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CONTAMINATION OF CHICKEN EGGSHELLS AND EGG CONTENTS WITH *SALMONELLA* SPECIES FROM SELECTED FARMS IN KOSGAMA, COLOMBO DISTRICT

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ABSTRACT

Salmonellosis is a common, widely distributed foodborne disease. Consumption of raw or undercooked chicken eggs infected with *Salmonella* has been reported in association with salmonellosis cases; however, minimum attention has been paid to regulate the quality of eggs released for consumption. This study aimed to investigate the presence of *Salmonella* in eggs collected from selected farms from Kosgama area and to compare the egg quality of backyard and commercial farms. Randomly purchased eggs from selected chicken farms were analyzed for the presence of *Salmonella*. Egg content was mixed thoroughly, and 25.0 mL was inoculated into 225.0 mL of 1% Buffered Peptone Water (BPW) and incubated at 35^{0}C (24h). From the pre-enriched specimen, 0.1 mL was added to 10.0 mL of Rappaport Vassiliadis broth and incubated at 42^{0}C (24h). The same procedure was followed for shells. Isolated cultures were streaked on Brilliant Green Agar (BGA) and Xylose Lysine Deoxycholate Agar (XLD) and incubated at 35^{0}C for 24h. Colonies were investigated with Gram staining, biochemical tests and serotyping was carried out to identify the species. Of 78 eggs, 35 were from backyard and 43 from commercial farms. Six specimens (4 from shell and 2 from content) yielded *Salmonella* (7.69 %). Four of the positive specimens were from backyard farms (4/35, 8.91%) and remaining two (2/43, 3.62%) were from commercial farms. Isolates were identified as *S.* Typhimurium and *S.* Enteriditis. The prevalence of *Salmonella* was 7.69 % (n=6). The proportion of *Salmonella* showed no significant difference (p=0.782) between backyard and commercial farms.

Keywords: *Salmonella*, egg content, eggshells, backyard, commercial

INTRODUCTION

Salmonellosis is a significant foodborne disease that has public health significance. This disease is caused by *Salmonella*, a pathogen that has a reported distribution in poultry and swine **(Kwang *et al.*, 1996)**. Foodborne salmonellosis a common type of food poisoning that results in 1.35 million infected, 26 500 hospitalizations and 420 deaths in the USA annually **(CDC, 2019)**. Human infections of *Salmonella* are generally caused by the consumption of foods that are typically eaten raw that have been exposed to *Salmonella* through cross-contamination or contaminated water **(Nguse *et al.*, 2015).** Overall, the poultry eggs and associated egg products are one of the most expected vehicles of *Salmonella* outbreaks. Salmonellosis symptoms usually occur within a few hours of food consumption, and infected people may experience stomach cramps, vomiting, diarrhoea and fever. Most people will recover independently without treatment, but some will develop serious complications **(CDC, 2019).** In Sri Lanka, eggs have been the most common animal protein due to the high availability of eggs throughout the country. However, minimal attention has been paid to regulate the microbiological quality of eggs released to the market. In Sri Lanka, published data or evidence on the presence of *Salmonella* in poultry farms are minimal **(Kalupahana *et al.*, 2016).** The objective of our study was to isolate and identify *Salmonella* serovars present in poultry eggs collected from selected chicken farms.

MATERIAL AND METHODS

Study area

Farms were selected from Kosgama in Colombo district, Western Province, Sri Lanka. Both backyard and commercial farms were enrolled in the research. Farms were selected covering the whole area of Kosgama, and the number of farms was calculated according to the proportion of total farms in Kosgama.

Study design

A cross-sectional study was undertaken to determine the contamination of chicken eggshell and egg contents with *Salmonella* spp. from selected farms in Kosgama from October 2019 to June 2020.

Sample size

The sample size was determined based on the available updated prevalence of 5.4% *Salmonella* reported from China in 2019 **(Xie *et al.*, 2019)**. For statistical analysis, a total of 78 eggs from selected farms, including backyard and commercial, were collected.

Sampling

First, the farms were listed down from the highest supplying farm to the lowest, and higher values were selected. The number of eggs collected from each farm were calculated considering the number of eggs each farm supply to the market per month, and eggs from each chicken farm were selected randomly. In total, 78 eggs were purchased from both backyard and commercial farms, transported to the laboratory within 2 hours of collection.

Isolation, identification of *Salmonella*

The eggshell and egg content of 78 eggs were examined separately for *Salmonella.* Isolation of *Salmonella* was performed according to the ISO 6579-1:2017 standard with some modifications. Briefly, each egg was broken aseptically using a sterile spatula and eggshell, and egg content was separated. The content was ground under aseptic conditions. After grinding, 25.0 mL of the content was added to 225.0 mL of 1% Buffered Peptone Water (BPW) (OXOID, UK). Eggshell was crushed using sterile mortar and pestle, and the crushed eggshell was immersed in a separate container of 1% BPW according to the proportion of shell's weight and BPW quantity **(Sodagari *et al.*, 2019)**.
Both specimens were incubated at 35ºC for 24 hours. After incubation, 0.1 mL of sample was inoculated to 10.0 mL of selective enrichment broth; Rappaport Vassiliadis (HIMEDIA, India) and incubated at 42ºC for 24 hours. After enrichment, each solution was streaked on BGA (HIMEDIA, India) and XLD (HIMEDIA, India) and incubated at 35ºC for 24 - 48 hours. The presumptive colonies were cultured on Nutrient agar and tested with Gram staining, subjected to biochemical tests including KIA pattern, urease test and indole test for further identification **(Sodagari *et al.*, 2019)**. The isolates were then sent to Enteric Reference Laboratory, Medical Research Institute, Sri Lanka, for serotyping.

Serotype identification

All the *Salmonella* isolates were tested at the Enteric Reference Laboratory, using the slide agglutination method with O and H antisera. According to the

Kauffmann-White scheme, serotypes were identified based on the reactions with O and H group antigens **(Poppoff & Le Minor, 2001)**.

Data Analysis

Data of the present study were analyzed using the SPSS version 23 statistic software. Prevalence of *Salmonella* by collecting eggs from both backyard and commercial farms were expressed as percentages. Fisher's exact test was used to compare the prevalence of *Salmonella* between the backyard and commercial farms.

RESULTS

In the present study, 78 chicken eggs were collected, 35 eggs were from five backyard farms and 43 eggs from four commercial farms. Out of the total 78 chicken eggs, six samples were positive for *Salmonella* (7.69 %). From the six positive samples, 4 were from backyard farms (4/35, 8.91%), and the remaining two (2/43, 3.62 %) were from commercial farms. The distribution of six isolates among eggshells and egg contents were four (4/78, 5.13%) and two (2/78, 2.56%), respectively. The statistical analysis indicated no significant difference ($p = 0.782$) between contamination of chicken eggs with *Salmonella* spp. in the backyard and commercial farms. As shown in (Table 1), all isolated serovars were distributed among two serovars, *Salmonella* enterica Typhimurium and *Salmonella* enterica Enteritidis. Of positive samples, three were *Salmonella* enterica Enteritidis, and three were *Salmonella* enterica Typhimurium. From the four eggshells of positive samples, three were contaminated with *Salmonella* serovar Enteritidis and one with *Salmonella* serovar Typhimurium, while two positive egg contents were contaminated with *Salmonella* Typhimurium. None of the specimens was positive for *Salmonella* in both eggshells and egg contents.

Table 1 Distribution of *Salmonella* serovars isolated from chicken eggs

Farm type	Specimen type	Serovar isolated
Backyard	Egg content	*Salmonella* Typhimurium
	Egg Shell	*Salmonella* Typhimurium
	Egg Shell	*Salmonella* Enteritidis
	Egg Sehll	*Salmonella* Enteritidis
Commercial	Egg contrent	*Salmonella* Typhimurium
	Egg Shell	*Salmonella* Enteritidis

DISCUSSION

The present study identified a 7.69% prevalence of *Salmonella* in chicken eggs produced in chicken egg farms from Kosgama. This prevalence indicates that eggs may be one of the sources of *Salmonella* transmission among consumers of eggs. A study conducted by **Kalupahana *et al.* (2016)** in the Kandy district of Sri Lanka tested 1000 eggs purchased from 100 different retail outlets for the presence of *Salmonella* spp. In this population, *Salmonella* was found on 15 eggs showing a 15% prevalence **(Kalupahana *et al.*, 2016)**. **Kalupahana *et al.* (2016)** note that the prevalence of *Salmonella* is 12% of a study done testing 128 boiler meat samples representing 4 provinces in the country **(Kalupahana *et al.*, 2016)**. Among foodborne diseases, salmonellosis is a leading cause worldwide, including in Sri Lanka. In Asian countries, the prevalence of salmonellosis has been reported as 5.4% in China **(Xie *et al.*, 2019)** and 20% in the Tamil Nadu region of India in 2019 **(Sangeetha *et al.*, 2019)**.

The present study highlighted a higher level of eggshell contamination (5.13%) than egg content (2.56%) with *Salmonella* spp. The deep litter open house system is the most commonly practiced method in Sri Lankan poultry farms. Eggshell contamination may happen due to this method since it has less bio-security and a high chance of *Salmonella* contamination. The outer eggshell appearance of eggs from the commercial and backyard farms were not clean, and they were contaminated with faecal matter. This implicates a high risk to the general population because unclean eggs can cross-contaminate other food, and consumption of raw or undercooked eggs can be a health risk to consumers. Egg contamination can occur through two possible transmission routes, namely vertical transmission and horizontal transmission **(Gantois *et al.*, 2009)**. This contamination of *Salmonella* in the egg content may be due to infection in the ovary of birds, while surface contamination of eggs can be through the feed, faecal material, storage material and may happen during handling of chicken eggs. Improving proper hygienic standards and practices in handling, transportation, and storage may reduce the *Salmonella* prevalence in poultry. After collection, all the eggs were stored in clean boxes, and cross-contamination was prevented during transportation until the examination of eggs. This ensured that there was no contamination of *Salmonella* in transported eggs during the transport process until that reached the laboratory.

Serotyping results of the present study revealed identified isolates, namely *Salmonella* enterica Typhimurium and *Salmonella* enterica Enteritidis, which were highly human pathogens. But the recent survey conducted by **Kalupahana *et al.* (2016)** in Kandy district, Sri Lanka, has isolated *Salmonella* serovars Mbandaka, Braenderup, Corvallis and Emek, implicating the absence of serovars Enteritidis and Typhimurium in raw table eggs in the Kandy district. This may be due to the implementation of strict *Salmonella* control programs, including the killed vaccine containing *S.* Enteritidis and *S.* Typhimurium for layer flocks **(Kalupahana *et al.*, 2016)**. In most countries, *Salmonella* enterica serovar Enteritidis and *Salmonella* enterica serovar Typhimurium are common causes for poultry associated foodborne illnesses in humans **(Galanis *et al.*, 2006; Hendriksen *et al.*, 2011),** while in Sri Lanka, *Salmonella* serovar Pullorum and *Salmonella* serovar Gallinarum are the commonly reported poultry specific serovars **(DAPH, 2008)** absent in the present study, and a recent study was done in 2016. This may also due to the vaccination program, which introduced as *Salmonella* control programs for breeder flock. A study carried out by **Weerasooriya *et al.* (2008)** show the presence of *S*. Typhimurium in a poultry processing plant in the Kandy district **(Weerasooriya *et al.*, 2008),** while a study done by Wijemanne report *S*. Enteritidis in a poultry breeder farm **(Wijemanna, 2008)**.

According to the annual report published by the Department of Animal Production and Health (DAPH) in 2014, all the breeder farms in Sri Lanka have been instructed to carry out regular screening programs followed by official verification by the relevant Veterinary Investigation Centers and the Veterinary Research Institute. During testing, *Salmonella* organism has been isolated from some of the hatcheries **(DAPH, 2014)**. Therefore, the killed *Salmonella* vaccine was allowed to be used in selected breeder farms to control the *Salmonella* infection. In Sri Lanka, some breeder farms maintain *Salmonella* free status using testing and culling of infected birds. As a part of the vaccination program in Sri Lanka, breeder farms and commercial layer farms are allowed to use SG 9R live vaccine, and *Salmonella* killed vaccine, respectively **(DAPH, 2014)**. Generally, the killed vaccine containing *Salmonella* serovars Enteritidis and Typhimurium and live vaccine with serovar Gallinarum has been shown to exert cross-protection **(Van Immerseel *et al.*, 2005)**. Developed countries have international poultry control programs, including on-farm monitoring, diverting contaminated eggs for processing, culling infected flocks, cleaning and disinfection of sheds, maintaining cold chain of eggs, and vaccination of flocks, and it resulted in a significant decrease in egg-related salmonellosis **(Moffatt & Musto, 2013)**.

Most of the countries have guidelines and regulations to ensure the quality of food they release to retails. According to the food safety guidelines in the UK, they have "best before date" (BBD) for eggs, and it must not exceed 28 days from the date of lay. Eggs must reach the final consumer (user) within 21 days from the date of lay **(O'Brien, 2013).** There is no available literature for our local setting with our temperature and humidity parameters and best before the date for eggs in retail shops. Sri Lankan Standard Institute (SLS/959:1992) has also recommended some specifications only for packing eggs put into the market, and a few are adhering to those. However, the local standards do not specify microbiological quality requirements for chicken eggs though maintaining microbiological quality in eggs is important to protect the consumer. In Sri Lanka, the egg consumption rate increases year by year **(DAPH, 2014)** with the high availability of table eggs throughout the country. Since the consumption has been increased, effective steps are needed to monitor the egg quality sold in local markets. Faecal contamination on the eggshell is the frequent route of infection **(Radkowski, 2001),** which may be able to contaminate egg contents by migration through the eggshell and membranes, but it can be effectively reduced by storing eggs at ambient temperature, cleaning and disinfection of the environment, and good production and handling practices.

In Sri Lanka, there are not enough epidemiological data on foodborne infections since it doesn't have an active surveillance system to monitor them, and it doesn't mean poultry of Sri Lanka are at the level of *Salmonella* free condition. However, identifying *Salmonella* prevalence as 7.69 % of chicken eggs purchased from farms is a serious concern. To minimize foodborne infections, consumers should be educated about risks associated with improper handling and consumption of table eggs. The data and findings revealed from this study are important to poultry farmers, veterinarians, public health policymakers and finally, consumers. This study suggests the importance of introducing new laws and regulations to ensure the microbiological quality of eggs, establishing good animal health

practices in poultry farms and maintaining refrigeration chain throughout egg transportation, storage and commercialization to prevent the contamination of chicken eggs. However, refrigeration is not a mandatory requirement in Sri Lanka.

CONCLUSION

The findings of this present survey provide a novel dataset of the prevalence of *Salmonella* spp. in chicken eggs at farms, including backyard and commercial in the Colombo district of Sri Lanka. Out of the 78 chicken eggs, 6 chicken eggs were contaminated with highly pathogenic *Salmonella* enterica Typhimurium and Enteriditis, representing a potential risk to consumers. Contamination of the eggs with *Salmonella* spp. happens either by penetration through the shell or by the passage from the hen's intestinal tract to the reproductive tract. The causes of egg contamination can be due to external contact with contaminated surfaces, poor hygienic practices, and insufficient knowledge of egg collectors about the possible diseases and ways of contamination. This a recent investigation of *Salmonella*'s epidemiology at the farm level and findings of this important to the knowledge of general consumers, poultry producers, veterinary officers, and public health inspectors.

To enhance the microbiological quality of chicken eggs, introducing proper hygienic practices and educational programs among food handlers, implementing *Salmonella* control programs among poultries are required. The control programs can include a proper vaccine schedule and routine checkups for *Salmonella* infections in hens. Another recommendation is its need to regulate active national and regional surveillance systems on foodborne diseases, including *Salmonella*. To increase the strength of the food safety system, proper rules, regulations for farm handlers and food safety standards should be implemented by the responsible authorities. Due to the pandemic of COVID-19, the process of sample collection and access to observe the egg production procedure, including hygienic practices of farmers, were limited. Since it is undergraduate research, the allocated period, resources and number of farms included in this study were limited. But if a comprehensive study was carried out to isolate and identify *Salmonella* with the farms' process, it will demonstrate a wide knowledge.

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Conflict of interest: The authors declare that there is no conflict of interest.

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