Incidence of Some Food-Borne Pathogens in Table Eggs in Assiut City, Egypt

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Abstract: Food-borne diseases are of major concern worldwide. Among the predominant bacteria involved in these diseases, Campylobacter species, Salmonella species and Listeria species. Eggs have been documented to be a source of food-borne diseases. To study the extent of contamination of table eggs with some food poisoning microorganisms, a total of 300 fresh eggs representing native and foreign breeds’ eggs were randomly collected in Assiut city, Egypt. Campylobacter spp., Salmonella spp. and Listeria spp were detected in 6.7%, 13.3% and 6.7% in native breed eggs and in 3.3%, 6.7% and 3.3% in foreign breeds’ eggs, respectively. The isolated bacteria were identified to be Campylobacter jejuni, Campylobacter coli, Salmonella enteritidis, Salmonella typhimurium, Salmonella Kentucky, Listeria monocytogenes and Listeria innocua. The incidence of these bacteria in eggs shells and contents were determined.

Keywords: Eggs, Campylobacter Spp., Salmonella Spp. And Listeria Monocytogenes.

I. INTRODUCTION

Table eggs are consumed worldwide in various dishes and are considered very nutritious and a cheap source of protein (MAFF, 2000). Eggs are considered a unique well-balanced source of nutrients and essential food elements for growth and maintenance of health in the human body of all ages. Besides the high nutrient contents of eggs, their low caloric values and ease of digestibility make them also valuable in many therapeutic diets for adults (Burley and Vadehra, 1989 & Bufano, 2000).

In spite of the antibacterial factors of eggs, they can be infected with different bacteria such as Salmonella spp., Listeria monocytogenes, Campylobacter jejuni (Adesiyun et al., 2005). Campylobacter is the most common identified cause of food-borne disease (Kaneko et al., 1999). Salmonella spp. is the second-most-common cause of food poisoning after Campylobacter spp. (EFSA, 2012). Campylobacter and Salmonella represent over 90% of reported cases of bacteria-related food poisoning worldwide. Poultry and poultry products have been involved in the majority of food-borne illnesses caused by these bacteria. Almost all cases of human listeriosis are related to food-borne contamination (Jackson et al., 2010).

Egg shells and egg contents can be contaminated by the bacteria in a variety of routes, such as during egg formation in the hen reproductive system or the environmental conditions. Campylobacter species cause mild to severe diarrhea with watery followed by bloody diarrhea (Nachamkin et al., 1992). Campylobacter jejuni has been identified as etiological agents in outbreaks and sporadic cases of gastroenteritis in developed countries (Podunton and Kaneene, 2002).

Contamination of eggs and eggshells has been identified as one of the major causes of food-borne Salmonella (Howard et al., 2012). Salmonella species induce self-limiting gastroenteritis characterized by diarrhea, abdominal pain, and vomiting in people of all ages (Foley and Lynne, 2008). The two most commonly identified causative agents of food-borne salmonellosis are S. typhimurium and S. enteritidis (Galis et al., 2013).

L. monocytogenes is the most pathogenic species of Listeria causing intense food-borne infections that leads to meningitis, encephalitis, and gastroenteritis (Dogany, 2003). L. monocytogenes constitutes a significant threat to public health. Listeria monocytogenes is the causative agent of listeriosis, where those considered being most susceptible are the elderly, immuno compromised, and pregnant women (Tsai et al., 2011).

Table eggs in Egypt include two types of eggs; eggs of native breed hens which are raised in farmers’ houses and eggs of foreign breed hens which are raised in poultry farms. This study was conducted to determine the extent of contamination of table eggs with some food-borne pathogens; Campylobacter spp., Salmonella spp. and Listeria spp. in Assiut City, Egypt.

II. MATERIALS AND METHODS

A. Collection of samples

A total of 300 fresh eggs samples representing native and foreign breed’s eggs (30 groups each) randomly collected from different markets, groceries, supermarkets and farmers’ houses in Assiut city, Egypt. Every 5 eggs constituted an egg pooled sample. Eggs were transported to the laboratory with a minimum of delay.

B. Preparation of samples

Each sample was represented by five eggs which were washed in a sterile plastic bag with 100 ml sterile saline. Egg shells were tested by the rinse method as described by Moats (1980). The egg contents were tested after egg evacuation according to Speck (1976).

C. Isolation & Identification of the selected pathogens

Campylobacter species: (Hunt et al., 2001)

Enrichment of each sample was done using Bolton broth plus 5% Laked horse blood (50ml/litre), and antibiotics (Cefoperazone 20.0 mg/litre, Vancomycin 20.0 mg/litre, Trimethoprim 20.0 mg/litre, Cycloheximide 50.0 mg/litre) and incubated under microaerophilic conditions at 37°C for 4 hrs followed by 24-48 hrs at 42°C. Modified Charcoal Cefoperazone Deoxycholate agar (mCCDA) was used for selective plating. The agar plates were incubated under microaerophilic conditions at 42°C for 24-48 hrs. All flat, mucoid, gram-negative colonies were confirmed to be Campylobacter by oxidase test; all Campylobacter spp. are oxidase-positive. C. coli and C. jejuni were differentiated biochemically by catalase reaction, hippurate hydrolysis and antibiotic sensitivity test. C. jejuni was hippurate positive, whereas C. coli was hippurate negative.

Salmonella species: (Andrews et al., 2016)
Preenrichment was done using Trypticase soy broth at 35°C for 24±2 h, followed by enrichment on Rappaport-Vassiliadis broth and incubated at 42°C ±0.2 °C for 24 h ±2 h. From each enrichment culture, a loopful was streaked onto Xylose Lysine Deoxycholate (XLD) agar at 35°C±1 for 24h±2. Salmonella was identified primarily by biochemical reactions. Furthermore, for typing of Salmonella, isolates were subjected to serological identification for the determination of somatic (O) and flagellar (H) antigens using Salmonella antiserum (Denka Seiken Co., Japan).

Listeria species: (Hitchins et al., 2017)

Enrichment was performed using Buffered Listeria Enrichment Broth (BLEB) supplemented with 10 mg/L acriflavrin, 40 mg/L cycloheximide and 50 mg/L sodium nalidixic acid and incubated at 30°C for 24-48 h, then BLEB enrichments were streaked onto Oxford agar at 35°C for 24 h incubation. Biochemical identification of Listeria spp. was done by CAMP test, motility, catalase test and carbohydrate fermentation. Differentiation of Listeria species was done by hemolysis and fermentation of dextrose, esculin, maltose, rhamnose, mannitol, and xylose.

**DISCUSSION**

Nutrient substances present in eggs make them excellent media for the development of bacteria including pathogenic bacteria (Stepień-Pyśniak, 2010). Contaminated eggs are a serious source of human diseases around the world and the significance of these diseases in humans varies from mild symptoms to life-threatening situation. Eggs can be infected with different bacteria such as Campylobacter spp., Salmonella spp. and Listeria spp. (Abdullah, 2010).

Results presented in Table 1 showed that eggs of the native breed were contaminated with Campylobacter spp. (6.7%), Salmonella spp. (13.3%) and Listeria spp. (6.7%). The eggs’ shells were the only source of Campylobacter spp. and Listeria spp., while Salmonella spp. were detected in the contents of eggs (3.3%). Regarding the eggs of the foreign breed, they were contaminated in the percentages of 3.3%, 6.7% and 3.3% for Campylobacter, Salmonella and Listeria, respectively. The eggs’ shells were the only source of such organisms, while the eggs’ contents were free from contamination.

Different results were reported by Safaei et al. (2011) who couldn’t isolate Campylobacter jejuni, Salmonella spp. and Listeria monocytogenes. Nearly similar results; 9% Salmonella spp. were isolated from egg shells by Hussien et al. (2009). Also, Rashwan (2013) isolated Salmonella spp. in 7.83% of egg shells. While El Sherif and Hassan (2013) detected Salmonella spp. in higher incidences; 26.7% of shells and 20% of contents of native breed, 26.7% in each of shells and contents of foreign breed eggs. Meanwhile, Mahdavi et al. (2012) couldn’t detect Listeria and Salmonella.

The higher incidence rate of contamination among egg shell than egg content could be attributed to the possibility of bacterial contamination of the shell after laying because shell contamination is the first line for penetration. The factors affecting bacterial penetration are intrinsic and extrinsic factors. The extrinsic factors are the influence of bacterial strain, number of organisms, temperature, moisture and storage conditions. The intrinsic factors are the presence of cuticle shell characteristics as shell quality, porosity, shell defects (Messens et al., 2005).

The eggs’ shells are contaminated with all the investigated organisms in a way that shells of native breed hens’ eggs are more contaminated than those of foreign breed hens’ eggs. This could be attributed to the fact that native breed hens are raised in the farmers’ houses with farm animals in the same way on Rappaport-Vassiliadis broth and incubated at 42°C ±0.2 °C for 24 h ±2 h. From each enrichment culture, a loopful was streaked onto Xylose Lysine Deoxycholate (XLD) agar at 35°C±1 for 24h±2. Salmonella was identified primarily by biochemical reactions. Furthermore, for typing of Salmonella, isolates were subjected to serological identification for the determination of somatic (O) and flagellar (H) antigens using Salmonella antiserum (Denka Seiken Co., Japan).

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#### Table 1: Incidence of the isolated food-borne pathogens in the examined eggs

<table>
<thead>
<tr>
<th>Isolated microorganisms</th>
<th>Whole egg</th>
<th>Egg shell</th>
<th>Egg content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Native breed eggs</td>
<td>Foreign breed eggs</td>
<td>Native breed eggs</td>
</tr>
<tr>
<td></td>
<td>No./30</td>
<td>%</td>
<td>No./30</td>
</tr>
<tr>
<td>Campylobacter spp.</td>
<td>2</td>
<td>6.7</td>
<td>1</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>4</td>
<td>13.3</td>
<td>2</td>
</tr>
<tr>
<td>Listeria spp.</td>
<td>2</td>
<td>6.7</td>
<td>1</td>
</tr>
</tbody>
</table>

Meanwhile, the lower incidence in the eggs of foreign breed hens which are raised in poultry farms may be due to the hygienic measures adopted in the commercial egg production and the use of antibiotics as prophylaxis. Although there are relatively low incidences of positive samples in foreign breed eggs, the pathogens represent a potential risk to consumers since they are food-borne pathogens.

The egg shell contamination may result from deposition of fecal material on the shell, oviduct and gut flora, egg crates, packing and storage, workers on farms, dust, transporting and marketing (Al-Bahry et al., 2012). It’s clear from the recorded results that the contents of foreign breed eggs are free of contamination, while the contents of native breed were contaminated with Salmonella spp. It’s worth to mention that the contamination of egg shells pose a risk to the public by giving rise to internal contamination following shell penetration or by being a source of cross-contamination during food preparation (Murchie et al., 2008). The rate of penetration is influenced by humidity and storage temperature at which the eggs are produced and stored (Cox et al., 2000).

It is obvious from Table 2 that C. jejuni and C. coli were detected in the shells of eggs of native breed (3.3% each), while only C. jejuni was detected in the shells of foreign breed eggs (3.3%). Poultry and poultry products have been implicated in the transmission of Campylobacter. Studies of
food-borne infection have estimated that 50%: 70% of Campylobacter illness is caused by poultry and poultry products (Allos, 2001). As a commensal organism in poultry, Campylobacter colonizes the intestinal mucous layer in the crypts of the intestinal epithelium (Beery et al., 1988). Campylobacter jejuni and Campylobacter coli account for the majority of human infections (Friedman et al., 2000).

Table 2: Incidence of C. jejuni and C. coli in egg shells

<table>
<thead>
<tr>
<th>Isolated species</th>
<th>Native breed eggs</th>
<th>Foreign breed eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No./30</td>
<td>%</td>
</tr>
<tr>
<td>C. jejuni</td>
<td>1</td>
<td>3.3</td>
</tr>
<tr>
<td>C. coli</td>
<td>1</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Similarly, other investigators couldn’t isolate Campylobacter from the egg contents (Doyle, 1984 and Bastawrows et al., 2002). A higher incidence was recorded by Hussein and El-Prince (2002) who isolated Campylobacter spp. from 15% of the examined contents of farm eggs. Lower incidence of C. jejuni (1%) was reported by Hedawey and Youssef (2014) in the egg shells, while they reported the same result as not detecting any in the egg contents.

Table 3: Incidence of Salmonella species in egg shells and contents

<table>
<thead>
<tr>
<th>Isolated species</th>
<th>Egg shell Native breed eggs</th>
<th>Egg shell Foreign breed eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No./30</td>
<td>%</td>
</tr>
<tr>
<td>S. enteritidis</td>
<td>2</td>
<td>6.7</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>1</td>
<td>3.3</td>
</tr>
<tr>
<td>S. kentucky</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*S. enteritidis* and *S. typhimurium* as shown in Table 3 were isolated from the shells of native breed eggs (6.7% and 3.3%) respectively and the contents were contaminated only with *S. enteritidis* (3.3%). *S. enteritidis* is known to have the ability to colonize the ovaries of hens and transmitted vertically to be found in the content of eggs (Shirato et al., 2001). Therefore, its presence in shells and contents was not surprising. Meanwhile, shells of foreign breed eggs were contaminated with *S. enteritidis* and *S. Kentucky* (3.3% each). Salmonella and in particular Salmonella enteritidis outbreaks in humans are linked to the consumption of contaminated eggs or food containing eggs (Collard et al., 2007).

Table 4: Incidence of Listeria species in egg shells

<table>
<thead>
<tr>
<th>Isolated species</th>
<th>Native breed eggs</th>
<th>Foreign breed eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No./30</td>
<td>%</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>1</td>
<td>3.3</td>
</tr>
<tr>
<td>Listeria innocua</td>
<td>1</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Listeria monocytogenes and Listeria innocua as presented in Table 4 were the identified Listeria species. Shells of native breed eggs were contaminated with both species (3.3% each), while the shells of foreign breed eggs were contaminated only with *Listeria innocua* (3.3%). A higher incidence of *Listeria monocytogenes* (17.36%) was reported by Rivoal et al. (2010), while lower incidence (0.4%) was reported by Miho et al. (2009). The variability in results may be due to the health status of hens, the extent of shell contamination, the different management system in poultry farms as well as the methodology of isolation.

Preventive measures should be adopted before and after the collection of eggs. This is important in order to reduce contamination of table eggs and hence the risk of food poisoning infection due to their consumption.

References


