BIOLOGIA (LAHORE)
June, 2020, 66 (I), 95-99

PKISSN 0006 – 3096 (Print)
ISSN 2313 – 206X (On-Line)

Detection of *Salmonella* in poultry eggs and egg storing trays collected from poultry farms and local stores of Mianwali, Pakistan

ZULNAIRAH REHMAN[1*], AZMATULLAH KHAN[2], ABDUL WAJID[3] & AYESHA MAQBOOL[4]

[1]*Department of Biology, Virtual University of Pakistan.*
[2]*Department of Food Science and Human Nutrition University of Veterinary and Animal Sciences Lahore.*
[3]*Department of Biotechnology, Virtual University of Pakistan.*
[4]*Department of Molecular Biology, Virtual University of Pakistan.*

ARTICLE INFORMAION

Received: 06-11-2019
Received in revised form: 13-05-2020
Accepted: 15-06-2020

***Corresponding Author:**

Zulnairah Rehman:
ms160200131@vu.edu.pk

Short Communication

ABSTRACT

Bacterial genus *Salmonella* causes food borne illness called salmonellosis. It is characterized by the typhoid fever and gastroenteritis. To investigate the prevalence of *Salmonella* 240 samples were collected from different areas of District Mianwali. Out of 240, eighty samples were of commercial eggs, eighty were of non-commercial eggs and eighty were of egg storing tray samples collected from local markets and house farms of District Mianwali. The selective media used for the *Salmonella* was hektoen enteric agar. The incidence of *Salmonella* in egg shells of commercial eggs and non-commercial eggs was 32.5% and 27.5%. The *Salmonella* prevalence in egg content was 15% and 12.5% in samples of commercial eggs and non-commercial eggs. The *Salmonella* incidence was 40% and 32.5% in egg storing tray samples that were collected from local markets and house farms respectively. *Salmonella* prevalence in albumin and yolk was 15%, 12.5% and 10% 5% in egg content of commercial eggs and non-commercial eggs respectively. Overall *Salmonella* contamination in local market samples and house farm samples was 29.17% and 24.17% respectively. The *Salmonella* prevalence in samples of house farms was lower than the local markets samples collected across Mianwali District.

Keywords: Prevalence. *Salmonella*, Commercial eggs, Non- commercial eggs, Food borne diseases.

INTRODUCTION

The poultry industry is an important sector in national GDP of Pakistan. The birds used for the meat and egg production are referred to as poultry i.e. chicken, guinea fowl, ducks, pheasants, turkeys, pigeons (Muazu *et al.,* 2008; Addis, 2014). Economically chicken dominates among all of them (Kamani *et al.,* 2010). From poultry eggs and egg products are the major nutritive components of food. Eggs are a major source of proteins as well as of many minerals and vitamins. However, improperly treated eggs cause food borne diseases like salmonellosis in humans. Salmonellosis is the bacterial infection caused by the *Salmonella* and is characterized by typhoid fever and gastroenteritis. *Salmonella* infects humans and lies in the intestinal tract of poultry (Khan *et al.,* 2010). It is the major zoonotic pathogen distributed worldwide and is the main source of food borne disease in Pakistan. Salmonellosis is a major zoonotic disease that causes huge economic losses (Hafez, 2011). As it is zoonotic disease, there is always a great risk for human to get pathogen through contaminated food (Sabuj *et al*., 2019). *Salmonella* causes a lot of problems to public health that result in economic consequences worldwide (Collard *et al.,* 2008; Jing *et al.,* 2014). *Salmonella* species contaminate poultry products and cause Salmonellosis (Messens *et al.,* 2007). The optimum temperature for their growth is 37℃ (Bierer *et al.,* 1961). Cross-contamination of *Salmonella* also occurred and is another threat to poultry, as it increases rate of food contamination. Contaminated surfaces are not directly involved in an outbreak, but these surfaces are source of cross-contamination of *Salmonella* when used for preparing foodstuffs (Slader *et al.,* 2002). *Salmonella* has ability to infect human and it

Author's Contribution: Z. R., Performed lab work; **A. U. K.,** Designed the study; **A. W. & A. M.,** Reviewed the study and article

causes gastroenteritis. Even one to ten cells of pathogen gets entry into the host and invades the intestinal lining and then symptoms of salmonellosis appear that include headache, vomiting, nausea, diarrhea, abdominal pain, chill, and sometimes fever. The aim of present study was to isolate the *Salmonella* from commercial eggs, non-commercial eggs and egg storing trays to determine its prevalence in District Mianwali.

MATERIALS AND METHOD

Sample size and collection

Research was carried out to investigate the contamination of *Salmonella* in commercial eggs, non-commercial eggs and egg storing trays in different areas of District Mianwali, Pakistan. Total 240 samples were examined. Out of 240, 80 samples of eggs were of eggshells, 80 samples were of egg content and 80 samples were of egg storing trays. Out of the samples of 80 commercial eggs, 40 were of eggshells and 40 were of egg content. Samples of 80 non-commercial eggs were collected from the houses, and from these 80 egg samples, 40 were of eggshells and 40 were of egg content. Out of 80 samples of egg storing trays, 40 samples were collected from trays used in local markets, and 40 samples were collected from trays used in house farms. Samples of eggs and trays were unwashed and collected in polythene bags. Aseptic method was used for collection of samples. The collected samples were labeled and transported to the Food Safety Laboratory Department of Food Science and Human Nutrition University of Veterinary and Animal Sciences Lahore for further examination.

Isolation of *Salmonella*

Following method was used for isolation of *Salmonella* (Shahzad *et al.,* 2012).

From egg storing trays

For isolation of *Salmonella* cotton swab were used. The sterilized normal saline solution was used to wet the swab. This wetted swab was swabbed on the tray surface. Then it was reabsorbed into 10ml normal saline solution. It was followed by the transmission to 90ml buffered peptone water at 37℃ for 18 hours.

From surface of egg shell

The egg shell surface was swabbed with sterile cotton swab. It was engrossed into a 10ml normal saline solution. It was then transferred to 90ml buffered peptone water for 18 hours at 37℃.

From egg content

Eggs were washed with soap and brush. Surface of egg was sterilized by immersing eggs into 70% ethyl alcohol for 30 minutes. Eggs were air dried for 10 minutes in sterile chamber and cracked with sterile knife. Egg yolk and albumin were examined separately. 5ml of each sample and 5 ml of normal saline solution were mixed. Then it was transferred to the 90ml of buffered peptone water for 18 hours at 37℃.

Identification of *Salmonella*

1ml of pre enriched sample was transferred into 10ml of tetra thionate broth (TTB). Cultures from the TTB were streaked on selective media Hektoen enteric agar (HEA) plates and were incubated at 37℃ for 24 hours. Biochemical tests such as Methyl Red, Indole, Triple Sugar Iron and Gram Staining were performed to identify the *Salmonella* from suspected colonies. Descriptive analysis was carried out to calculate prevalence.

RESULTS

Colonies having black centers were observed for *Salmonella* from the 240 samples of commercial eggs, non-commercial eggs and trays used for egg storage. Out of 240 samples, 160 samples were of eggs and 80 samples were of egg storing trays. 80 samples of eggs were of commercial eggs and 80 samples were of non-commercial eggs. The overall prevalence of *Salmonella* was 29.17% in samples collected from local markets and was 24.17% in samples collected from house farms (Fig.1). Prevalence of *Salmonella* in eggshells was 32.50%, 27.50%, in egg content was 15%, 12.50% and in egg storing trays was 40%, 32.50% in samples collected from local markets and house farms respectively (Fig. 2). Out of 80 commercial egg samples, 40 samples were of eggshells and 40 samples were of egg content. In commercial eggs, 13 samples of eggshells out of 40 were positive for *Salmonella* and prevalence was 32.50%. In egg content samples, 6 samples out of 40 were positive for *Salmonella* and prevalence was 15%. From 40 egg storing tray samples, 16 samples were positive for *Salmonella* and prevalence was 40% (Table I).

Out of 80 non-commercial egg samples, 40 samples were of eggshells and 40 samples were of egg content. 11 samples out of 40 samples of eggshell were found to be positive for *Salmonella* and prevalence was 27.50%. 5 samples of egg content out of 40 were positive and prevalence was 12.50%. From 40 egg storing tray samples, 13 were positive for *Salmonella* and prevalence was 32.50% (Table II).

From egg content samples of commercial eggs collected from local markets, *Salmonella* was present in 6 samples. In these 6 samples, 6 out of 40 samples of albumin were having *Salmonella* and 4 samples out of 40 of yolk were positive. The prevalence of *Salmonella* in egg albumin and yolk was 15% and 10%, respectively (Table III).

From egg content samples of non-commercial eggs collected from house farms, overall 5 samples were positive for *Salmonella*. In these 5 samples, 5 samples out of 40 samples of egg albumin were positive for *Salmonella* and 2 out of 40 samples of egg yolk were having *Salmonella*. The prevalence of *Salmonella* in egg albumin and egg yolk was 12.50% and 5%, respectively (Table IV).

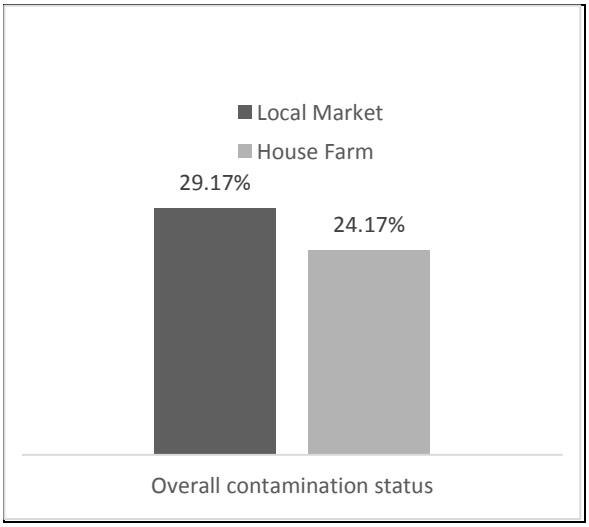

Fig. 1: Overall *Salmonella* prevalence in samples collected from local markets and house farms.

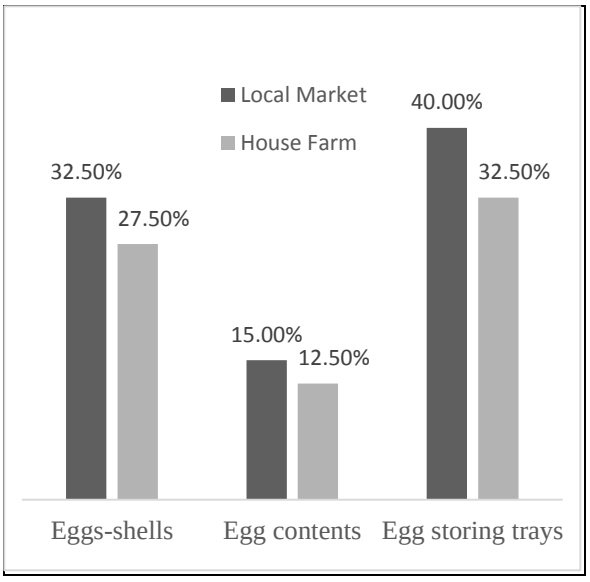

Fig. 2: Overall *Salmonella* prevalence in egg shells samples, egg content samples and egg storing trays samples collected from local markets and house farms.

Table I: *Salmonella* incidence in egg shells, egg content and egg storing trays in local markets samples.

Sample type	Number of sample analyzed	Number of samples tested +ve for *Salmonella*	Percentage of Incidence
Egg shells	40	13	32.50%
Egg contents	40	6	15%
Egg storing trays	40	16	40%

Table II: *Salmonella* incidence in egg shell, egg content and egg storing trays in samples from house farms.

Sample type	Number of sample analyzed	Number of samples tested +ve for *Salmonella*	Percentage of Incidence
Egg shells	40	11	27.50%
Egg contents	40	5	12.50%
Egg storing trays	40	13	32.50%

Table III: Incidence of *Salmonella* in egg content (albumin and yolk separately) in local market egg samples.

Sample type	Number of sample analyzed	Number of samples tested +ve for *Salmonella*	Percentage of Incidence
Egg contents	40	6	15.00%
Egg Albumin	40	6	15.00%
Egg Yolk	40	4	10.00%

Table IV: Incidence of *Salmonella* in egg content (albumin and yolk separately) in house farms samples.

Sample type	Number of sample analyzed	Number of samples tested +ve for *Salmonella*	Percentage of Incidence
Egg contents	40	5	12.50%
Egg Albumin	40	5	12.50%
Egg Yolk	40	2	5.00%

DISCUSSION

When eggs get in touch with fecal material, insects, nourish, during transportation and handling, eggshells become contaminated with *Salmonella*. According to the results of present study *Salmonella* prevalence in egg shell was 32.50% and 27.50% in local markets and house farms respectively. The reason of higher prevalence in eggshell is the fecal material that comes in contact with eggs, unhygienic conditions in farms and poor transportation. The presence of *Salmonella* in egg content was 15% and 12.50% in local markets and house farms respectively. Egg content gets *Salmonella* contamination due to infected ovaries of hen (Barnhart *et al.,* 1991). In the present study *Salmonella* prevalence is higher in egg shell than in egg content and similar findings have already been reported (Shahzad *et al.,* 2012; Tsegaye *et al*., 2016). Prevalence of *Salmonella* in egg storing trays was 40% and 32.50% in samples collected from local markets and house farms, respectively. The trays were contaminated by fecal material. The prevalence of *Salmonella* in trays was due to fecal material or environmental conditions (Bangrakulnonth *et al.,* 2004). The cause of high *Salmonella* occurrence in egg storing trays was reusing trays without disinfecting. The percentage of *Salmonella* was reported to be 45.8% in eggshells surface, 13.3% in egg content and 57.2% in egg storing trays in a study carried out in Chittagong city, Bangladesh (Mahmud *et al.,* 2013). A previous study in Faisalabad Pakistan reported the *Salmonella* prevalence 38.88%, 29.36% in eggshells, 15.07%, 10.31% in egg content and 43.93%, 28.78% in egg storing trays in samples collected from marketing outlets and poultry farms, respectively (Shahzad *et al*., 2012).The *Salmonella* prevalence was 40% in eggshell samples and 8.33% in egg content samples in Pakistan (Akhtar *et al.,* 2010).

REFERENCES

Addis, G., 2014. Review on Ethiopian poultry origin, domestication, classification and characterization of its production systems. *Middle East Journal of Scientific Research*, 22: 1025-1032.

Akhtar, F., Hussain, I., Khan, A., and Rahman, S. U., 2010. Prevalence and antibiogram studies of Salmonella enteritidis isolated from human and poultry sources. *Pak. Vet. J*, 30: 25-28.

Bangtrakulnonth, A., Pornreongwong, S., Pulsrikarn, C., Sawanpany alert, P., Hendriksen, K., and Aarestrup, F. M., 2004.Salmonella serovars in ovaries of layer hens at a time of slaughter. *J. Food Prot*, 54: 488-491.

Barnhart, H.M., Dreesen, D. W., Bastien, R., and Pancorbo, O. C., 1991. Prevalence of Salmonella enteritidis and other serovars in ovaries of layer hens at time of slaughter. *Journal of Food Protection*, 54: 488-491.

Bierer, B. W., Barnett, B. D., and Valentine, H. D., 1961.Experimentally killing Salmonella Typhimurium on egg shells by washing. *Poultry Science*, 40: 1009-1014.

Collard, J. M., Bertrand, S., Dierick, K., Godard, C.,Wildemauwe, C.,

Vermeersch, K. andQuinet, C., 2008. Drastic decrease of Salmonella Enteritidis isolated from humans in Belgium in 2005, shift in phage types and influence on foodborne outbreaks. *Epidemiology & Infection*, 136: 771-781.

Hafez, H. M., 2011.Enteric diseases of poultry with special attention to Clostridium perfringens.

Jing, Y. Y., Li, Y. S., Xin, J. K., & Chai, J. Q. (2014).Co-infection of ALV-J and Salmonella pullorum in laying hens. *Pak Vet J*, *34*(3), 372-6.

Kamani, J., Meseko, C. A., Oladokun, A. T., Tafarki, A. E., Abba, I., andDogo, G. I., 2010.Tetrameres infection in local poultry in Katsina State, Nigeria.

Khan, W. A., Khan, M. Z., Khan, A., andHussain, I., 2010.Pathological effects of aflatoxin and their amelioration by vitamin E in White Leghorn layers. *Pak. Vet. J*, 30: 155-162.

Mahmud, T., Hassan, M. M., Alam, M., Khan, M. M., Bari, M. S., and Islam, A., 2013.Prevalence and multidrug-resistant pattern of Salmonella from the eggs and egg-storing trays of retail markets of Bangladesh. *Population*.

Messens, W., Grijspeerdt, K., De Reu, K., De Ketelaere, B., Mertens, K., Bamelis, F., and Herman, L., 2007. Eggshell penetration of various types of hens' eggs by Salmonella entericaserovar Enteritidis. *Journal of Food Protection*, 70: 623-628.

Muazu, A., Masdooq, A. A., Ngbede, J., Salihu, A. E.,Haruna, G., Habu, A. K., and Jamilu, H., 2008.Prevalence and identification of species of Eimeria causing coccidiosisin poultry within Vom, Plateau State, Nigeria. *International Journal of Poultry Science*, 7: 917-918.

Sabuj, A. A. M., Rahman, M., Barua, N., Haque, Z. F., Pondit, A., Islam, K., & Hassan, M. M. (2019).Seroprevalence of Salmonella Infection in Commercial Layer Chickens in Cox's Bazar District, Bangladesh. *American Journal of Microbiological Research*, *7*(1), 19-23.

Shahzad, A., Mahmood, M. S., Hussain, I., Siddique, F., and Abbas, R. Z., 2012.Prevalence of salmonella species in hen eggs and egg storing-trays collected from poultry farms and marketing outlets of Faisalabad, Pakistan. *Pak J AgriSci*, 49: 565-8.

Slader, J., Domingue, G., Jørgensen, F., McAlpine, K., Owen, R. J., Bolton, F. J., and Humphrey, T. J., 2002.Impact of transport crate reuse and of catching and processing on Campylobacter and Salmonella contamination of broiler chickens. *Appl. Environ. Microbiol,* 68: 713-719.

Tsegaye, S., Beyene, W., Tesfaye, B., Tesfaye, S., &Feleke, A.(2016). Prevalence and antimicrobial susceptibility pattern of Salmonella species from exotic chicken eggs in Alage, Ziway and Shashemene, Ethiopia. *Africa Journal of Basic and Applied Science*, *8*(3),180-4.

Utrarachkij, F., Pornraungwong, S., Siripanichgon, K., Nakajima, C., Suzuki, Y., andSuthienkul, O., 2012.Possible horizontal transmission of Salmonella via reusable egg trays in Thailand. *International*