of pathogens both on the eggshell and on the contents, such as Escherichia coli, *Yersinia enterocolitica*, and *Salmonella* (Ricke et al., 2001). Food poisoning related to egg-borne pathogens may cause severe morbidity or mortality with diarrhoea, vomiting, nausea, and abdominal cramps.

Coliforms are faecal bacteria that indicate some kind of faecal contamination of the food and give an index of poor sanitation. *E. coli* is one of the coliform bacteria that naturally inhabit the gastrointestinal tract of all warm-blooded animals. *E. coli* is commonly used as an indicator of faecal contamination of food, although most of its strains are not considered pathogenic (Willey et al., 2009). *E. coli* strains are enterohaemorrahagic, enteropathogenic, enterotoxigenic, enteroaggregative, shiga toxin-secreting, and haemolytic- diarrhea. Some strains can cause food poisoning due to their pathogenicity. Pathogenic strains such as O157:H7 are highly virulent and may have an infective dose as low as ten organisms (Foodborne Pathogenic Microorganisms & Natural Toxins Handbook 2009).

Shiga toxin-producing *E. coli* (STEC) is a bacterium that can cause serious foodborne diseases. In most cases, the illness is self-limiting but can progress to a life-threatening condition, including haemolytic uraemic syndrome (HUS), particularly in young children and the elderly. HUS is characterised by acute renal failure, haemolytic anaemia, and thrombocytopenia (low platelet count). STEC produces toxins known as Shiga-toxins (Stx1 & Stx2) because of their similarity to the toxins produced by Shigella dysenteriae. These are potent bacterial toxins that cause severe damage to the lining of the intestine and are also known as Vero toxins, or previously as Shiga-like toxins (Melton-Celsa, 2014).

STEC is destroyed by cooking the food thoroughly until the temperature of all parts reaches 70 °C or higher. Although *E. coli* O157: H7 is the most important STEC serotype in terms of public health, other serotypes have been implicated in sporadic outbreaks and cases.

Salmonellosis is a zoonotic infection transmitted to humans by contact with the bird itself or its eggs (Willey et al., 2009). Eggs can become contaminated by exposure to contaminants such as dust or droppings that are found in the nest or on the littered floor. Salmonellosis can cause gastrointestinal illness in humans (Perry, 2004).

Y. enterocolitica is a zoonotic enterobacterium that causes enterocolitis and other clinical manifestations in humans, including immunological signs (Bottone, 1997). Yersiniosis is often characterised by symptoms such as diarrhoea and gastroenteritis with vomiting. However, the hallmark symptoms are fever and abdominal pain. Yersinia infection mimics appendicitis and mesenteric lymphadenitis, but the bacteria can also cause infections in other sites such as joints, wounds, and the urinary tract. (Foodborne Pathogenic Microorganisms and Natural Toxins Handbook, 2009).

Eggshell quality is of primary importance to the egg industry worldwide. Eggshells need to be firmly intact throughout the chain from when the egg is laid until it is used by the consumer (Roberts, 2010). Cracks in the eggshells affect the quality, as eggs with cracks spoil faster than intact eggs (Gietema, 2005). Duck eggs are more highly contaminated than hen's eggs as they are laid near damp places and due to the rapid deterioration of the antibacterial activity of albumen by the unfavourable surroundings. Bahout (2001) studied the public health implications resulting from the consumption of duck's and hen's eggs.

Due to the risk of spreading diseases, hygiene is not only important for health and production performance but also for food safety (Vucemilo et al., 2010). The target of the present investigation was to study the incidence of some enteric pathogens in table eggs, with particular reference to *E. coli* O157: H7, which were collected from poultry farms, street vendors, groceries, and supermarkets located in Fayoum city, Egypt.

2. Materials and Methods

2.1. Collection of samples

A total of 250 table egg samples (75 Baladi hen 's, 75 white farm hen 's, 75 brown farm hen 's, and 25 duck

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eggs) were collected randomly from poultry farms, groceries, supermarkets, and street vendors in El Fayoum city, Egypt. Each Baladi hen's egg sample is represented by five eggs, while each farm hen's and duck eggs are represented by three eggs. Each sample was put in a sterile plastic bag and immediately taken to the laboratory, where they were prepared and examined microbiologically.

2.2. Preparation of samples: as described by Wehr, H. M., & Frank, J. F. (2012)

Eggshell: The eggshell was washed by a surface rinse method.
Egg content: The eggs were prepared to evacuate their contents.

2.3. Microbiological examination

2.3.1. Enumeration of total coliform count: (Most Probable Number)

This was done using lauryl sulphate tryptose broth (LST) with inverted Durham 's tubes according to (Wehr, H. M., & Frank, J. F. 2012).

2.3.2. Enumeration of faecal coliform count (Most Probable Number)

A loopful from each LST-positive broth was inoculated into sterile tubes of *E. coli* broth (EC broth). The inoculated and control tubes were incubated in a thermostatically controlled water bath at 44.5 °C for 48 h. Positive tubes showing gas production were recorded according to Wehr, H. M., & Frank, J. F. 2012.

2.3.3. Enumeration, isolation, biochemical identification and serology of Escherichia coli of true faecal type

A loopful from each EC-positive broth tube was streaked onto Eosin Methylene Blue (EMB) (Oxoid, Ltd, Basingstoke, UK). The inoculated and control plates were incubated at 35+1 °C for 24 hrs. The plates were examined for the presence of typical nuclear colonies with a dark centre and a green metallic sheen. Positive EMB plates for *E. coli* were recorded. The numbers of Escherichia coli/ml. or gm. were calculated after the IMViC pattern from the most probable number (MPN) tables for the three-tube method. Agar slants were prepared from EMB for further biochemical identification (Wehr, H. M., & Frank, J. F. , 2012).

The serological characterization of *E. coli* isolates by the slide agglutination method was performed using polyvalent and monovalent antisera. The isolates were tested first with OK polyvalent antisera. Substantially, two separate glass slides were used. A saline solution was added to the slide glass, followed by some of the colonies from the suspicious culture, mixed to form a smooth, dense suspension. To the first glass slide (control), only a drop of saline was added and mixed. To the second, an undiluted antiserum was added and then tilted forward and backward for one minute. Agglutination was noticed using indirect lighting over a dark background. When a colony agglutinated strongly positive with one of the polyvalent serum pools, a further part was inoculated onto a nutrient agar slant (Oxoid, Ltd, Basingstoke, Hampshire, UK) and incubated at 37°C for 24 hours to grow as a culture before testing with O monovalent antisera for serogroups O26, O44, O86, O111, O114, O126, O142, O157 and O158. The strains were members of the same serogroups and isolated from the same samples were reported only once. Positive control strains gained from the Animal Health research institute, Dokki, Giza, Egypt, were involved in each experimental run.

2.3.4. Isolation of Escherichia coli O157:H7

Twenty-five ml. /gm. of each sample was separately homogenised with 225 ml of modified tryptone soy broth supplemented by Novobiocin (20 mg / l) for 2 minutes using a sterile homogenizer (universal Laboratory Aid, Poland). The inoculated broth was incubated at 37 °C for 24 hours. A loopful from the incubated broth was streaked onto a Tellurite Cefixime Sorbitol MacConkey agar plate and incubated at 37 °C for 24 hours. Sorbitol-negative colonies (colourless) were picked up and purified, and then examined biochemically and serologically (De-Boer & Heuvelink, 2000).

2.3.5. Molecular identification of Shiga-Like Toxins (Stx1&Stx2)

2.3.5.1. Extraction of DNA

DNA was extracted using QIAamp DNA Mini K (Qia-

gen, Hilden, Germany). Briefly, 1.5 ml of an overnight broth culture of *E. coli* grown in MacConke broth at 37°C was centrifuged at 8000 rpm for 5 min and the supernatant was discarded. The cell pellet was resuspended in phosphate-buffered saline (PBS) to a final volume of 200 ml. Twenty ml of QIAGEN protease were put into the bottom of a 1.5 ml microcentrifuge tube, then 200 ml of the sample followed by 200 ml of buffer A were added and mixed by pulse vortexing for 15 seconds. The mixture was then incubated at 56˚Cfor 10 min and centrifugated to remove droplets from inside the lid. Then 200 ml of ethanol (96%) was added and mixed again for 1 second. After that, centrifugation was done to discard droplets from inside the lid. The mixture was gently applied to the QIAamp Mini spin column (in a 2 ml collecting tube) for DNA extraction. The DNA concentration was weighted using a spectrophotometer (DU530, Beckman, CA). An average of 10 mg of DNA was gained.

2.3.5.2. Cycling conditions of the primers during PCR

The Stx1 and Stx2 genes for *E. coli* were amplified by duplex PCR as described by Dipineto et al. (2006). 6 ml of template DNA was tested in a reaction mixture containing 25 µL of Emerald Amp GT PCR master mix (2x premix), 15 µL of PCR grade water, and 1 ml of both forwarding and reverse primer (20 pmol) according to Emerald Amp GT PCR master mix (Takara), code number RR310AKit. The primary denaturation for Stx1 and Stx2 was for 5 min at 94°C followed by 35 cycles at 94°C for the 30 s, 58°C for 40 s, then 72°C for 45 s, and a final extension at 72°C for 10 min. Twenty ml of the reaction product were subjected to running gel electrophoresis in a 1.5% agarose gel (AppliChem, Ottoweg 4, Darmstadt, Germany) at 1–5 volts/cm of the tank length for 30 min, and the gel was sent to a UV cabinet and photographed using a gel documentation system. The data were analysed using the computer software Automatic Image Capture Software, Protein Simple formerly Cell Biosciences, the USA at the reference lab for veterinary quality control on poultry production, Animal health research institute, Dokki, Giza, Egypt (Sambrook et al., 1989).

2.3.6. Isolation, identification, and serology of *Salmonella typhimurium*

Twenty-five ml./gm. of prepared samples, both of eggs rinsing solution and homogenous eggs contents, were added septically to 225 ml of sterile buffered peptone water and incubated at 37 °C for 24 ±2 hours, one ml of the incubated pre-enriched broth was inoculated into 10 ml Rappaport Vassiliadis broth tube, after that the tube was incubated at 41.5 ± 0.5 °C for 24 hours. Loops from the inoculated tubes were streaked separately onto Xylose lysine deoxycholate agar (XLD) agar medium and incubated at 37 °C for 24 hrs. Suspected colonies were red with or without black centres. The suspected colonies were sub-cultured onto a nutrient agar plate and incubated at 37 °C for 24 hours. The purified isolates were identified morphologically, biochemically (IMViC, Urea hydrolysis and Triple sugar iron agar) and serologically by using polyvalent group and specific antisera for the determination of somatic (O) and flagellar (H) antigens at the serology unit, Animal Health Research Institute, Ministry of Agriculture; Dokki, Giza, Egypt (FDA, 2010).

2.3.7. Isolation of *Yersinia enterocolitica*

Twenty-five ml/gm of samples was transferred aseptically to 225 ml tryptic soy broth (TSB, Biolife, 1996), mixed thoroughly and incubated at 22 °C for 24 hours (Scheimann & Wauters, 1992). Then loops from the incubated broth were streaked directly onto Cefsulodin- Irgasan-Novobiocin (CIN) agar media plates (Difco, 1997) and were incubated at 25 °C for 48 hours. The colonies of *Yersinia enterocolitica* having a characteristic appearance (bull-eye like, dark red centre surrounded by a translucent zone) were picked and streaked onto Trypticase Soy agar (TSA) slants and incubated at 25 °C for 24 hours for further identification (morphologically, motility test and biochemically as Urease test and sugar fermentation reaction) (Walker & Glimour,1986).

3. Results

3.1. Statistical analytical results and frequency distribution of coliform counts.

Table 1 & Figure 1 show that coliforms were found in 19 (25.33%) of Baladi hens' eggshells samples in the counts ranging from 4 CFU / ml to 4.3×10 CFU / ml with a mean count of 1.34×10 CFU / ml and the highest frequency distribution, 10 (52.6%), lies within

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the range of 3- < 10 CFU/ ml., while coliforms were detected in 4 (5.33%) of Baladi hens' egg contents in counts ranging from 4 CFU/gm to 9 CFU/gm with a mean count of 6 CFU/gm with a frequency distribution of 100% that lies within the range of 3- < 10 CFU/ gm. Four(5.33%) of the examined white poultry farms' eggshells were contaminated with coliforms in counts ranging from 4 CFU/ml to 2.1×10 CFU/ml with a mean count of 1.03×10 CFU/ml and the highest frequency distribution of 3(75%) that lies in the range of 3 -< 10 CFU/ml, while one (1.33%) of the examined brown poultry farms' eggshells were contaminated with coliforms with a mean count of 7 CFU/ml and the highest frequency distribution 1(100%) of positive samples lies between 3 -< 10 CFU/ml, while coliforms couldn't be detected from all samples of poultry farms' egg contents. Concerning duck egg samples, coliforms were found in 11 (44%) of ducks' eggshells samples with the counts ranging from 9 CFU/ml to 1.5×10^2CFU/ml with a mean count of 5.03×10 CFU/ml with the highest frequency distribution of positive samples was 8 (72.7%) that lies between 10- < 10 2 CFU/ml, while coliforms were detected in 2 (8 %) of ducks' egg contents in counts ranging from 7 CFU/gm to 9 CFU/gm with a mean count of 8 CFU/gm with the highest frequency distribution (100%) that lies between 3 -< 10 CFU/gm.

3.2. Statistical analytical results and frequency distribution of faecal coliform count.

Table 2 & Figure 2 illustrate that faecal coliforms were found in 6 (8%) of Baladi hens' eggshells samples in counts ranging from 4 CFU/ml to 9 CFU/ml with a mean count of 6.7 CFU/ml and 100% of the positive samples contained faecal coliforms within the range of 3 -< 10 CFU/ml. Faecal coliforms were found in 2 (2.7%) of white poultry farms' eggshells samples in counts ranging from 4 CFU/ml to 7 CFU/ml with a mean count of 5.5 CFU/ml. 100% of the positive

Table1: Statistical analytical results of coliform count in the examined samples of shells and contents of Baladi, poultry farm (white and brown), and duck eggs:

Examined samples	No. of examined samples	Positive samples		Minimum	Maximum	Mean	± SEM
		No.	%				
Baladi hens' eggshells	75	19	25.33	4	4.3×10	1.34×10	2.23
White poultry farms' eggshells	75	4	5.33	4	2.1×10	1.03×10	3.73
Brown poultry farms' eggshells	75	1	1.33	7	7	7	0
Ducks' eggshells	25	11	44	9	1.5×10^2	5.03×10	1.65×10
Baladi hens' egg contents	75	4	5.33	4	9	6	1.23
White poultry farms' egg contents	75	0	0	0	0	0	0
Brown poultry farms' egg contents	75	0	0	0	0	0	0
Ducks' egg contents	25	2	8	7	9	8	1

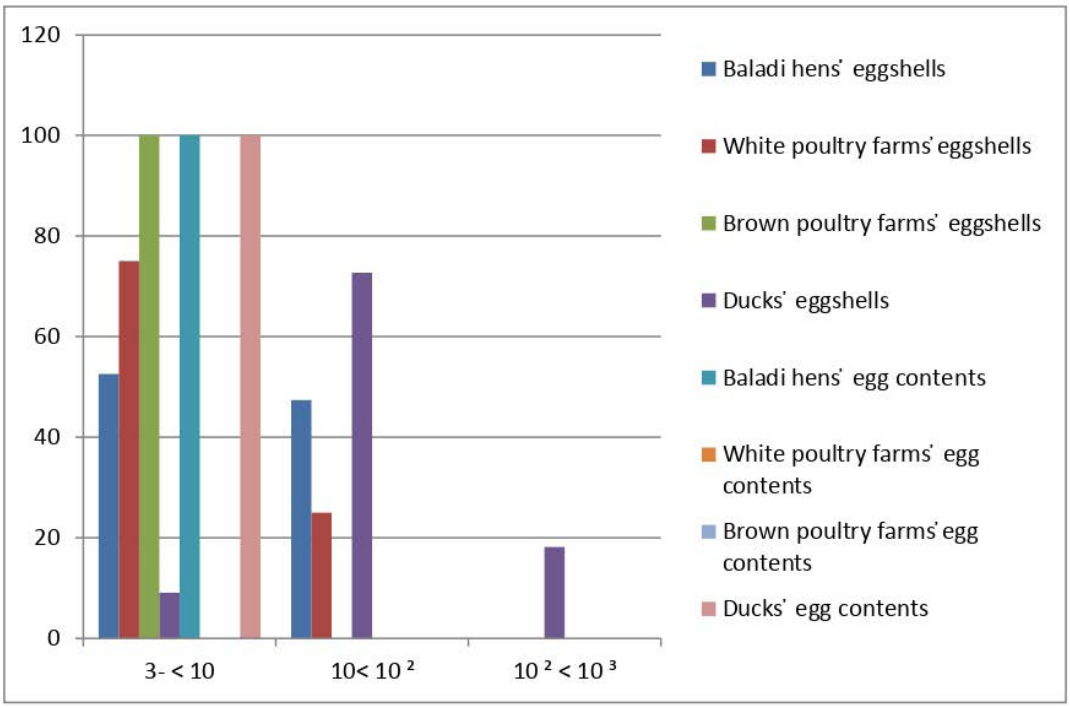

Figure 1. Frequency distribution of coliform count in the examined samples of shells and contents of Baladi, farm (white and brown), and duck eggs.

samples were contaminated with faecal coliforms within the range of 3 -< 10 CFU/ml. However, no faecal coliforms were detected in brown poultry farms ' eggshells, whereas 5(20%) of the examined ducks ' eggshells samples were contaminated with faecal coliforms in the counts ranging from 4 CFU/ml to 23CFU /ml with a mean count of 12.6 CFU/ml. From the positive samples, 3(60%) were found in the range of 3 -< 10 CFU/ml., and 2 (40%) within 10- < 10 2 CFU/ml. On the other hand, faecal coliforms couldn't be detected from all examined content samples of Baladi hens', poultry farms', and ducks 'eggs.

3.3. Statistical analytical results and frequency distribution of *E. coli* count.

Table 3 & Figure 3 show that *E. coli* was found in Baladi hens' eggshells at an incidence of two (2.7%) in counts ranging from 7 CFU/ml to 9 CFU/ml with a mean count of 8 CFU/ml. All the positive samples, two (100%), were found in the range of 3 -< 10 CFU/ml, but only one (1.3%) *E. coli* isolate was found in white Poultry farm 's eggshell sample, which lies within the range of 3 -< 10 CFU/ml with a mean count of 7 CFU/ml, while *E. coli* was detected in two (8%) of examined ducks ' eggshells samples in counts ranging from 2×10 CFU/ml to 2.3×10 CFU/ml with a mean count of 2.15×10 CFU/ml and the highest frequency distribution two (100%) lies between the range 10- < 10 2 CFU/ml.

On the other hand, *E. coli* couldn't be found in brown poultry farms ' eggshells and all examined content samples of Baladi hens', poultry farms', and duck eggs.

3.4. Molecular identification of Shiga-like toxins (Stx1 & Stx2) from the recovered *E. coli* strains of the examined egg samples.

It is apparent in Figure 4, PCR results for Shiga like toxins 1 & 2 genes. Stx1 (614 bp) was found in one Baladi hen's eggshell sample, and Stx2 (779 bp) was detected in one duck' s eggshell.

3.5. Incidence of *Salmonella typhimurium* and *Yersinia enterocolitica* in the examined samples.

Table (4) shows that only one (4%) isolate was identified as S. typhimurium, which was isolated from a duck's eggshell sample, while S. typhimurium couldn't be detected in Baladi hens' and poultry farms' eggshells and contents samples, no S. typhimurium was found in ducks' egg contents samples. *Yersinia enterocolitica* was not found in all the samples tested.

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Table 2. Statistical analytical results of faecal coliform count in the examined samples of shells and contents of Baladi, poultry farm (white and brown), and duck eggs:

Examined samples	No. of examined samples	Positive samples		Minimum	Maximum	Mean	± SEM
		No.	%				
Baladi hens' eggshells	75	6	8	4	9	6.7	0.92
White poultry farms' eggshells	75	2	2.7	4	7	5.5	1.5
Brown poultry farms' eggshells	75	0	0	0	0	0	0
Ducks' eggshells	25	5	20	4	23	12.6	3.8
Baladi hens' egg contents	75	0	0	0	0	0	0
White poultry farms' egg contents	75	0	0	0	0	0	0
Brown poultry farms' egg contents	75	0	0	0	0	0	0
Ducks' egg contents	25	0	0	0	0	0	0

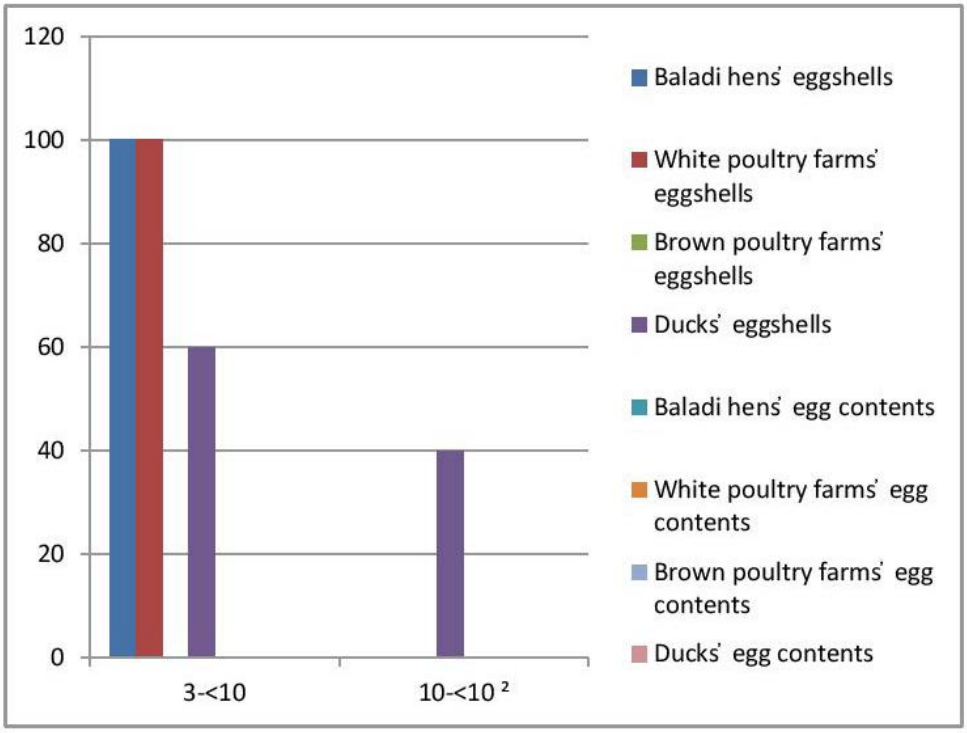

Figure 2. Frequency distribution of faecal coliform count in the examined samples of shells and contents of Baladi, poultry farm (white and brown), and duck eggs samples.

Table 3. Statistical analytical results of *E. coli* count in the examined samples of shells and contents of Baladi, poultry farm (white and brown), and duck eggs

Examined samples	No. of examined samples	Positive Samples		Minimum	Maximum	Mean	± SEM
		No.	%				
Baladi hens' eggshells	75	2	2.7	7	9	8	1
White poultry farms' eggshells	75	1	1.3	7	7	7	0
Brown poultry farms' eggshells	75	0	0	0	0	0	0
Ducks' eggshells	25	2	8	2×10	2.3×10	2.15×10	1.5
Baladi hens' egg contents	75	0	0	0	0	0	0
White poultry farms' egg contents	75	0	0	0	0	0	0
Brown poultry farms' egg contents	75	0	0	0	0	0	0
Ducks' egg contents	25	0	0	0	0	0	0

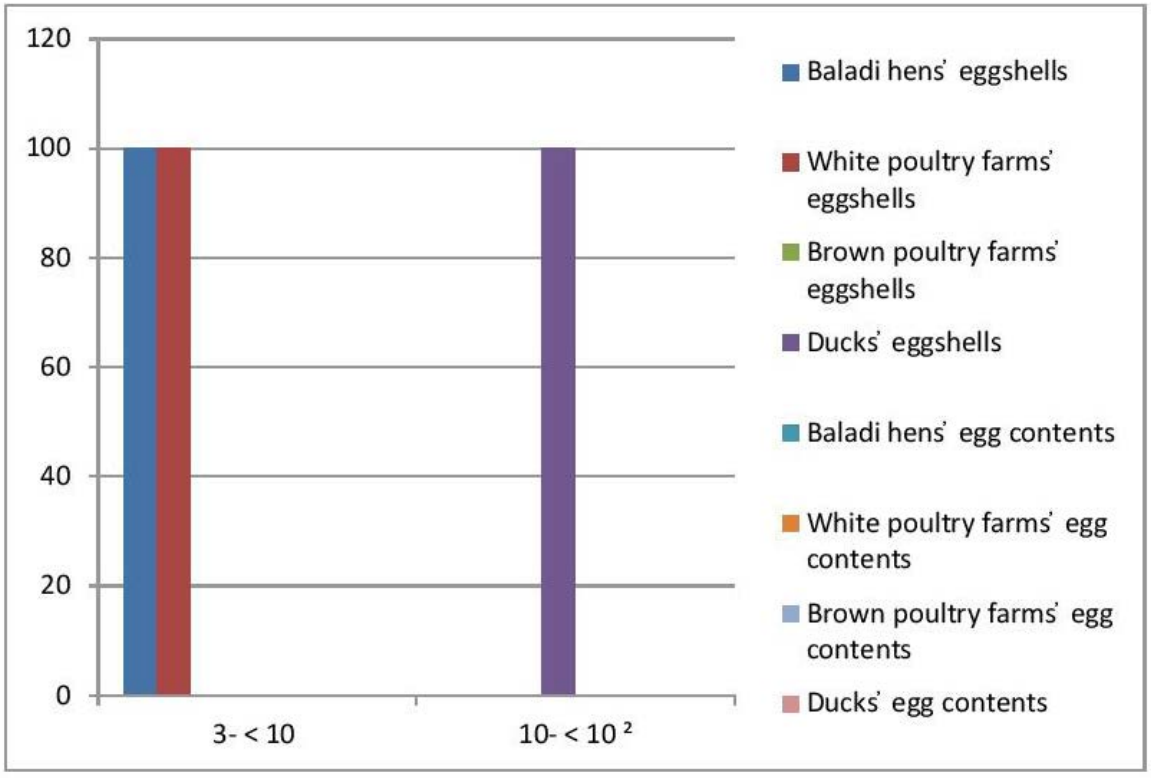

Figure 3. Frequency distribution of *E. coli* count in the examined samples of shells and contents of Baladi, farm (white and brown), and duck's eggs.

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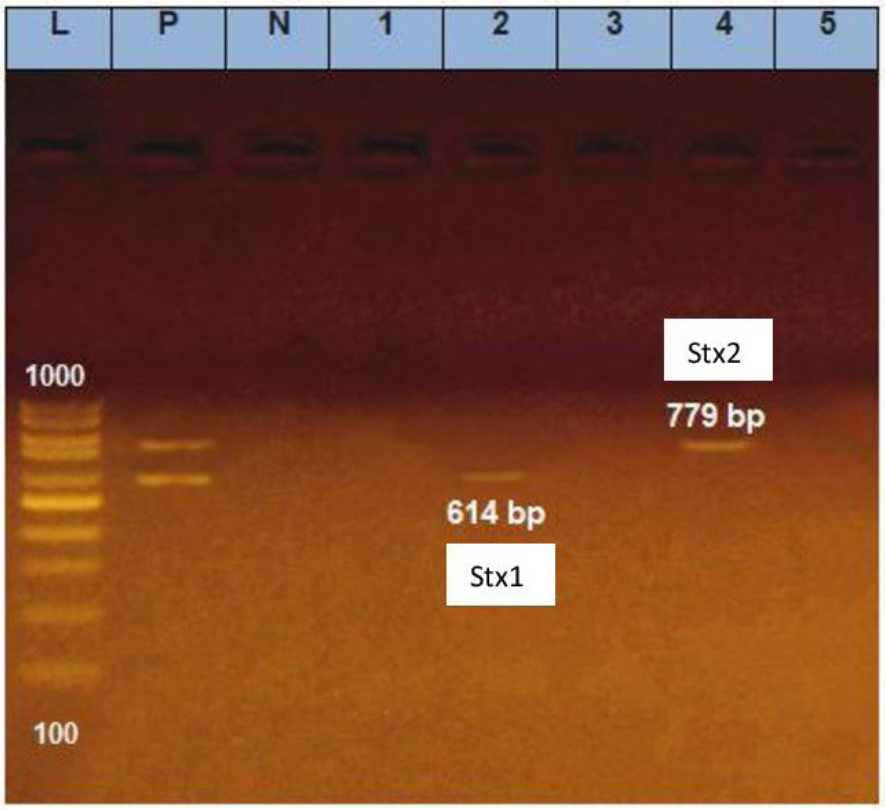

Figure 4. PCR results for Shiga-like toxins 1 & 2 genes, Stx1 (614 bp) and Stx2 (779 bp) from *E. coli* strains. Lane L: DNA ladder, Lane Pos.; control +ve, Lane Neg.; control –ve, Lane 2 (+ve Stx1) and Lane 4 (+ve Stx2).

Table 4. Incidence of *Salmonella typhimurium* & *Yersinia enterocolitica* in the examined samples of shells and contents of Baladi, poultry farm (white and brown), and duck eggs:

Examined samples	No. of examined samples	*Salmonella typhimurium*		*Yersinia enterocolitica*	
		No. of positive samples	%	No. of positive samples	%
Baladi hens' eggshells	75	0	0	0	0
White poultry farms' eggshells	75	0	0	0	0
Brown poultry farms' eggshells	75	0	0	0	0
Ducks' eggshells	25	1	4	0	0
Baladi hens' egg contents	75	0	0	0	0
White poultry farms' egg contents	75	0	0	0	0
Brown poultry farms' egg contents	75	0	0	0	0
Ducks' egg contents	25	0	0	0	0
Total	500	1	0.2	0	0

4. Discussion

4.1. Coliform counts

4.1.1. Baladi hens' eggs

Results recorded in Table 1& Figure 1, showed that coliforms were found in 19 (25.33%) Baladi hens' eggshells samples with a mean count of 1.34×10 CFU/ml and the highest frequency distribution, 10 (52.6%), lies within the range of 3- < 10 CFU/ ml.

Higher results were recorded by El-Leboudy & El-Mossalami (2006), Refaat (2009), El-Kholy (2014), Sadek et al. (2016), and El-Kholy et al. (2020), and lower results were obtained by Bahobail et al. (2012). On the other hand, coliforms could be detected in 4(5.33%) of Baladi hens 'egg contents with a mean count of 6 CFU / gm and a frequency distribution of 100% that lies in the range of 3- < 10 CFU/gm. Higher incidences were reported by El-Leboudy & El-Mossalami (2006), El-Kholy (2014), and Sadek et al. (2016). Moreover, our result is nearly similar to that obtained by El-Kholy et al. (2020), while Refaat (2009) couldn't find coliforms in Baladi hens' egg contents.

4.1.2. Poultry farms' eggs

According to the findings in Table 1 & Figure 1, 4(5.33%) of the examined white poultry farms' eggshells were contaminated with coliforms with a mean count of 1.03×10 CFU/ml with the highest frequency distribution of 3(75%) that lies in the range of 3 -< 10 CFU/ml, while 1(1.33%) of the examined brown poultry farms ' eggshells were contaminated with coliforms with a mean count of 7 CFU / ml. The highest frequency distribution 1(100%) of positive samples lies between 3 -< 10 CFU/ml.

Higher results of 33.3, 37.1, 30, 47.06, and 10% were estimated by El-Leboudy & El-Mossalami (2006), Refaat (2009), El-Leboudy et al. (2011), El-Kholy et al. (2014), and Sadek et al. (2016), respectively. A slightly lower result (4%) was found by El-Kholy et al. (2020). On the other hand, coliforms couldn't be detected from all samples of poultry farm's egg contents. Similar results were obtained by Refaat (2009), Sadek et al. (2016), and El-Kholy et al. (2020), while higher results were detected by El-Leboudy & El-Mossalami (2006), and El-Kholy et al. (2014).

4.1.3. Ducks ' eggs:

From the data presented in Table 1 & Figure 1, coliforms were detected in 11(44%) of ducks ' eggshell samples with a mean count of 5.03×10 CFU / ml. The highest frequency distribution of positive samples was 8 (72.7%) which lies between 10- < 10 2 CFU/ml. A lower result was obtained by Refaat (2009).

On the other hand, coliforms were detected in 2 (8 %) of ducks ' egg contents with a mean count of 8 CFU / gm, and the highest frequency distribution (100%) lies between 3 -< 10 CFU/gm. Higher results were found by El-Leboudy & El-Mossalami (2006) and Awny et al. (2018), while Refaat (2009) couldn't find coliform organisms in ducks egg contents.

Coliforms are an intestinal and non-intestinal inhabitant, so coliform count is a traditional indicator of faecal contamination, microbial quality, and reflects food hygiene standards (Musgrove et al., 2008). The existence of coliforms in Baladi hens 'eggs and ducks eggs is an indicator of poor hygiene. Therefore, eggs that contain a high percentage of coliforms are of economic and public health importance (Sabreen, 2001), while the lower rate of contamination of poultry farm eggs is due to the egg cleaning process before marketing and its hygienic handling. Also, eggshell surface disinfection has an important role in preventing egg spoilage and egg-related diseases (De Reu et al., 2006).

4.2. Faecal coliform counts

4.2.1. Baladi hens' eggs

As recorded in Table 2 & Figure 2, faecal coliforms were found on 6(8%) of Baladi hens' eggshells samples, and 100% of positive samples contained faecal coliforms with a mean count of 6.7 CFU / ml within the range of 3 -< 10 CFU/ml. Our results disagreed with the results estimated by Refaat (2009), El-Kholy (2014), and Sadek et al. (2016), who detected faecal coliforms with higher as 22.9, 57.1, and 73.3%, respectively.

On the other hand, faecal coliforms couldn't be detected from all samples of Baladi hens' egg contents.

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This result was in harmony with those obtained by Refaat (2009), who mentioned that faecal coliforms couldn't be detected from all samples of Baladi hens 'egg contents, while higher incidences were estimated by El-Kholy (2014) and Sadek et al. (2016).

4.2.2. Poultry farms' eggs

According to the data reported in Table 2& Figure 2, faecal coliforms were found in 2 (2.7%) of the white poultry farms' eggshells samples with a mean count of 5.5 CFU/ml. 100% of the positive samples contained faecal coliforms within the range of 3 -< 10 CFU/ml, while no faecal coliforms were detected in brown poultry farms' eggshells.

Higher incidences of 11.4, 20.59, and 6.7 % were estimated by Refaat (2009), El-Kholy et al. (2014), and Sadek et al. (2016), respectively.

On the other hand, faecal coliforms couldn't be detected in the poultry farms ' egg contents in this study. Our results agreed with those reported by Refaat (2009), and Sadek et al. (2016), while faecal coliforms were detected in 20.59% of poultry farms 'egg contents by El-Kholy et al. (2014).

4.2.3. Ducks' eggs

The summarised results in Table 2 & Figure 2 showed that 5 (20%) of the examined ducks' eggshell samples were contaminated with faecal coliforms with a mean count of 12.6 CFU/ml. From the positive samples, 3 (60%) were found in the range of 3 -< 10 CFU/ml., and 2 (40%) within 10- < 10 2 CFU/ml.

A lower result of 11.4% was recorded by Refaat (2009). Regarding the examined ducks ' egg contents samples, no faecal coliforms were detected, and this result agreed with that estimated by Refaat (2009).

4.3. *E. coli* counts

4.3.1. Baladi hens' eggs

The results presented in Table 3 & Figure 3 revealed that *E. coli* was found in Baladi hens' eggshells in an incidence of 2 (2.7%) with a mean count of 8 CFU/ml. All the positive samples, 2 (100%), were found in the range of 3 -< 10 CFU/ml. Higher results of 32, 42.8, 44, and 53.3 were recorded by Al-Ashmawy (2013), El-Kholy (2014), Ibrahim et al. (2014) and Sadek et al. (2016), respectively, while *E. coli* couldn't be detected by Refaat (2009).

On the other hand, in our study, *E. coli* couldn't be found in Baladi hens' egg contents samples. A similar result was recorded by Refaat (2009). Higher incidences of 23, 19, and 6.7% were found by Al-Ashmawy (2013), Ibrahim et al. (2014), and Sadek et al. (2016), respectively.

4.3.2. Poultry farms' eggs

Table 3 & Figure 3 show that only 1(1.3%) *E. coli* isolate was found in a white poultry farm's eggshell sample with a mean count of 7 CFU/ml, which lies within the range of 3 -< 10 CFU/ml, whereas *E. coli* couldn't be isolated from brown farms ' eggshells samples and all samples of farms ' egg contents.

Concerning poultry farms' eggshells, higher incidences of 5.7, 14.71, 27.5, and 6.7 % were reported by Refaat (2009), El-Kholy et al. (2014), Ibrahim et al. (2014), and Sadek et al. (2016), respectively.

Regarding the farms' egg content samples, many authors failed to isolate *E. coli* as Refaat (2009), Al-Ashmawy (2013), El-Malt (2015), and Sadek et al. (2016), while El-Kholy et al. (2014) detected *E. coli* in 11.76%.

4.3.3. Ducks' eggs

E. coli was detected in 2 (8%) of examined ducks eggshells samples with a mean count of 2.15×10 CFU / ml as reported in Table 3, and the highest frequency distribution, as shown in Figure 3, 2 (100%), lies between the range 10- < 10 2 CFU/ml. A lower incidence of 5.7% was recorded by Refaat (2009).

Concerning ducks ' egg contents, *E. coli* couldn't be isolated from all examined samples. A higher result was obtained by El-Leboudy & El-Mossalami (2006). According to Tables (2&3), no faecal coliforms and *E. coli* were found in all examined samples of contents of Baladi, poultry farm hens, and ducks 'eggs. This may be due to the internal antimicrobial defence mechanisms of eggs and the use of antibiotics in farms.

Serological identification of the suspected *E. coli* isolates was done. Moreover, molecular identification of Shiga-like toxins (Stx1 & Stx2) from the recovered *E. coli* strains of the examined egg samples was done. It is apparent in Figure 4, PCR results for Shiga-like toxins 1 & 2 genes, Stx1 (614 bp) was found in one Baladi hen's eggshell sample, and Stx2 (779 bp) was detected in one duck's eggshell.

Shiga toxin-producing *E. coli* (STEC) can cause intense foodborne illnesses and haemolytic uraemic syndrome (HUS), which is characterised by acute renal failure, haemolytic anaemia, and thrombocytopenia (low platelet count). Although *E. coli* O157:H7 is the most critical STEC serotype in terms of public health, other serotypes have been implicated in sporadic cases and outbreaks.

In the current study, *E. coli* O157:H7 failed to be detected.

Generally, the presence of *E. coli* in eggs is an excellent indicator of faecal pollution and the presence of some enteric pathogens, which may lead to foodborne infection and intoxication. It constitutes a public health hazard to humans and a significant economic menace to the poultry industry (Quiroga et al., 2000).

4.4. Isolation of *Salmonella typhimurium*

4.4.1. Baladi hens' eggs

As shown in Table (4), S. typhimurium couldn't be detected in Baladi hen's eggshells and contents samples. Higher results were reported by Refaat (2009) and El-Kholy (2014) for eggshells and contents.

4.4.2. Poultry farms' eggs

It is evident from the results recorded in Table (4), that S. typhimurium couldn't be detected in all examined poultry farms' eggshells and contents samples. Several investigators failed to isolate *Salmonella* Spp. from table eggs, such as El-Kholy et al. (2014), Awny et al. (2018), and Mahdavi et al. (2012). This result in poultry farms' eggs may return to the strict hygienic measures applied in egg production and prophylactic treatment against pathogens. Also, the use of probiotics in the ration of layer poultry farms to establish beneficial gut microflora may reduce colonisation by pathogenic organisms like *Salmonella* by competitive exclusion. It represents a potential risk to consumers because all *Salmonella* are potentially pathogenic (Kabir, 2009).

4.4.3. Ducks' egg

In the present study, 1 (4%) isolate was identified as S. typhimurium as shown in Table (4), which was isolated from ducks' eggshell samples. The presence of *Salmonella* on eggshells indicates contamination with duck faeces. Our result is nearly similar to the incidence of 4.29% that was estimated by Harsha et al. (2011), while higher values were demonstrated by Korashy et al. (2008) and Suksangawong (2008). However, Adzitey et al. (2012), and Sedeek & Aioub (2014) couldn't detect *Salmonella* in all of the examined ducks' eggshell samples.

On the other hand, it couldn't be detected in ducks' egg contents in our study. Also, Sedeek & Aioub (2014) failed to detect *Salmonella* in ducks' egg contents. Ducks' eggs were associated with S. typhimurium outbreaks in Germany between 1974 and 1996 (Rabsch et al., 2002).

4.5. Isolation of *Yersinia enterocolitica*

4.5.1. Baladi hens' eggs

As recorded in Table (4), Y. enterocolitica failed to be detected in our study from both the shells and contents of Baladi hen's eggs. A higher result was obtained by Abdel-Haleem & Ali (2005), who isolated the pathogen in incidences of 6.7% and 20% from eggshells and contents, respectively.

4.5.2. Poultry farms' eggs

In the present study, as reported in Table (4), we failed to isolate Y. enterocolitica from the examined samples of both shells and contents of poultry farm hens' eggs (white and brown). Also, other investigators couldn't isolate Y. enterocolitica from poultry farm hens' eggs (shells and contents), such as Abdel-Haleem & Ali (2005), while Favier et al. (2005) found Y. enterocolitica at a percentage of 2.27% on eggshells.

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4.5.3. Ducks' eggs

From Table (4), it is clear that no Y. enterocolitica was found in all examined samples of both shells and contents of ducks' eggs, while the investigation carried out by Korashy et al. (2008) pointed out that Y. enterocolitica could be detected in 10% and 6.7% of eggshells and contents, respectively.

5. Conclusion

This study revealed that Baladi hens' and ducks' eggs have a higher microbial load than poultry farm hens' eggs. There are higher incidences of coliforms, faecal coliforms, and *E. coli* organisms in Baladi hens' and ducks' eggs than those from poultry farm hens' eggs, and the presence of S. typhimurium on ducks' eggshells.

Duck eggs contain a relatively high contamination percentage as they lay their eggs nearer to damp places (ponds) with high moisture and pick up flies and other infective materials. On the other hand, the antibacterial activity of their egg albumen (Con albumen) deteriorates rapidly on storage, and the eggshell is thinner than that of a hen's egg (Burley & Vadehra, 1989).

Therefore, hygienic measures should be applied to home-produced hens and ducks to lower the bacterial load in their eggshells and subsequently in their egg contents. In addition, strict sanitary measures should be implemented in farms to safeguard egg consumers from infection and to save eggs from deteriorating. Also, egg preservation, handling, and distribution should be done with care. Thorough cooking and preparation of eggs and egg-containing foods should be applied to safeguard human beings from being infected with pathogenic organisms.

Future recommendations include the following: Routine microbiological screening, control programs, and prompt vaccination for Baladi, poultry, and duck farms should be adopted to reduce herd infections. Make sure the egg-laying areas are clean and perform frequent egg collection to minimise egg contamination. Prevention of egg washing and application of eggshell sanitation or fumigation programs properly. From laying to consumption, eggs should be stored at a temperature of less than 4 °C and at a relative humidity of 70% to 80%. Prevention of eating raw and half-cooked eggs to avoid the risk of food poisoning.

Conflict of interest

The authors declare no conflict of interest.

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