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PREVALENCE OF SALMONELLA SPECIES IN HEN EGGS AND EGG STORING-TRAYS COLLECTED FROM POULTRY FARMS AND MARKETING OUTLETS OF FAISALABAD, PAKISTAN

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Salmonellosis is a food-borne bacterial disease having a zoonotic importance in global perspective. A huge range of foodstuffs have been involved in salmonellosis, yet animal originated foods typically from poultry products including eggs have been consistently involved in outbreaks of disease. To study the prevalence of Salmonella species a total of 384 samples were collected including hen eggs (252) and egg storing trays (132) from various poultry farms and marketing outlets of Faisalabad. A sterile cotton swab was used for sampling the surface of eggs and egg storing trays. Egg yolk and albumin was also checked for the presence of Salmonella spp. Hektoen enteric agar was used as selective media for isolation of Salmonella species and to identify Salmonella serovars polyvalent antisera were used. Prevalence was observed 29.36 and 38.88% in eggshells, 10.31 and 15.07% in egg contents and 28.78 and 43.93% in egg storing trays from poultry farms and marketing outlets, respectively. The occurrence of Salmonella spp. was lower in eggs collected from poultry farms and in egg storing paper trays as compared to that of eggs collected from market outlets and in egg storing plastic trays.
Keywords: Salmonella spp., hen eggs, egg trays, egg contents, bacterial diseases

INTRODUCTION

Poultry products especially eggs and egg products are nutritive food items and a vital constituent of human food in Pakistan. They are rich in protein, phosphorous, selenium, choline, riboflavin and vitamin B12. Moreover, they are also comprises with folic acid, zinc, pantothenic acid and vitamins A, D, E, and K (Anonymous, 2004). However, inaccurately treated eggs can cause food-borne illness. The intestinal tract of poultry is the major reservoir of Salmonella spp. which infects the humans (Roberts *et al*., 2005; Khan *et al*., 2010). Among food-borne bacterial zoonotic diseases salmonellosis causes huge economic losses in terms of massive mortality and morbidity (Hafez, 2011). Huge ranges of poultry products have been contaminated with Salmonella spp. leading to outbreaks of salmonellosis (Messens *et al*., 2007). Different species of Salmonella including *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella bongori*, *Salmonella typhi*, *Salmonella paratyphi* and *Salmonella typhimurium* causes GIT and typhoid fever. *Salmonella* spp. causes typhoid fever to 16 million people, gastroenteritis to 1.3 billion people and causes 3 million deaths annually worldwide (Bhunia, 2008). Salmonella spp. contamination in egg farms and market outlets may arise at any production stage by horizontal or vertical transmission. Vertical transmission means contamination of egg yolk, albumin, membranes or eggshells. While in horizontal transmission disease is penetrated during or after ovipositor through the egg shell from the gut or contaminated feces (Aoust *et al*., 2000). The cause and the method of spread of Salmonella spp. between the egg items and the customers should be recognized to influence the illness manage. Salmonella spp. contamination may be prevalent in farm environment. One possible cause of Salmonella contamination in developing countries is reusable egg trays (Utrarachkij *et al*., 2012). Outbreaks and sporadic cases of salmonellosis are frequently associated with the intake of infected hen eggs with Salmonella spp. Therefore awareness of the prevalence of Salmonella spp. is essential to commence a control programmed. The present study aimed to determine the prevalence of Salmonella spp. in hen eggs and egg storing trays collected from poultry farms and marketing outlets in Faisalabad, Pakistan.

MATERIALS AND METHODS

***Sample size and collection*:** A total of 384 eggs and egg storing trays were collected from 10 poultry farms and 10 marketing outlets in Faisalabad during the period of October 2011 to May 2012. Out of 384 samples, fresh hen egg samples were 252 and 132 samples were paper and plastic egg storing trays. The samples were then carried to laboratory of the Institute of Microbiology in University of Agriculture Faisalabad, under sterile conditions and

processed immediately for the isolation of Salmonella spp. Simple random sampling was used for the sample collection and the sample size was estimated by the formula as described by Thrusfield (2008).

***Isolation of* Salmonella:**

***Egg storing trays*:** A sterile cotton swab was used for isolation purpose where first of all this swab was wetted in sterilized normal saline solution to swabbed on egg storing tray surface and then re-immersed in 10 ml normal saline solution followed by transmission to 90 ml of buffered peptone water and incubation at 37°C for 18 hours.

***Egg shell surface*:** A sterile cotton swab, soaked in sterilized normal saline was swabbed on egg surface and immersed in 10 ml normal saline solution followed by transmission to 90 ml of buffered peptone water then incubated at 37°C for 18 hours (Singh *et al*., 2010).

***Egg albumin and yolk*:** Samples of egg yolks and egg albumins were examined separately as 5 ml of each sample was mixed with 5 ml of normal saline solution. Afterwards, the solution was transferred to 90 ml of buffered peptone water and incubated at 37°C for 18 hours.

***Identification of* Salmonella:**

One ml pre-enriched sample was added in 10 ml of tetra thionate broth for all samples individually and incubated at 37°C for 24 hours and then streaking was done on hektoen enteric agar and incubation at 37°C for 24 hours. It is worth to mention that buffered peptone water and tetrathionate broth were used for enrichment purpose. Hektoen enteric agar was used for selective isolation of Salmonella spp.

Suspected colonies were identified by using chemical tests including Gram staining, Indole, Methyl Red, Voges Proskauer, Citrate Utilization, Triple Sugar Iron, Lysine Decarboxylation (Agarwal *et al.,* 2003). Subsequently, Salmonella serovars were identified by using polyvalent antisera.

RESULTS

For detection of Salmonella spp. from eggs and egg storing trays collected from poultry farms and market outlets colonies with black centers were observed. Out of 384 samples (252 fresh eggs and 132 egg storing trays), eggs were used to examine the incidence of Salmonella spp. in egg-shells and egg contents, while egg storing trays were examined for the prevalence of same pathogen (Fig. 1). The total prevalence of positive Salmonella rates were, eggshells 86 (34.12%), egg contents 32 (12.69%). and egg storing trays 48 (36.36%). Out of 34.12% occurrence of Salmonella in egg shells and 12.69% in egg contents, the incidence of the pathogen was 29.36 and 38.88 % in egg shells and 10.31 and 15.07 % in egg contents of eggs collected from poultry farms and market outlets, respectively. The prevalence of Salmonella also varied among freshly and stored collected eggs, i.e. 31.74 and 46.02% in eggshells and 11.11 and 19.04% in egg contents as eggs collected from poultry farms and market outlets, respectively (Fig. 3).

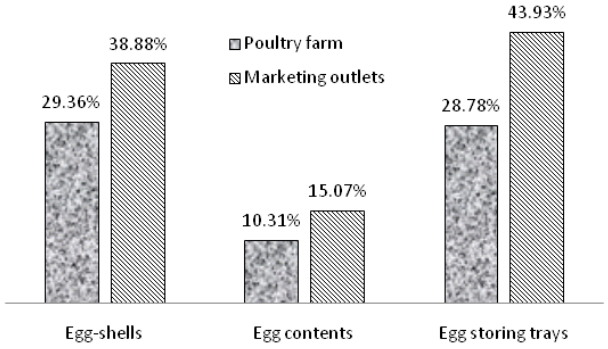

Figure 1. Total prevalence of Salmonella in egg shells, egg contents and in egg storing-trays collected from poultry farms and marketing outlets

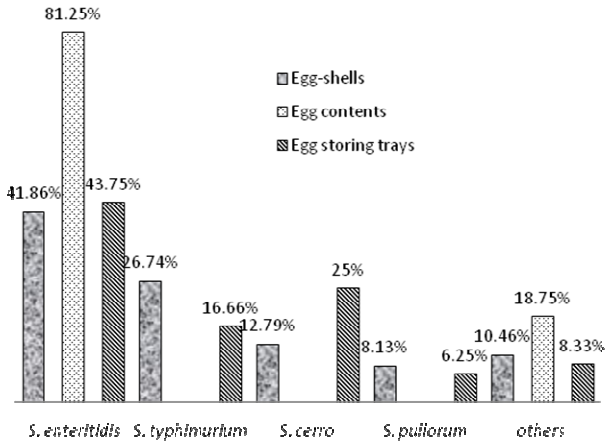

Figure 2. Prevalence of Salmonella serovars isolated from egg shells, egg contents and egg storing-trays

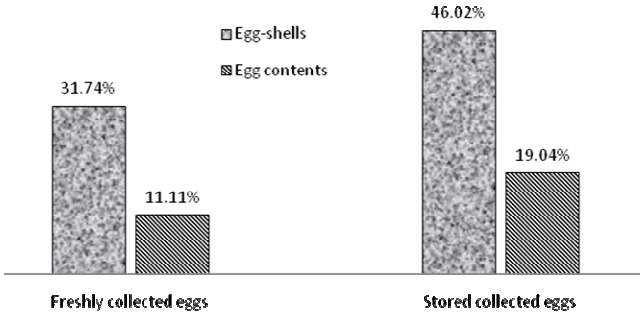

Figure 3. Prevalence of Salmonella in freshly collected and stored eggs

The egg storing trays also have varied Salmonella spp. Rates, i.e. 28.78 and 43.93% as trays collected from poultry

farms and market outlets, respectively. Moreover, among egg storing trays the prevalence of Salmonella spp. was 30.30 and 42.42% in paper trays and plastic trays, respectively (Fig. 4). From the total positive samples of Salmonella from eggshells serovars were isolated, *S. enteritidis* (41.86%), *S. typhimurium* (26.74%), *S. cerro* (12.79%) and *S. pullorum* (8.13%); from egg contents 81.25% *S. enteritidis* and finally from the, egg trays total positive samples were isolated as 43.75% *S. enteritidis,* 25% *S. cerro,* 16.66% *S. typhimurium* and 6.25% *S. pullorum* (Fig. 2).

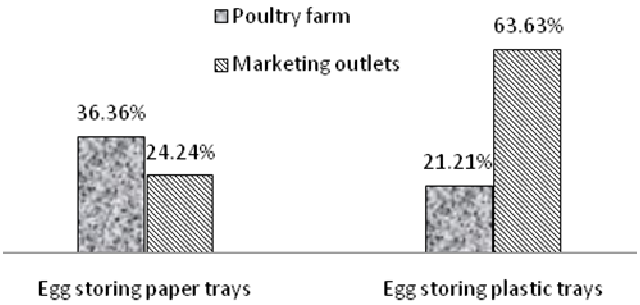

Figure 4. Prevalence of Salmonella in paper and plastic egg-storing trays

DISCUSSION

***Salmonella incidence in eggs*:** In poultry the most significant zoonotic bacterium is Salmonella (Gupta *et al*., 1999) the extreme importance is to evaluate the level and place of contamination for making a decision to control the Salmonella infection in chickens. Salmonella contamination within the egg contents can occur with the infected ovary of the hen (Barnhart *et al*., 1991). While, eggshell contamination can occur through egg contact with fecal material, insects, feed or even through transportation, storage or during handling. Our results indicated that egg shell contamination with the fecal material was significantly higher with that of egg contents. Similar observation was reported (Davies and Breslin, 2004). The present study signified that occurrence of Salmonella spp. in farm eggs; in egg-shells, egg contents and in egg storing-trays 9.52, 4.76 and 15.15% lower, respectively as evaluated to eggs collected from the market outlets. Out of 252 egg-shells total 86 (34.12%), out of 252 egg-contents total 32 (12.69%) and out of 132 egg-trays total 48 (36.36%) were positive for Salmonealla spp. Out of these positive samples prevalence was recorded 29.36 and 38.88% in eggshells, 10.31 and 15.07% in egg contents and 28.78 and 43.93% in egg storing trays from poultry farms and marketing outlets, respectively. Prevalence was reported 40 and 8.33% in eggshells and in egg contents, respectively in a previous study that was carried out in Pakistan (Akhtar *et al*., 2010). In the present study, the prevalence of Salmonella spp. in egg contents was slightly higher than that of previously conducted studies in other countries. The prevalence of Salmonella spp. was recorded to be 6.1% in egg shells, 1.8% in egg contents and 7.7% in retail eggs in South India (Suresh *et al*., 2006). The prevalence of disease was recorded 1% in egg shells in Spain (Perales and Audicana, 1989) and 7% in UK (Evans *et al*., 1998). The higher prevalence of Salmonella spp. infecting eggs collected from market outlets could be explained as contamination of eggshells with fecal material, insects, and feed or contamination during transportation, storage or handling.

Salmonella *incidence in egg-storing trays*: One possible cause of Salmonella contamination in developing countries is reusable egg trays (Utrarachkij *et al*., 2012). Egg storing-trays contamination might be due to chicken fecal material or due to the environment (Bangtrakulnonth *et al*., 2004). Our data indicated higher prevalence of Salmonella spp. in egg storing-trays, 30.30 and 42.42% in egg storing paper and plastic trays, respectively. Salmonella spp. prevalence is higher in egg storing plastic trays as compared to egg storing paper trays (as Fig. 4); this may be due to the use of plastic trays for long duration without disinfecting.

Salmonella *incidence during storage*: When eggs contaminated through vitelline membrane, Salmonella get entrance to egg yolk and increases their number due to favorable environmental conditions for its growth (Humphrey, 2004). The integrity of vitelline membrane depends mainly on the temperature and time of storage (Anonymous, 2002). Salmonella spp. in egg albumen can rise at 20°C; it grows quickly in egg yolk at 25°C. The weakening of the vitelline membrane discharges nutrients and iron in the albumin from the yolk and invites the Salmonella spp. towards the egg yolk (Gantois*et al*., 2009). Egg storage temperature between 18°C to 30°C increases the bacterial number inside the eggs just after 7 to 10 days of storage (Humphrey and Whitehead, 1993). In the present study, prevalence was recorded 31.74 and 46.02% in egg shells and 11.11 and 19.04% in egg contents from fresh and stored collected eggs, respectively. Salmonella spp. occurrence is significantly higher in stored egg as compared to freshly collected eggs this might be due to the poor storage conditions.

***Conclusions*:** The results of the present study showed that prevalence of *Salmonella* spp. in farm eggs is lower than that of eggs collected from the market outlets. Secondly, higher incidence of Salmonella spp. was found in stored eggs as compared to fresh eggs. This difference in occurrence of Salmonella spp. showed that there is contamination of eggs during their supply from poultry farms to wholesale and retail markets. The poor storage and handling of eggs at the site of sale could be a source of contamination. The prevalence of Salmonella spp. was higher in egg storing

plastic trays than that of paper trays because of using of plastic trays for longer durations without being disinfected.

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