

Isolation and identification of *Salmonella* from marketing eggs

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عزل وتشخيص جراثيم السالمونيلا من بيض الاسواق

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المستخلص

أجريت هذه الدراسة في مدينة الكوت (العراق) لمعرفة تلوث بيض المائدة المحلي والمستورد بجراثيم السالمونيلا، جمعت العينات (120) بيضة وبواقع (60) بيضة محلية و (60) بيضة مستوردة من الأسواق المحلية في مدينة الكوت خلال الفترة من شهر شباط الى شهر ايار 2012، أظهرت الدراسة تواجد جراثيم السالمونيلا في اربع وخمسون عينة من مجموع (120) (45%) عينة في المستورد والمحلي، وبواقع (50%) (30) من مجموع 60 عينة محلي، و (40%) (24) من مجموع 60 مستورد. تواجد جراثيم السالمونيلا في عينات المحلية والمستوردة قد تتراوح بين أشهر السنة، اذ كانت اعلى معدلات تواجده في ايار (68.7%) في المحلي و (62.5) في البيض المستورد. أظهرت نتائج الدراسة وجود فرق معنوي ($P < 0.05$) في نسبة تواجد جراثيم السالمونيلا بين البيض المحلي والمستورد، وكذلك هنالك فرق معنوي ($P < 0.05$) في تواجد الجراثيم بالبيض المحلي والمستورد بين اشهر الدراسة، كما اثبتت الدراسة أن البيض مصدر هام للتسمم الغذائي البكتيري الذي يهدد الصحة العامة في العراق.

Abstract

A study was done in Kut City (Iraq), for detection incidence of *Salmonella* spp. in local and imported eggs samples. For that purpose 120 samples of eggs, (60) local, (60) imported were obtained from local markets during the period from the February up to May 2012. As results, *Salmonella* spp. was detected in 54 of 120(45%) local and imported eggs: 30 of 60 (50%) local eggs and 24 of 60 (40%) imported eggs. The prevalence of *Salmonella* spp. in local and imported samples varied between year months with the highest prevalence occurring in May (68.7%) in local and (62.5) in imported eggs. The results of statistical analysis showed significant differences ($P < 0.05$) between local and imported samples of eggs, and there was statistically significant difference ($P < 0.05$) between the months year in prevalence of *Salmonella* in imported and local eggs. The results of this study showed that eggs are a significant source for Bacterial food poisoning that concerns the public health in Iraq.

Introduction

Food poisoning is acute enteritis caused by the ingestion of food characterized by diarrhea, vomiting, with or without fever and abdominal pains. Food poisoning is normally associated with the small and large intestine. Certain types of food poisoning are described as intoxications and others as infection. The CDC (CDC, 2012) has reported during 2011 that there are around 48 million foodborne illnesses each year in the United States. That means that, on average, one of every six people becomes ill every year. These foodborne illnesses result in about 128,000

hospitalizations and 3,000 deaths. Many of the germs are familiar because they've caused outbreaks that have been in the news; for example, *Salmonella* and *E. coli* O157. About 90 percent of all illnesses, hospitalizations, and deaths caused by germs that we know about are caused by just seven germs. *Salmonella* species are responsible for the highest number of documents cases of food poisoning in the developed country (Karmali, 1999).

The *Salmonella* is food-borne pathogens of major public health concern in the United States, A variety of foods, including poultry, eggs, meat, milk, fruits, and vegetables, have been implicated as vehicles of one or more of these pathogens in outbreaks of food-borne illness (Beuchat, 1995). Infection by *Salmonella typhimurium* is an important cause of morbidity and mortality in poultry, and infection with *Salmonella enteritis* is a major cause of food-borne illness, *Salmonella* is an enterinvasive bacterium and causes infections that may have one of five different clinical presentations (Goldberg and Rubin, 1988). Gastroenteritis is the most common presentation in industrial countries and is considering as an emergent food-borne pathogen caused disease it's a self-limited illness of brief duration, usually characterized by diarrhea and fever, *Salmonella enterica* serotype Typhimurium are associated with gastroenteritis, while serotype Choleraesuis and serotype Dublin are associated with bacteremia, Enteric fever, or typhoid fever, is due to serotype Typhi (which is adapted to human hosts; animals do not serve as a reservoir)(Suresh *et al.*,2006). but may also be caused by other serotypes (mainly paratyphoid A, B, and C) (Rodriguez, *et al.* 1998). The aim of this study was examination of local and imported eggs for detection of *Salmonella spp.* And know the effect of Weather temperature on the Prevalence of *Salmonella* in eggs.

Materials and Methods

Sample collection and analysis

One hundred and twenty samples of eggs, (60) local, (60) imported were obtained from local markets in kut city in sterile plastic bags for four months from February up to May,2012 and stored at – 4 °C until analyzed (Agarwal *et al.*,2003).

Enrichment and isolation

Salmonella. Pre-enrichments were performed as standard method for isolation of *Salmonella* from food eggs sample, 1 ml were added to 9 ml buffer peptone water (BPW) (Oxoid) , then Dropping of 0.1 ml of pre-enrichment on to salmonella-Shigella ager (SS) (Oxoid) and incubated for 24 h at 37°C(HPA,1998).

Selective diagnostic isolation

Sub-culturing of positive samples on to modified brilliant green agar (MBGA)(Oxoid) and xylose-lysine-desoxycholate agar (XLDA) (Oxoid) and incubated for 24h at 37 °C.

Biochemical and serological confirmation

Presumptive *Salmonella* colonies were sub-cultured on tryptic soya agar (TSA) (Difco) plates for 24 to 48 h at 37 °C in order to obtain pure culture according to (Stefanovicova *et al.*,1998). Biochemical tests carried out by Api-20 E Kits (BioMerieux).

Results and Discussion

Isolation and diagnosis *Salmonella* in eggs

Since *Salmonella* is an important zoonotic bacterium with poultry as largest single reservoir of *Salmonella*, the assessment of the contamination level and site of contamination is of utmost importance in deciding the control strategies against *Salmonella* in chickens. The contamination of *Salmonella* in the internal content of chicken eggs can be due to infection in the ovary of birds while surface contamination of eggs can be through feces, feed, insects, or through handling, transport or storage material (Gupta *et al.*, 1999). A total 120 local and imported eggs samples, collected from local markets, Kut city, Iraq. Fifty four (45%) were positive for *Salmonella* spp. (Fig.1). The results indicated that, eggs samples were the most vehicles of *Salmonella* under investigation. The prevalence found in this study shows the similarity to those reported by (Bajaj *et al.* 2003) who found the prevalence the *Salmonella* in chicken eggs was 25% (50 out of 200). The same finding were reported by Tietjen and Fung (2005) they reported that, eggs vehicles of food borne salmonellosis because the raw product is initially contaminated with *Salmonella* cells when it is delivered to the consumer or due to subsequent undercooking, cross contamination, or improper thawing, and *Salmonella* caused a large number of cases of food poisoning in developed countries, which are transmitted by many types of food such as meat poultry, eggs, meat, milk, vegetables, fruit.

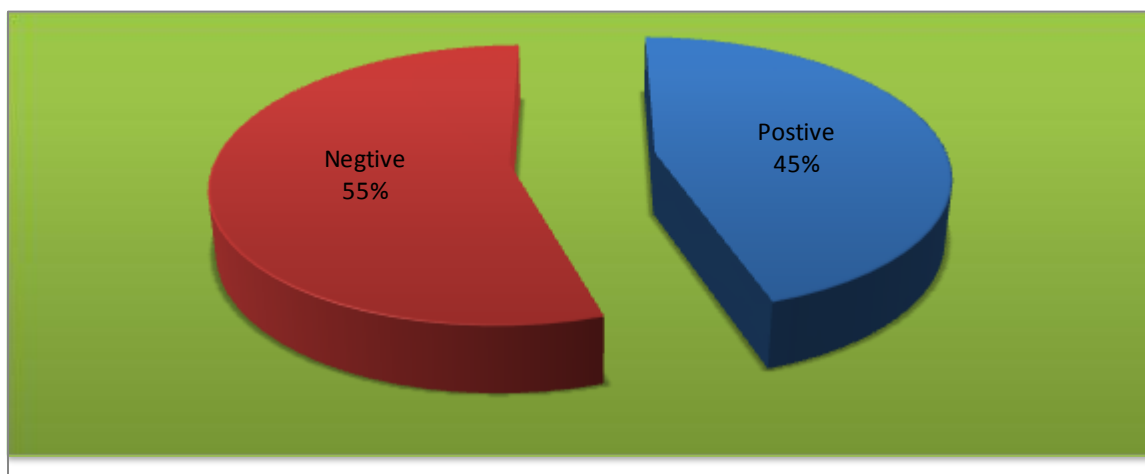


Fig.(1) Incidence of *Salmonella* in local and imported eggs

Incidence of *Salmonella* in local eggs

A total of 60 local eggs samples were collected from local markets in Kut city during four months, Thirty (50%) were positive for *Salmonella* spp. The results of Statistical analysis showed significant differences ($P < 0.05$) between the months years in prevalence of *Salmonella* in local eggs (Table1). This results agree with Surveys conducted in England and Wales have also shown egg contamination level of *Salmonella* to be 10.2% to 10.6% (De Louvois, 1994). Also This results agree with Jubouri (2001), who isolate *S. typhimurium* from people suffering from severe diarrhea result eating eggs contaminated with *Salmonella* in a hospital in Mosul city, Eggs contamination with *Salmonella* occurs either before

oviposition, where intervention germ to chicken through gastrointestinal tract, When entering *salmonella* through the mouth of the surrounding environment, it greatly multiply in the digestive system, especially in the crop and intestines. Then penetrates the mucosal epithelial cells and lead to a large spread in the internal organs, especially the female reproductive system, through the colonies in the ovary (where mature yolk and excretion) and oviduct (an albumin secretion) *Salmonella* can go through the internal components of the egg, or after oviposition through contaminated with poultry residues (Feng, 1992).

Table (1): Incidence of Salmonella in local eggs

Month	No. Samples	Positive	Negative	Percentage of infection (%)
Feb.	14	4	10	29
Mar.	16	6	10	38
Apr.	14	9	5	64.3
May.	16	11	5	68.7
Total	60	30	30	50

RLSD=1.301(P<0.05)

Incidence of *Salmonella* in imported eggs

The higher incidence of *Salmonella* in the market eggs may be due to surface contamination during handling, storage and transportation while contamination in the internal contents points to infection of hen. A total of 60 imported eggs samples were collected from the markets in Kut city during four months, twenty four (40%) were positive for *Salmonella* spp. The results of Statistical analysis showed significant differences (P<0.05) between the months years in prevalence of *Salmonella* in imported eggs (Table 2). Similarly, out of 200 samples from marketing eggs, 10 (35.29%) were positive for *S. Typhimurium*, (Murugka *et al.*, 2005). Isolation of *S. Typhimurium*, in marketing eggs was also reported by (Otomo *et al.*, 2007). This results also agree with Karimi (2011), that reported (35%) of marketing eggs contaminated with *Salmonella* spp. in Iran. The high level of contamination imported eggs due to Weak of control at border crossing points, which facilitated the entry of large quantities of eggs without a laboratory tests that confirm the safety of imported products and suitability for consumed human, and the length of storage at temperatures inappropriate often leads to high incidence of germs and particularly when the lack of storage conditions health for food

and non-followers conditions necessary to prevent pollution (LingandWang,2001).

Table (2): Incidence of *Salmonella* in imported egg

Month	No. Samples	Positive	Negative	Percentage of infection (%)
Feb.	14	1	13	7.5
Mar.	16	5	11	32
Apr.	14	8	6	58
May.	16	10	6	62.5
Total	60	24	36	40

RLSD=1.601(P<0.05)

Prevalence of *Salmonella* during Study Months

There are different ways in which weather conditions can affect the incidence of food borne diseases. Firstly, the prevalence of specific pathogenic organisms in animals may increase with higher temperatures. Secondly, the food cooling chain is harder to maintain in higher temperatures and prolonged warm weather increases the risk of mistakes in food handling. Thirdly, higher air temperatures may speed up the replication cycles of foodborne pathogenic organisms, which lead to a higher degree of contamination. Higher temperatures, in interaction with inadequate hygiene conditions, improper food handling, and lack of hand-washing, may lead to an increased number of epidemics resulting from consumption of unsafe food (Vladimir and Dragan, 2010). The prevalence of *salmonella* in eggs varied between study months with the highest prevalence occurring in May (68.7%) in local and (62.5%) imported eggs, while the lowest prevalence occurring in February (7.5%), (25%) respectively (Fig. 2). This results is agree with (Zhao *et al.*, 2006) that reported the incidence of *Salmonella* cases among humans in word population varies seasonally, and may be expected to be change in response to global climate changes. During the review period, the highest values of the Seasonal Index for *Salmonella* cases were registered in the summer months, i.e. June, July, August and September. Also the same finding was reported in by Karimi (2011) he reported that, the prevalence of *salmonella* in summer (35%) followed by fall (24%), while in winter was (2%). The recent studies on foodborne diseases show that disease episodes

caused by *Salmonella* bacteria increase by 5-10% per each degree Celsius rise in temperature. During 1991–2008, 6969 cases of salmonellosis were reported in the Republic of Macedonia increasing trend a, with total morbidity of 340.3 per 100 000, or an average of 387 cases a year, with an in recent years (Bryan and Doyle, 2009).

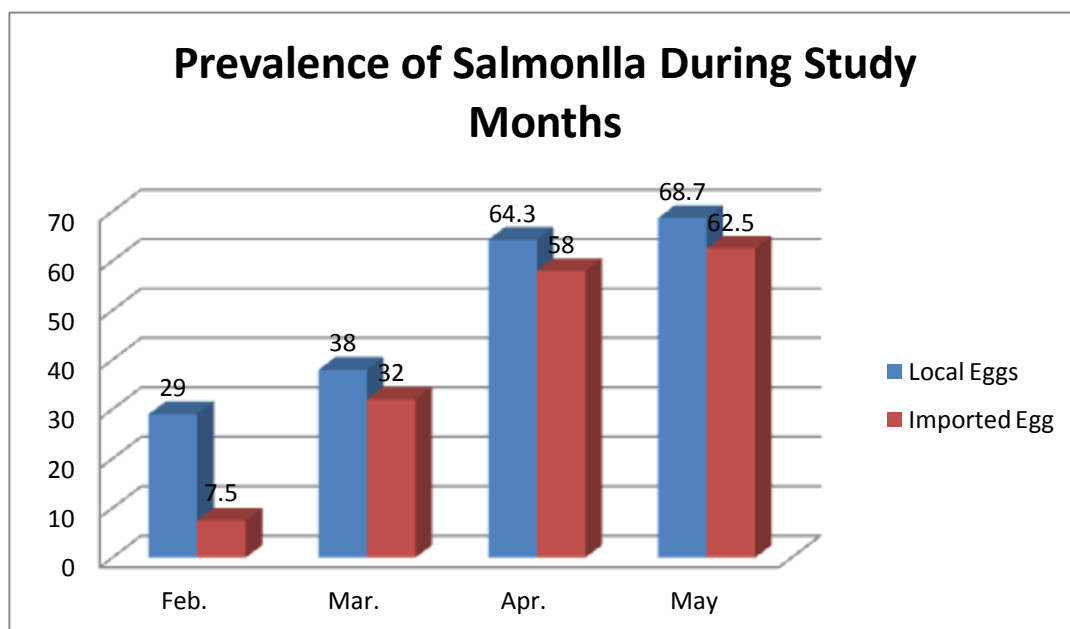


Fig (2): Prevalence of *Salmonella* during study months

Conclusions

1. The local and imported eggs are a significant source for *salmonella* food poisoning that concerns the public health in Iraq.
2. The prevalence of *Salmonella* in marketing eggs was high belonging to Weak of control at border crossing points, and the bad conditions of storage.
3. The prevalence of *salmonella* in eggs increasing with increasing the temperatures of weather.

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