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RESEARCH ARTICLE

CONTAMINATION OF THE SHELLS OF LEBANESE FREE RANGE CHICKEN EGGS WITH POTENTIAL PATHOGENS.

Julian Maamari and *Tarek Nawas.
Natural Sciences Department, Lebanese American University, Beirut – Lebanon.

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Abstract

Local free-range eggs in Lebanon are favored by consumers, but are often sold without prior cleaning or processing. Eggs were proved to be a major source of many enteric pathogens that can cause varying episodes of gastroenteritis, this study was designed to investigate the surface contamination of free range egg shells. The tested eggs were purchased from 11 stores located in different regions of Lebanon. The results revealed that many members of the family *Enterobacteriaceae*, that are present in chicken feces, including *Escherichia coli*, *Enterobacter cloacae* and others, were isolated, however it was noted that *Salmonella* spp. were not among the isolates. The presence of these organisms, in the eggs obtained from different areas, confirmed that there was a trend not to clean or process these eggs in any way before sending them to the stores for sale. The mere isolation of fecal organisms, indicated that the eggs can, at any time, be contaminated with *Salmonella* spp. and other dangerous enteric bacteria, and thus endanger the health of the customers. It is required that public health protective measures, be enforced immediately to protect the consumers from what is currently an obvious health hazard.

Introduction:-

Birds have a common excretory and reproductive system. As such, it is known that chicken egg shells are often contaminated with a wide spectrum of different enteric Gram positive and Gram negative bacteria. Thus, fecal contamination of eggs results in the presence of many members of the family *Enterobacteriaceae*, including potentially pathogenic species such as *Salmonella spp.* and *Escherichia spp*. (Gole et al., 2013). The contamination of eggs with *Salmonella* Enteritidis (*Salmonella enterica*, subspecies *enterica*, serovar Enteritidis), has been of major concern, being the leading cause of Salmonellosis in both

humans and animals in the USA, Europe and many other countries around the world including Lebanon (JAMA, 1992; Mishu et al., 1994; Barbour et al., 1999; Poppe, 1999; WHO, 2000; FDA, 2016a). SE infections can be very serious, even life-threatening, especially to the very young, the elderly, and people with weakened immune systems. Infected people may experience general enteric symptoms, but some infected people may suffer from severe illness, arthritis, or even death (FDA, 2016a). Eggs can become contaminated on the farm because a laying hen can become infected with SE and pass the bacteria into the egg before it is laid. If the egg was not refrigerated, the bacteria can grow inside the uncracked, whole egg (FDA, 2016a). In fact, *Salmonella spp.* such as SE, have shown the ability to

Corresponding Author:- Tarek Nawas.
Address**:-** Natural Sciences Department, Lebanese American University, Beirut – Lebanon.

penetrate egg shells through pores on their surface and enter to the egg yolk where they can multiply significantly faster (Gast et al., 2005). For this reason, the FDA imposed a set of strict regulations on egg producers to prevent and reduce SE infections (FDA, 2017). Today, modern commercial processing successfully decreased microbial contamination of egg shells in many countries all over the globe (Musgrove et al., 2005).

In Lebanon, some stores proudly advertise that they sell local free-range eggs, transported “fresh” from a farm. These eggs are perceived by the public as being healthier and less subject to the chemicals used during commercial egg production, and are thus desired by a large segment of the Lebanese society. Since there are no standard washing or processing procedures performed by the farmers, these eggs are often not cleaned and present a higher risk of contamination by numerous members of the family *Enterobacteriaceae* (Hannah et al., 2011). The estimated incidence of Salmonellosis in Lebanon is reported at 13.34 cases/100,000 individuals mostly in the age group of 20-39 years old (Malaeb et al., 2016). The incidence is, however, believed to be higher for many reasons including: the general symptoms in most infection (diarrhea, fever, abdominal cramps, headache, nausea and vomiting) that, often, go unreported, in addition to, the lack of regular reporting, especially for the less developed areas and the absence of a central office that compiles the information (Malaeb et al., 2016). *Salmonella* infections in Lebanon have been, however, noted to become increasingly resistant to antibiotics (Moubareck et al., 2005). This study was designed to gather information about the contamination of the surfaces of the shells of free-range chicken eggs, obtained from stores located in different areas of Lebanon, with members of the family *Enterobacteriaceae* and specifically *Salmonella* spp.

Materials and Methods:-

Samples tested:-

From 11 different stores located in different parts of Lebanon (Figure 1), 2 “free-range” and “fresh” eggs were purchased from each. The sample eggs were purchased between January and March, 2017, from the stores and not the producers themselves, to ensure that the eggs were received just as a regular consumer would. The “freshest” eggs were chosen and transported to the microbiology laboratory at room temperature and in the shortest period of time not exceeding 24 hours. The eggs were transported to the laboratory in the same bag that they were packed in, simulating what a consumer would actually take home.

Figure 1:- A map of Lebanon, showing the sites from where the samples, in this study, were purchased

Processing of samples:-

Upon arrival to the microbiology laboratory, separate sterile swabs were dipped in Selenite Cystine Broth (SCB) and used to swab the entire shell surface of each egg. Each of the swabs was used to inoculate: Tryptic Soy Agar (TSA), MacConkey Agar (MCA), and Salmonella-Shigella Agar (SSA) plates in that order. Following inoculation, the swabs were re-inserted into the tubes and the plates were properly streaked. All plates and tubes were then incubated at 35°C for 20-24 hours. After the incubation period, the surfaces of the plates were checked for bacterial growth and all colonies that appeared on the different media were isolated, checked for purity and preserved on TSA slants at 4°C for the next step of identification. The SCB tubes were further used to inoculate the same set of plates again. The new plates were incubated for 20-24 hours at 35°C. The purpose of using SC broth was to inhibit the growth of Gram positive bacteria and aid in the recovery of *Salmonella* spp. and/or *Shigella* spp. in case they were present in small amounts (Hammack et al., 1999).

Identification of isolates:-

After Gram staining, as only Gram-negative bacilli were sought, the following preliminary tests were performed on each isolate: Oxidase production using oxidase test strips and Glucose and lactose fermentation and gas and H2S production using Kligler's Iron Agar (KIA) done as recommended (Jorgensen and Pfaller, 2015). Definitive identification of the isolates, however, was done using the API 20E kits (Biomerieux-France) as recommended by the manufacturer.

Results:-

Table 1, shows that, except for the eggs purchased from two stores in Zahle and coastal Akkar (numbered 7 and 10), the eggs from the other locations showed positive bacterial growth. Of the 18 tested eggs, 16 grew Gram negative bacilli, 15 of which were members of the family *Enterobacteriaceae* while the last was a non-fermenter from the family *Moraxellaceae.*

Table 1:-The isolates from the shells of the eggs; reported by location of stores they were purchased from.

Sample Number	Source Location	Egg Number	Growth on study media	Organisms isolated
1	Majdelyoun	1	-	-
		2	+	*Enterobacter cloacae*
2	Bchamoun	1	+	*Escherichia coli*
		2	+	*Pantoea* sp.
3	Kfarshima	1	+	*Citrobacter freundii*
		2	+	*Citrobacter braakii*
4	Hamra	1	-	-
		2	+	*Pantoea* sp.
5	Jdaide	1	+	*Escherichia coli*
		2	+	*Citrobacter freundii*
6	Mazraat Yachouh	1	+	*Enterobacter cloacae*
		2	+	*Enterobacter cloacae*
7	Zahle	1	-	-
		2	-	-
8	Koura	1	+	*Citrobacter freundii*
		2	+	*Pantoea* sp.
9	Tripoli	1	+	*Enterobacter cloacae*
		2	+	*Enterobacter cloacae*
10	Akkar (coast)	1	-	-
		2	-	-
11	Akkar (inland)	1	+	*Moraxella* sp.
		2	+	*Escherichia coli*

The frequencies of the *Enterobacteriaceae* isolates from the 15 eggs were: *Enterobacter cloacae* (33.3%), *Escherichia coli*, *Pantoea* spp. *Citrobacter freundii* (20 % each) and *Citrobacter braakii* (6.7 %). No *Salmonella* spp. were isolated from any of the egg shells.

Discussion and Conclusion:-

Checking the surface contamination of the shells of the free-range eggs tested in this study, was done using media that usually enhances the growth of Gram negative bacteria. The broth used to collect the samples from the surface of the shells was selenite cysteine broth, as the cystine formulation was first proposed by the US Food and Drug Administration (FDA), as an enrichment medium for detecting *Salmonella* in food materials. It was proved useful in detecting *Salmonella* when low numbers of organisms were present in stools (Jorgensen and Pfaller, 2015), and it was also recommended for use in detecting *Salmonella* in food and water (Speck, 1984; Greenberg, 1985). The sodium selenite in the medium is an inhibitor of Gram positive bacteria.

No *Salmonella* spp. were isolated from the samples in this study, for reasons, that for the investigators, were obvious and will be discussed later. The results in Table 1, however, clearly show that the organisms isolated indicate that the surfaces of the eggs were contaminated with fecal material. Moreover, all the isolated organisms are described to be potential pathogens. They may not be primary pathogens, but pose a higher risk of infection for the young, elderly and immunocompromised members of the community. *Enterobacter* and *Pantoea* species for instance, were reported to cause several hospital-acquired infections and were noted to have become increasingly multi-resistant (Davin-Regli and Pagã¨s, 2015; Dutkiewiez et al., 2016). *E. coli* is also known to cause infection upon ingestion, even in small numbers (Mayo Clinic, 2014). Shiga toxin-producing *E. coli* strains which were detected in living hens (CDC, 2015), and the dangerous O157:H7 strain were linked to many outbreaks of gastrointestinal infections (Dipineto et al. 2006). Moreover, it was also proved that *E. coli* is capable of facilitating the penetration of *Staphylococcus aureus* into the egg content (Al-Natour et al., 2011), another reason for the increased interest in controlling fecal contamination, of the surface of egg shells. Although the *Citrobacter* isolates are generally not considered as primary pathogens as some of the other isolates in the study, yet some of the members of this genus, have been reported to cause urinary tract infections, upper respiratory tract infections (Mossad, 2013) otitis media in children (Doran, 1999) and infant meningitis (Samonis et al., 2008), but mostly in patients who have at least 1 underlying illness (Mossad, 2013). The isolate from the genus *Moraxella* belongs to a genus that is also known to have many members that act as opportunistic pathogens that cause many infections including upper respiratory tract infections (Bernhard, 2012).

The WHO predicts that opportunistic infections will become more dangerous in the 21st century, further increasing the importance of restricting the spread of the bacteria that cause these infections. What adds to the problem, is the fact that most members of the family *Enterobacteriaceae* have become increasingly multi-resistant to antibiotics, as seen through the emergence of the Extended Spectrum β-Lactamase producing (ESBL) bacteria all over the world and the isolation of the first ESBL *Salmonella* Typhimurium in Lebanon in 2006 (Moubareck et al., 2005), and around the world (Mishu et al., 1994; WHO, 2000).

The absence of *Salmonella* spp. from the isolates is instigated by many factors. It is a known for a fact that *Salmonella* spp. are much more sensitive to external conditions than most other fecal organisms, and their survival is influenced by several different factors (Kim et al., 1989; Garcia et al., 2010; CDC, 2016). The samples tested in this study were collected in the winter (between January and March) from the different regions in Lebanon, which are known to have different weather conditions. The request from the merchant to provide the investigators with the "freshest free range eggs" in the market, may not have been respected and the samples may have been older eggs that were present in the store. Another factor is the long distances that these eggs had to be transported through, from the farm to the market and later to the laboratory, without refrigeration; This probably led to the loss of any *Salmonella* spp. or any other sensitive organism that may have initially been present on the shells. This is confirmed by noting that the two samples (7 and 10 in Table 1) purchased from the markets that are farthest away from the microbiology laboratory, located in the capital Beirut, did not grow any organism.

Knowing that not all chicken carry *Salmonella* as part of their microflora, the fecal contamination that was obvious on the shells of the tested eggs, however, reveals a health hazard to the consumers. Considering these findings, it is recommended that Lebanese egg producers implement the accepted international guidelines in order to reduce contamination by these potentially dangerous bacteria. The FDA (2016b, 2017), introduced a set of prevention methods and recommendations which could be easily implemented by the Lebanese producers, although there were previous recommendations to that effect (Barbour et al., 2001). Moreover, pasteurization and dry heat treatment can also be used as they were proved to be effective in decreasing SE contamination (Arnold et al., 2014), in addition to that vaccination against certain strains of *Salmonella* spp., also showed effectiveness in decreasing SE contamination on egg shells (Barker et al., 2003). However, perhaps the most important recommendation is for the consumer, as the risk of infection by SE or any of the *Enterobacteriaceae* can be tremendously decreased by making sure that eggs are not eaten raw, as is common in the Lebanese countryside, and that they are well-cooked. Consumers must also wash all kitchen utensils, benches, and hands exposed to these eggs using proper detergents to diminish any chance of cross-contamination (Barker et al., 2003). The role of public health authorities in adopting cleaning and processing regulations and monitoring their implementation, should also be affirmed.

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