PJHRD Philippine Journal of Health Research and Development

RESEARCH COMMUNICATION

Detection of putative *Salmonella enterica* in retail chicken egg from a selected public market in the City of Manila

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ABSTRACT

Background: Salmonellosis is one of the most reported bacterial foodborne illnesses worldwide. *Salmonella* outbreaks are also prevalent in the Philippines, with egg-containing food and feces of chicken as implicated sources. The presence of *Salmonella* in eggshells and in egg content poses a significant threat to public health. Hence, this study aimed to determine the presence of *S. enterica* from different parts of chicken eggs sold in a public market in the City of Manila.

Methodology: A descriptive study design was employed to detect the presence of *Salmonella* spp. in different parts of retail chicken eggs. A total of 72 egg samples from 24 stalls were included. The methodology for isolation and identification of *Salmonella* followed the guidelines set by the US Food and Drug Administration as seen in the Bacteriological Analytical Manual with some additions and modifications.

Results: Contaminated eggs were found in 21 (87%) of the 24 stalls. A total of 29 (40%) out of 72 eggs were identified as the source of putative *Salmonella* isolates. Nineteen (66%) eggs had putative *Salmonella* isolates from the eggshell, while 7 (24%) had putative *Salmonella* isolates from the egg content. There were three (10%) eggs with both eggshell and egg content possibly contaminated with *Salmonella*.

Conclusion: The presence of putative *Salmonella* and *Enterobacteriaceae* highlight the need to strengthen food safety at the production and distribution levels of retail chicken eggs. There is also a need to establish a national surveillance system along with strengthened diagnostic capacity for *S. enterica* in the Philippines.

Keywords: *Salmonella, Salmonella enterica serotypes, chicken eggs, public market*

Introduction

Salmonellosis is one of the most reported bacterial foodborne illnesses worldwide. It usually manifests as gastroenteritis or enteric fever, accounting for 93.8 million cases and 26 million cases, respectively, each year [1] . *Salmonella* outbreaks are also prevalent in the Philippines (Carmela Reyes-Estrope, 2011; GMA News TV, 2008; Medlineplus, 2017), with eggs containing food and feces of chicken as implicated sources (Carmela Reyes-Estrope, 2011; Medlineplus, 2017). Contrary to the smaller values of isolated *Salmonella* in various countries in Asia which ranged from 1 to 5%, a study in the Philippines showed that 33% of samples from eggshells tested positive for *Salmonella* (Palmes *et al.*, 2017).

S. enterica, commonly found in retail egg, is cited as one of the primary causes of foodborne-disease outbreaks (Foley *et al.*, 2011). The presence of *S. enterica* in eggshells and in egg content poses a significant threat to public health. Hence, this study aimed to determine the presence of putative *S. enterica* by biochemical testing from different parts of chicken eggs sold in a public market in the City of Manila.

Methodology

A descriptive study design was employed to detect the presence of *Salmonella* spp. in different parts of retail chicken eggs sold in a public market in the City of Manila, the Philippines. Total sampling was done at the market level as all stalls in the public market were included. An egg from each stall was obtained and triplicate sampling was done by obtaining an egg at three different instances. A total of 72 egg samples from 24 stalls were included.

The preparation, pre-enrichment, selective enrichment, isolation, and identification of *Salmonella* primarily followed the guidelines set by the US Food and Drug Administration as seen in the Bacteriological Analytical Manual (BAM) (Andrews *et al.*, 2007) with some additions and modifications. Some procedures were adopted from a similar study by Suresh and colleagues (Suresh *et al.*, 2006). All samples were examined within 6 hours upon purchase.

Preparation and Pre-enrichment

Eggshell

Sterile cotton swabs with Tryptic Soy broth (TSB) were used to swab the entire surface of the shell. Samples were inoculated in 10 mL TSB in cotton-plugged tubes, and were incubated for 24±2 hours at 35°C.

Egg content

The eggs were submerged in 3:1 disinfection solution consisting of 3 parts 70% isopropyl alcohol to 1 part iodine/potassium iodide for 10 seconds. Eggs were cracked aseptically and samples were placed in a Stomacher Circulator for about 60 seconds until yolks were completely mixed with the albumen. Afterwards, 25 mL of the mixture was inoculated in 225 mL of TSB. It was allowed to settle for 60±5 minutes at room temperature then mixed by swirling with the pH maintained at 7. Samples were incubated for 24±2 hours at 35°C.

Selective Enrichment

For both eggshell and egg content, 1 mL and 0.1 mL of TSB cultures were transferred to 10 mL of Tetrathionate broth (TTB) and 10 mL Rappaport-Vassiliadis (RV) medium, respectively. Both were incubated by water bath for 24±2 hours at 42-44°C.

Isolation of Salmonella spp.

After 24 hours, the incubated TTB and RV medium were mixed, and 3 mm loopful or 10 µL were streaked on Xylose Lysine Deoxycholate (XLD) agar and Bismuth Sulfite agar (BSA). The plates were then incubated for 24±2 hours at 35°C.

Negative controls including *Citrobacter koseri*, *Citrobacter freundii*, *Enterobacter faecalis*, *Enterobacter cloacae*, *Escherichia coli*, and *Shigella sonnei* were used.

After incubation, the typical colonies present in XLD were picked, stored, and coded in Brain Heart Infusion Agar (BHIA) slants as back-up. In the absence of typical colonies from XLD, atypical colonies were picked instead. Atypical *Salmonella* colonies present as yellow colonies with or without black centers in XLD, or green color with little to no darkening of the surrounding medium in BSA.

Identification of Salmonella Isolates

The putative colonies from the BSA and XLD plates were inoculated into the Triple Sugar Iron (TSI) agar, Motility Indole Lysine (MIL) medium, and Urea broth, and were incubated for 24±2 hours at 35°C. All cultures with a urease-positive reaction, alkaline butt, or A/A reaction in TSI and LDC-negative reaction in MIL were regarded as negative for *Salmonella* spp. and were excluded from further biochemical tests.

The remaining colonies were inoculated in Simmons Citrate Agar, Phenol Red Lactose Broth (PRLB), Phenol Red Sucrose Broth (PRSB), and Methyl Red-Voges Proskauer (MR-VP) broth, and were incubated at 35oC. The Simmons Citrate Agar, PRLB, and PRSB were examined after 24 hours, while the MR-VP was examined after 48-60 hours. All cultures that presented a positive reaction to phenol red lactose and/or sucrose broth were regarded as negative for *Salmonella* spp. The discard criteria are shown in Table 1.

Results

All TSB inoculated separately with the chicken egg samples revealed growth of bacteria after 24±2 hours of incubation at 35oC aerobically. On selective isolation media (XLD and BSA), a total of 184 colonies were detected from chicken egg samples. After biochemical tests, 144 (78%)

Table 1. *Criteria for Discarding Non-Salmonella Cultures [9]*

Test	Results
Triple sugar iron (TSI) and Lysine decarboxylase (LDC)	alkaline (K) butt or A/A in TSI and negative (yellow bottom) LDC
Urease	positive (purple red color)
Phenol Red Lactose Broth	positive (yellow color and/or gas)
Phenol Red Sucrose Broth	positive (yellow color and/or gas)

colonies were identified as non-*Salmonella*. Of the remaining 40 colonies, 4 were identified to be non-*Salmonella* (2 *Providencia* spp. [1%] and 2 *Shigella* spp. [1%]).

The 36 (20%) remaining isolates presented atypical reactions, which did not correspond to any *S. enterica* serotypes identifiable through the biochemical tests performed. However, these isolates may remain to be putative *Salmonella* (Figure 1).

Contaminated eggs were found in 21 (87%) of the 24 stalls. A total of 29 (40%) out of 72 eggs were identified as the source of putative *Salmonella* isolates. Nineteen (66%) eggs had putative *Salmonella* colonies from the egg shell, while 7 (24%) had putative *Salmonella* isolates from the egg content. There were 3 (10%) eggs with both eggshell and egg content possibly contaminated with *Salmonella*.

Discussion

The high percentage of stalls with possible *Salmonella*-contaminated eggs may indicate the lack of sanitary conditions in certain poultry farms which allows *Salmonella* to persist. These include conditions such as infection of chickens prior to egg formation (vertical transmission (Soto-Arias *et al.*, 2014)) or the fecal-oral transmission (horizontal transmission (Food and Environmental Hygiene Department, 2004; Seockmo *et al.*, 2016; Soto-Arias *et al*., 2014)) from the excreta of infected rodents, pets, or farmers, with the latter reported as the most common route for *Salmonella* contamination. Farms with environmental contamination and higher rodent densities directly correlate with the production of contaminated eggs, highlighting the need for prevention of infection among laying hens to reduce *Salmonella* outbreaks linked to the consumption of chicken eggs (Trampel *et al.*, 2014). There may also be poor processing practices in farms including the lack of cleaning and disinfection of eggs. Although the Food and Agriculture Organization has released guidelines on washing and disinfection procedure for eggs after sorting and handling (Dunn & Martin, 1971), compliance with these measures remain questionable. Some post-collection methods (e.g., pasteurization) have demonstrated reduction of *Salmonella* contamination, while others continue to be debated on washing of eggs as it may possibly introduce *Salmonella* from the shell to the content (Whiley & Ross, 2015). Studies have also suggested the penetration of *Salmonella* from the shell to the egg content, as a result of (1) the long duration of opened pores after oviposition linked to egg cuticle immaturity and/or the (2) exposure of eggs to a cooler environment that may lead to the development of negative pressure or cuticle dehydration (Food and Environmental

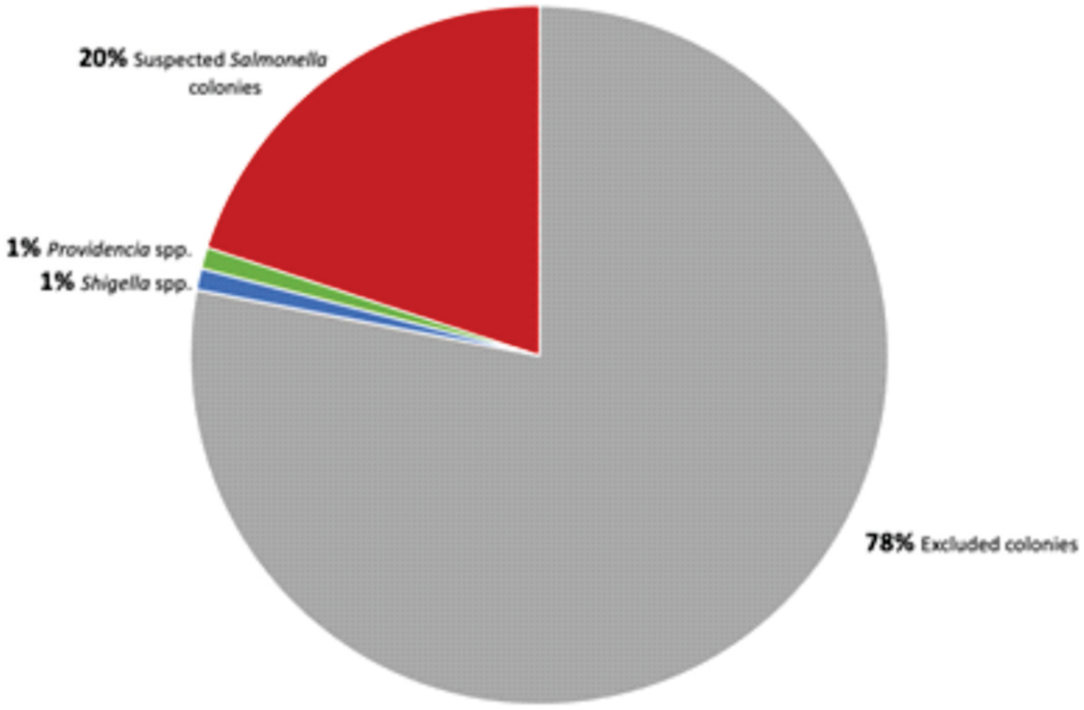

Figure 1. *Isolates identified based on their biochemical profile (n=184), January 2018*

Hygiene Department, 2004). Several studies support the putative contamination of chicken eggs with *Salmonella*. Studies in Bangkok and Iran revealed the presence of *Salmonella* contamination in eggshells by Polymerase Chain Reaction (PCR) (Food and Environmental Hygiene Department, 2004; Gantois *et al.*, 2009). In the Philippines, a recent study to determine the level and distribution of *Salmonella* spp. in chicken eggs in Metro Manila revealed that 13 (12.75) of 102 pooled eggshell samples (20 eggs/pool) were positive for *Salmonella* (Aguila & Umali, 2021).

Among 2,463 *Salmonella* serotypes, 2,443 are under *S. enterica*, with only a limited number being identifiable through biochemical means. This may account for the occurrence of undetermined colonies (Wu *et al.*, 2016). It is also noteworthy that 60% of the *S. enterica* serotypes are under subsp. enterica, which is the only type found in warm-blooded animals such as chickens and humans (White, 2010). Although hydrogen sulfide production may play a crucial role in the detection and identification of *S. enterica* serotypes, in this study, none of the colonies were considered as non-*Salmonella* based on their production of hydrogen sulfide. Based on the biochemical tests, all 36 undetermined colonies were negative for hydrogen sulfide production, which may point to the presence of *S.* Paratyphi, which does not produce hydrogen sulfide. Other studies have also reported the existence of *Salmonella* strains that exhibit atypical biochemical reactions (Andino & Hanning, 2015; Barbour *et al.*, 1984; Brenner *et al.*, 2000; Yamasaki *et al.*, 2007).

A recent study noted strains that did not produce hydrogen sulfide despite its well-known characteristic of hydrogen sulfide production (WHO Global Foodborne Infections Network, 2010). These strains included *S*. Sentfenberg, *S*. Derby, *S.* Heidelberg, *S*. Typhimurium, and *S*. Enteritidis, which are serovars that have been documented to be present in eggs and other poultry products.18 Additionally, a study had also reported that some strains of *S*. enteritidis were negative for lysine decarboxylase (Yamasaki *et al.*, 2007), while another study recorded the existence of non-motile *S*. Typhimurium (Barbour *et al.*, 1984).

However, findings on these atypical *Salmonella* strains still warrant further research, especially regarding their prevalence in the Philippines. This is contingent on the need to strengthen diagnostic capacity and establish a national surveillance system for *S. enterica* in the Philippines, which may help establish the prevalence of *Salmonella* in different poultry products and monitor outbreaks associated with *Salmonella* foodborne infections. Such surveillance systems may not only aid in future research on *Salmonella* but may also inform policies and programs on preventing *S. enterica* infections. Since the possibility that the undetermined colonies in this study may potentially be *S. enterica*, further testing (e.g., serotyping and molecular assays) is recommended in future surveys to confirm the presence or absence.

Additionally, the *Enterobacteriaceae* species were also identified such as *Providencia* spp. and *Shigella* spp. A number of *Providencia* species such as *P. alcalifaciens* have been reported to be associated with diarrhea among children and travellers (Stepień-Pyśniak, 2010). Similarly, *Providencia* was among one of the *Enterobacteriaceae* found in the yolks of commercially-available chicken eggs in a study conducted in the West Indies (Albert *et al.*, 1998). Another study in Poland reported the presence of P. stuartii and *Salmonella* spp. in both the yolks and shells of examined chicken eggs (Sabarinath *et al.*, 2009). On the other hand, although humans and primates are the natural reservoirs of *Shigella* spp., a study suggests the existence of new hosts which include chickens (Shi *et al.*, 2014). *Shigella* spp. contamination must also be addressed as it may cause severe inflammatory colitis in affected individuals. The presence of putative *Salmonella* and *Enterobacteriaceae* highlight the need to strengthen the implementation of food safety practices at the production and distribution levels of retail chicken eggs.

Some limitations of the study include time and financial constraints and availability of the media, resulting in unperformed tests such as KCN broth, malonate broth, and flagellar tests. The completion of all biochemical tests prescribed by BAM (Andrews *et al.*, 2007) could have further narrowed down the putative *Salmonella* isolates.

Conclusions

The study revealed that some egg samples are putative to be contaminated with *Salmonella*, with majority of putative isolates obtained from eggshells. Furthermore, most of the stalls in the study had putative Salmonella-contaminated eggs. Further testing such as serotyping and molecular assays are needed to guarantee the presence of *S. enterica* in the examined chicken eggs. This highlights the need to establish a national surveillance system along with strengthened diagnostic capacity for *S. enterica* in the Philippines, which may help establish the prevalence of *Salmonella* in different poultry products and monitor outbreaks associated with *Salmonella* foodborne infections. The presence of putative *Salmonella* and *Enterobacteriaceae* highlight the need to strengthen the

implementation of food safety practices at the production and distribution levels of retail chicken eggs.

References

1. Elhadi N, Aljindan R, Aljeldah M. (2013) Prevalence of nontyphoidal *Salmonella* serogroups and their antimicrobial resistance patterns in a university teaching hospital in Eastern Province of Saudi Arabia. Infection and Drug Resistance, 6:199–205. https://doi.org/10.2147/IDR.S51184
2. Judd MC, Grass JE, Mintz ED, Bicknese A, Mahon BE. (2015) *Salmonella enterica* Paratyphi A Infections in Travelers Returning from Cambodia, United States. In Emerg Infect Dis. Blackwell Publishing Ltd. https://doi.org/10.1111/j.1469-0691.2012.03821.x
3. Medlineplus. (2017). Salmonella Infections. National Library of Medicine. https://medlineplus.gov/salmonellainfections.html
4. GMA News TV. (2008). *Salmonella* found in 18 pupils in Manila food poisoning. GMA News Online. https://www.gmanetwork.com/news/news/nation/122931/salmonella-found-in-18-pupils-in-manila-food-poisoning/story/
5. Carmela Reyes-Estrope. (2011). Salmonella found cause of Bulacan food poisoning. Inquirer. https://newsinfo.inquirer.net/17168/salmonella-found-cause-of-bulacan-food-poisoning
6. Palmes A, Kahulugan P, Enal M. (2017) Prevalence and antibiotic resistance of *Salmonella* spp. from chicken eggshell. http://scinet.dost.gov.ph/union/ShowSearchResult.php?s=2&f=&p=&x=&page=&sid=1&id=Prevalence+and+antibiotic+resistance+of+%3Cem%3ESalmonella%3C%2Fem%3E+spp.+from+chicken+eggshell&Mtype=ANALYTICS
7. Foley SL, Nayak R, Hanning IB, Johnson TJ, Han J, Ricke SC. (2011) Population dynamics of *Salmonella enterica* serotypes in commercial egg and poultry production. Applied and Environmental Microbiology, 77(13):4273–4279. https://doi.org/10.1128/AEM.00598-11/FORMAT/EPUB
8. Andrews W, Wang H, Jacobson A, Hammack T, Hua Wang AJBGGZ, TH. (2007) Bacteriological Analytical Manual: Salmonella. https://www.fda.gov/media/83330/download
9. Suresh T, Hatha AAM, Sreenivasan D, Sangeetha N, & Lashmanaperumalsamy P. (2006) Prevalence and antimicrobial resistance of *Salmonella enteritidis* and other salmonellas in the eggs and egg-storing trays from retails markets of Coimbatore, South India. Food Microbiology, 23(3):294–299. https://doi.org/10.1016/j.fm.2005.04.001
10. Soto-Arias JP, Groves RL, Barak JD. (2014) Transmission and retention of *Salmonella enterica* by phytophagous hemipteran insects. Applied and Environmental Microbiology, 80(17), 5447–5456. https://doi.org/10.1128/AEM.01444-14
11. Food and Environmental Hygiene Department. (2004) Risk Assessment Studies *Salmonella* in Eggs and Egg Products.
12. Seockmo K, Eduardo X, Thomas K, Ladisch MR. (2016) Salmonella in Shell Eggs: Mechanisms, Prevention and Detection. Journal of Nutrition & Food Sciences, 06(01). https://doi.org/10.4172/2155-9600.1000455
13. Trampel DW, Holder TG, Gast RK. (2014) Integrated farm management to prevent *Salmonella Enteritidis* contamination of eggs. Journal of Applied Poultry Research, 23(2):353–365. https://doi.org/10.3382/japr.2014-00944
14. Dunn C, Martin WJ. (1971) Comparison of Media for Isolation of *Salmonellae* and Shigellae from Fecal Specimens. Applied Microbiology, 22(1), 17–22.
15. Whiley H, Ross K. (2015) *Salmonella* and eggs: From production to plate. International Journal of Environmental Research and Public Health, 12(3): 2543–2556. https://doi.org/10.3390/ijerph120302543
16. Gantois I, Ducatelle R, Pasmans F, Haesebrouck F, Gast R, Humphrey TJ, van Immerseel F. (2009) Mechanisms of egg contamination by *Salmonella Enteritidis*: Review article. In FEMS Microbiology Reviews 33(4):718–738. https://doi.org/10.1111/j.1574-6976.2008.00161.x
17. Aguila SRB, Umali Dv. (2021) Isolation and genetic characterization of *Salmonella* spp. In chicken eggs from public wet markets in Metro Manila, Philippines. Philippine Journal of Veterinary Medicine, 677:74–82.
18. Wu F, Xu X, Xie J, Yi S, Wang J, Yang X, Yang C, Liang B, Ma Q, Li H, Song H, Qiu S. (2016) Molecular characterization of *Salmonella enterica* serovar Aberdeen negative for H2S production in China. PLoS ONE, 11(8). https://doi.org/10.1371/journal.pone.0161352
19. White NJ. (2010) *Salmonella* typhi (Typhoid Fever) and *S*. paratyphi (Paratyphoid Fever).
20. Brenner FW, Villar RG., Angulo, F. J., Tauxe, R., & Swaminathan, B. (2000). *Salmonella Nomenclature*. In Journal of Clinical Microbiology 38(7).
21. Andino A, Hanning I. (2015) *Salmonella enterica*: Survival, colonization, and virulence differences among serovars. In Scientific World Journal (Vol. 2015). Hindawi Publishing Corporation. https://doi.org/10.1155/2015/520179

22. Yamasaki S, Hara K, Izumiya H, Watanabe H, Misawa N, Okamoto K, Takase K. (2007) Lysine Decarboxylase-Negative *Salmonella enterica* Serovar Enteritidis: Antibiotic Susceptibility, Phage and PFGE Typing. In J. Vet. Med. Sci 69(8).
23. Barbour EK, Nabbut NH, Al-Nakhli HM. (1984) A non-motile *salmonella* mutant (6, 7:-:-) and two serological variants encountered in Saudi Arabia. Zentralblatt Fur Bakteriologie Mikrobiologie Und Hygiene - Abt. 1 Orig. A, 256(4):548–552. https://doi.org/10.1016/s0174-3031(84)80033-5
24. WHO Global Foodborne Infections Network. (2010). Laboratory Protocol: "Isolation of Salmonella spp. From Food and Animal Faeces" 5th Ed. June 2010.
25. Stepień-Pyśniak D. (2010). Occurrence of gram-negative bacteria in hens' eggs depending on their source and storage conditions. Polish Journal of Veterinary Sciences, 13(3):507–513, 13(3):507–513.
26. Albert MJ, Faruque ASG, Mahalanabis D. (1998) Association of Providencia alcalifaciens with Diarrhea in Children. In Journal of Clinical Microbiology, 36(5). https://journals.asm.org/journal/jcm
27. Sabarinath A, Guillaume V, Guillaume B, Mathew V, DeAllie C, Nath Sharma R. (2009) 4 Bacterial contamination of commercial chicken eggs in Grenada. In West Indies West Indian Veterinary Journal, 9(2).
28. Shi R, Yang X, Chen L, Chang HT, Liu HY, Zhao J, Wang XW, Wang CQ. (2014) Pathogenicity of Shigella in chickens. PLoS ONE, 9(6). https://doi.org/10.1371/journal.pone.0100264