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Prevalence of Salmonella species in poultry eggs sold in Yenagoa metropolis Bayelsa state

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Abstract

Salmonella remains one of the most notable food-borne bacterial pathogens. It is also associated with poultry and poultry products including eggs. This study investigated *Salmonella* prevalence in poultry egg shell, its content and the possible risk factors driving contamination in Yenagoa metropolis, Bayelsa State, Nigeria. Three different study sites were surveyed which includes NDU Poultry Farm, Opolo Market outlet and Tombia Market outlet. A total of 150 eggs were collected and culturally examined. A total of 880 *Salmonella* isolates spread across 10 serovars were obtained from the egg samples; 269 (30.5%) isolates were obtained from NDU Poultry Farm, 297 (33.7%) isolates were obtained from Opolo Market outlet and 314 (35.6%) isolates were obtained from Tombia Market outlet. Sales of eggs in the market appear to be a strong factor encouraging contamination in addition to poor bio-security and unhygienic handling of eggs on the farm.

Keywords: Salmonella; Poultry; Egg; Biosecurity; Nigeria.

Introduction

Poultry eggs are high valuable foodstuffs consumed by humans, they provide a significant amount of animal protein, they are cheap, available and having little or no limitations in acceptance across the sociocultural and religious divide (Idowu *et al*., 2010; Bettridge *et al*., 2014). Poultry is considered an important source of food contamination.

Nigeria produces an average of 3.8 million poultry eggs annually with a population of approximately 180 million. Recently, a national survey reported Salmonella prevalence to be 43.6% among commercial poultry farms in Nigeria (Fagbamila, 2017).

Food-borne diseases and illness are associated with the consumption of contaminated eggs. Poultry eggs and its products when improperly handled can be a source of food-borne diseases such as Salmonellosis.

Salmonellosis is an important public health burden in most developing countries and

constitutes a major food-borne pathogen in the developed world (Zhang*et al*., 2015).

The term Salmonella refers to a group of bacteria that cause Salmonella infection, or Salmonellosis, in the intestinal tract. Salmonella are gram-negative, rod-shaped bacilli that can cause Salmonellosis, a diarrhoea illness in humans. It is a Gram-negative bacterium that usually has a cell wall composed of a thin layer of peptidoglycan, covered by a membrane. The bacteria live in the gut of infected humans and animals. Some animal and human strains can make humans sick. Salmonella is a major cause of human bacterial infections in the world. According to the Centers for Disease Control and Prevention (CDC), it affects thousands of people every year, leading to 19,000 hospitalizations and 380 deaths. Salmonella infection is often linked to contaminated water or foods, especially meat, poultry, and eggs. Symptoms include abdominal cramps and vomiting, which tend to appear after infection. Most people recover after 4 to 7 days without treatment, but a person with severe diarrhea may need hospital treatment (Oyadougha *et al*., 2021).

There are 16 million annual cases of typhoid fever, 1.3 billion of gastroenteritis and 3 million deaths worldwide due to Salmonella (Bhunia, 2007). Salmonella infection is the most frequent food-borne gastrointestinal disease transmitted from animals to humans mainly through water, meat and poultry droppings (Riyaz-Ul-Hassan *et al*., 2004). Large numbers of Salmonella serotypes that can cause a variety of diseases in different hosts exists. Some serotypes are host adapted (*Salmonella gallinarum*) while others like *Salmonella typhimurium* and *Salmonella enteritidis* may cause diseases in a large variety of hosts. *Salmonella* serotypes associated with poultry reproductive tissues that are of public health concern includes *Salmonella typhimurium* and *Salmonella enteritidis*. Among the different serotypes, *Salmonella enteritidis* posses the ability to achieve invasion and as a consequence may be found more frequently in reproductive tissues (Gantois *et al*., 2009).

There are two possible routes of bacterial contamination of egg shells which are either vertical or horizontal routes (DeReu *et al*., 2006). Vertical transmission takes place in the trans-ovarian route where the yolk, albumen and membranes are directly contaminated as a result of bacterial shedding from the infection of the hen's reproductive organs, which takes place before the shell covers the eggs (Messens *et al*., 2005). Horizontal contamination begins with the passage of the eggs through the highly contaminated cloaca area at the moment of laying and leads to the shells been penetrated by microorganisms (DeReu *et al*., 2006). Egg shells may additionally become contaminated from any surface with which it comes into contact with. Consequent to the above, this study seeks to establish:(i) a baseline survey of Salmonella prevalence in poultry eggs, (ii) determine the circulating *Salmonella serovars*, (iii) determine possible risk factors that may be driving *Salmonella* contamination of eggs.

Materials and Methods

Sample collection

A total of 150 poultry eggs were collected aseptically in polythene bags from NDU Poultry Farm, Opolo Market outlet and Tombia Market outlet respectively in Yenagoa metropolis in Bayelsa State. The samples were taken to the Microbiology laboratory in Niger Delta University and laboratory activities were carried out immediately under sterile conditions for the isolation of *Salmonella* species.

Sample preparation

Egg shell surface: A sterile cotton swab soaked in sterilized normal saline was used to swab the egg surface and immersed in 10ml normal saline solution followed by transmission, 1ml into 9ml distilled water applying 10 fold serial dilution respectively.

Media preparation: Media was prepared with the appropriate manufacturers description and autoclave for 121^{o}C for 15 minutes. Thereafter,

the media was transferred to the water bath to control the temperatures to safeguard the in-habitation of culturable microorganisms. In addition, 1ml of each diluted samples was evenly inoculated into the labeled petridishes and incubated at 37^{o}C for 24 hours. Growth of the isolates was observed and examined thoroughly before biochemical examinations was carried out.

Identification of isolates: The isolates were identified according to criteria. This includes the organism's morphology, Gram's staining reactions, growth conditions, colonies characteristics on different media.

Biochemical identification

The identification was based on biochemical test based international software available at http://www.tgw1916.net/bacteria_logare_desktop.html. Selected biochemical tests was conducted and the results fed into the software and collected.

Microscopic examination: A smear was made from each type of primary cultures and form colonies, fixed by heating and stained by Gram's stain. The stained smears were examined microscopically by oil immersion lens. The smears were examined for cell morphology and arrangement, presence of capsule and staining reactions.

Results

Colonies of Salmonella were isolated and counted from each petridishes according to the sources of the samples collected. In this study, a total of 880 *Salmonella* isolates was recovered; 10 different Salmonella serovars were identified, 5 different Salmonella serovars were identified from eggs predominantly sold in the market outlets and 5 different Salmonella serovars were identified from NDU Poultry Farm. The isolates include *Salmonella agama*, *Salmonella bradford*, *Salmonella derby*, *Salmonella durham* and *Salmonella kentucky* for eggs sold in market outlets while isolates obtained from NDU Poultry Farm include *Salmonella colorado*, *Salmonella carro*, *Salmonella alachua*, *Salmonella kingston* and *Salmonella lattenkemp*. Only serovar *Salmonella kentucky* was common in samples from the market and farms (table 1).

Out of 150 egg samples, a total of 880 isolates were obtained. 269(30.5%) were gotten from NDU Poultry Farm, 297(33.7%) isolates were gotten from Opolo Market outlet and 314(35.6%) isolates were gotten from Tombia Market outlet respectively. All Salmonella isolates were obtained from eggshell except one (*Salmonella alachua*) which was obtained from egg contents.

Table 1. Zonal distribution of *Salmonella serovars* in eggs according to sources of samples and types.

Zone	Farm	Salmonella serovars	colonies	CFU/ml
Amassoma	NDU	*Salmonella colorado*	59	5.9×10^{-5}
		Salmonella carro	38	3.8×10^{-5}
		Salmonella kingston	61	6.1×10^{-5}
		Salmonella kentucky	63	6.3×10^{-5}
		Salmonella alachua	48	4.8×10^{-5}
		Total	269	

Table 2. Zonal distribution of *Salmonella serovars* in eggs according to sources of samples and types.

Zone	Market	Salmonella serovars	colonies	CFU/ml
Yenagoa	Opolo Market outlet	*Salmonella agama*	57	5.7×10^{-5}
		Salmonella bradford	49	4.9×10^{-5}
		Salmonella derby	54	5.4×10^{-5}
		Salmonella durham	67	6.7×10^{-5}
		Salmonella kentucky	70	7.0×10^{-5}
		Total	297	

Table 3. Zonal distribution of *Salmonella serovars* in eggs according to sources of samples and types.

Zone	Market	Salmonella serovars	colonies	CFU/ml
Yenagoa	Tombia Market outlet	*Salmonella agama*	59	5.9×10^{-5}
		Salmonella bradford	48	4.8×10^{-5}
		Salmonella derby	64	6.4×10^{-5}
		Salmonella durham	62	6.2×10^{-5}
		Salmonella kentucky	81	8.1×10^{-5}
		Total	314	

Biosecurity Practices in the Production and Handling of Eggs

The questionnaire results indicated a predominant cage system (56.5%) operations, compared to the deep litter system (23.6%). Eighty percent (45/55) of the farms were less than 500m away from other farms and the tendency for farms to be visited by wild birds.

Wide biosecurity concerns exist across most farms with only about 24.7% (16/55) of farm operations involve in personal protective equipment (PPEs) where necessary. About half of the responding farms shared tools with other farms, thereby encouraging pathogen transfer. All respondents (100%) did not clean their eggs in any form before selling (Table 4).

Table 4. Husbandry and Biosecurity practices in the study area.

Items	Response	Frequency	Percentage
Husbandry system	Cage	40	70.0
	Deep litter	15	23.3
Presence of other farms <500 m away	Yes	45	78.0
	No	10	17.0
Presence of wild birds and rodents around the farm	Yes	50	85.0
	No	5	9.0
Sanitation			
Wearing protective clothing	Yes	15	18.3
	No	40	75.0
Sharing of tools with other farms	Yes	30	50.0
	No	25	43.3
Cleaning of eggs before sale	Yes	0	0.0
	No		
Inclusion of antibiotics in feed	Yes	55	97.0
	No		
Traffific control	Farm	15	17.0
	premises	40	77.0
Point of sale of eggs	Off-farm	40	65.0
	premises	20	28.3

Discussion

In this study, a prevalence of 16% non-typhoidal Salmonella was detected from pooled poultry egg samples. To the best of our knowledge, this study provides the first detailed comparison of Salmonella serovars profile sold on-farm and in the open market. Our results corroborate Salmonella presence in eggs in Nigeria as previously reported (Obi and Igbokwe 2007). A previous national study reported a 24.5% prevalence of Non-typhoidal Salmonella in poultry environments in Ogun State (Junaid *et al*., 2010). The differences in the prevalence of the two studies may be attributed to the sample types investigated. While the national study employed a matrix of five samples (dust, litter, feces, feed, and water) from poultry environments, the current study focused on pooled poultry egg samples.

The occurrence of Salmonella in eggs from markets and egg shell was significantly higher. Contamination of eggs may occur during packing, grading, transporting and sales in the market, as multiple buyers visually inspect, touch, and select eggs during sales in the study area (Rouger *et al*., 2017). In the present study, unhygienic egg handling practices were common in all farms and markets involved. Also, all farms involved in the questionnaire survey have no egg sanitation programs in place. Data from this study further highlight the potentials continuous relevance of poultry eggs as an important transmission reservoir of Salmonella in humans. Nine out of the 10 Salmonella serovars identified in this study were found on the eggshells and may suggest fecal, environmental, or handling contamination (Ferreira *et al*., 2020). Only one Salmonella serovar (*Salmonella alachua*) was detected in egg content, but, the route of contamination was not investigated. *Salmonella alachua* was recently reported from fecal samples in the Northern part of Nigeria (Jibril *et al*., 2020). Further study will be required to determine if *Salmonella alachua* was an accidental finding in the egg content, vertically transmissible, or can penetrate eggshells into the contents.

The 880 Salmonella isolates identified in this study were spread across 10 serovars, which depicts high serovar diversity. Studies across Nigeria have reported similar observations (Agbaje *et al*., 2019). Plausible reasons for this findings are the indiscriminate importation of poultry birds and eggs with no coordinated national screening and control program in place for salmonellosis. *Salmonella kentucky* was the most occurring in this study with 63 isolates gotten were from NDU farm, 70 isolates gotten from Opolo market and 81 isolates gotten from Tombia market respectively. It is then possible that they are colonially related, although clonal relatedness was not explored in this study. (Fagbamila *et al*., 2017) reported *Salmonella kentucky* in 11 states out of the 12 that were sampled in Nigeria. Other studies have similarly reported *Salmonella kentucky* across Nigeria, thereby suggesting this serovar is widely circulating in Nigeria (Okeke *et al*., 2020). *Salmonella kentucky* has a worldwide distribution and was previously thought to be endemic in Africa with public health significance (Le Hello *et al*., 2013). Notably in this study, Salmonella serovars commonly associated with foodborne infections (*Salmonella enteritidis* and *Salmonella typhimurium*) (Freitas *et al*., 2010) were absent. Serovar diversity may be attributable to a number of reasons but not limited to poor sanitary and biosecurity conditions, indiscriminate importation of poultry chickens and eggs without adequate screening for Salmonella, and lack of focused national Salmonella surveillance and control program.

In this study, data from the questionnaire indicated about 80% of the farms in the study area are accessed by wild birds and rodents. Similar to our results, a study involving three Caribbean countries also reported rodents in 80% of contaminated farms (Adesiyun *et al*., 2014). Investigations in Australia have demonstrated the role of environmental vectors in the epidemiology of Salmonella in farm settlements (Sodagari *et al*., 2020). High and unchecked rodent populations have been associated with increased Salmonella shedding in the environment (Lapuz *et al*., 2012) and are the most effective in the spread of Salmonella pathogen around farms (Wales *et al*., 2007). It is then imperative to initiate robust vector prevention programs in farmhouses which may include secured access doors and windows, sealing of holes, repairs of torn wire net, and the use of baits to help control contamination of farmhouses (Trampel *et al*., 2014).

Furthermore, our results revealed certain practices which may encourage Salmonella occurrence and/or persistence in farms. Poor adherence to strict biosecurity measures on farms. The use of protective clothing as a barrier to infectious agents was unpopular among the majority of the farmers and may contribute to increased chances of contamination.

Also, certain high-risk cross-contamination practices such as unhygienic picking of eggs with bare hands, sharing of tools with nearby farms (mostly <500 m away), and sales of eggs on the farm were observed. These practices all increase the risk of contamination and transfer of pathogens (Okeke *et al*., 2020).

The findings in this study are subject to at least two limitations. First, the pooling of samples ultimately reduced the sample size. While investigating individual egg samples will have provided more detailed data, pooled samples are considered more effective for the successful detection of Salmonella in the context of this study (McLaren *et al*., 2008). Second, was our inability to match individual samples collected with corresponding husbandry and biosecurity questionnaires in the data analyses. Considering that the positivity rate of Salmonella was 16%, it was considered that analyzing data as per the region will be more informative than individual sample-farm analysis.

Conclusion

This study demonstrated the presence of diverse NT Salmonella serovars in eggs. Sales of eggs in the market seem to promote the risk of Salmonella contamination as well as other unhygienic biosecurity practices on the farm.

References

Adebowale, O.O., Adeyemo, O.K., Awoyomi, O., Dada, R. and Adebowale, O. (2016). Antibiotic use and practices in commercial poultry laying hens in Ogun State Nigeria. *Rev. Elev. Med. Vet. Pays Trop*. **69**, 41–45.

Adesiyun, A., Webb, L., Musai, L., Louison, B., Joseph, G., Stewart-Johnson, A., Samlal, S. and Rodrigo, S. (2014). Survey of Salmonella contamination in chicken layer farms in three Caribbean countries. *J. Food Prot*. **77**, 1471–1480.

Agbaje, M., Lettini, A.A., Olufemi, O.E., Longo, A., Marafifin, E., Antonello, K., Zavagnin, P., Oluwasile, B.B., Omoshaba, E.O. and Dipeolu, M.A. (2019). Antimicrobial Resistance Profiles of Salmonella serovars Isolated from Dressed Chicken Meat at Slaughter in Kaduna, Nigeria. *Elev. Med. Vet. Pays Trop*.**72**.

Bettridge, J.M., Lunch, S.E., Brena, M.C., Melese, K., Dessie, T., Terfa, Z.G., Desta, T., Rushton, S., Hanotte, O. and Kaiser, P. (2014). Infection-interactions in Ethiopian village chickens. *Prev. Vet. Med.***117**, 358–366.

Bhunia, A. (2007). Food-borne microbial pathogens: mechanisms and pathogenesis: Springer Science and Business Media.

Carrique-Mas, J.J., Breslin, M., Sayers, A.R., McLaren, I., Arnold, M. and Davies, R. (2008). Comparison of environmental sampling methods for detecting Salmonella in commercial laying flocks in the UK. *Lett. Appl. Microbio*l. **47**, 514–519.

De Reu, k., Grijspeerdt, k., Messens, W., Heyndrickx, M., Uyttendaele, M., Debevere, J. and Herman, L. (2006). Eggshell factors influencing eggshell penetration and whole egg contamination by different bacteria including *Salmonella enteritidis*. *International Journal of Food Microbiology*, **112**(3), 253-260.

Fagbamila, I.O., Barco, L., Mancin, M., Kwaga, J., Ngulukun, S.S., Zavagnin, P., Lettini, A.A., Lorenzetto, M., Abdu, P.A. and Kabir, J. (2017). Salmonella serovars and their distribution in Nigerian commercial chicken layer farms. *PLoS* ONE 12, e0173097.

Ferreira, V., Cardoso, M.J., Magalhães, R., Maia, R., Neagu, C., Dumitra¸scu, L. and Teixeira, P. (2020). Occurrence of Salmonella spp. in eggs from backyard chicken flflocks in Portugal and Romania Results of a preliminary study. *Food Control*. **113**, 107–180.

Food and Agricultural Organization (FAO) (2006). The Structure and Importance of the Commercial and Village Based Poultry Industry in Nigeria. Available online: http://www.fao.org/docs/eims/upload/214281/.

Freitas, N.O.C., Penha, F.R.A.C., Barrow, P. and Berchieri, J.A. (2010). Sources of human non-typhoid salmonellosis: *A review*. *Braz. J. Poult. Sci.***12**, 1–11.

Gantois, I., Ducatelle, R., Pasmans, F., Haesebrouck, F., Gast, R. and Humphrey, T.J. (2009). Mechanisms of egg contamination by *Salmonella enteritidis*. *FEMS Microbiol*. Rev. **33**:718-738.

Grimont, P.A.D. and Weill, F.X. (2007). Antigenic Formulae of the Salmonella Serovars, 9th ed.; World Health Organization Collaborating Center for Reference and Research on Salmonella; Institut Pasteur: Paris, France; **pp**. 1–166.

Herikstad, H., Motarjemi, Y. and Tauxe, R.V. (2002). Salmonella surveillance: A global survey of public health serotyping. *Epidemiol*. Infect. **129**, 1–8.

Ibrahim, G.M. and Morin, P.M. (2018). Salmonella serotyping using whole genome sequencing. *Front. Microbiol*. **9**, 2993.

Idowu, F., Junaid, K., Paul, A., Gabriel, O., Paul, A., Sati, N., Maryam, M. and Jarlath, U. (2010). Antimicrobial screening of commercial eggs and determination of Tetracycline residue using two microbiological methods. *Int. J. Poult. Sci*. **9**, 959–962.

Jibril, A.H., Okeke, I.N., Dalsgaard, A., Kudirkiene, E., Akinlabi, O.C., Bello, M.B. and Olsen, J.E. (2020). Prevalence and risk factors of Salmonella in commercial poultry farms in Nigeria. PLoS ONE **15**, e0238190.

Lapuz, R.R., Umali, D.V., Suzuki, T., Shirota, K. and Katoh, H. (2012). Comparison of the prevalence of Salmonella infection in layer hens from commercial layer farms with high and low rodent densities. *Avian Dis*. **56**, 29–34.

Le Hello, S., Harrois, D., Bouchrif, B., Sontag, L., Elhani, D., Guibert, V., Zerouali, K. and Weill, F.X. (2013). Highly drug-resistant *Salmonella enterica* serotype Kentucky ST198-X1: *A microbiological study. Lancet Infect. Dis.***13**, 672–679.

Messens, W., Grijspeerdt, K. and Herman, L. (2005). Eggshell penetration by Salmonella: a review. *World's poultry science journal*, **61**(01), 71-86.

Namata, H., Welby, S., Aerts, M., Faes, C., Abrahantes, J.C., Imberechts, H., Vermeersch, K., Hooyberghs, J., Méroc, E. and Mintiens, K. (2009). Identifification of risk factors for the prevalence and persistence of Salmonella in Belgian broiler chicken flocks. *Prev. Vet. Med*. **90**, 211–222.

Obi, C.N. and Igbokwe, A.J. (2007). Microbiological analyses of freshly laid and stored domestic poultry eggs in selected poultry farms in Umuahia, Abia State, Nigeria. *Res. J. Biol. Sci*. **2**, 161–166.

Orji, M.C., Onuigbo, H.C. and Mbata, T.I. (2005). Serological survey of mycoplasmosis and pullorum disease in Plateau State of Nigeria. *Int. J. Infect. Dis*. **9**, 86–89.

Oyadougha, T.W., Braide W. and Chinakwe E.C. (2021). Modelling the Effects of Local Food Spices on the Growth of *Salmonella typhimurium*. *International Journal of Advanced Research in Biological Science*s. **8**(11): 46–54.

Riyaz-UI-Hassan, S., Verma, V. and Qazi, G.N. (2004). Rapid detection of Salmonella by polymerase chain reaction PCR. *Mol Cell Probes*, **18**(5), 333-339.

Rouger, A., Tresse, O. and Zagorec, M. (2017). Bacterial Contaminants of Poultry Meat: Sources, Species, and Dynamics. Microorganisms. **5**, 50.

Sodagari, H.R., Habib, I., Whiddon, S., Wang, P. Mohammed, A.B., Robertson, I. and Goodchild, S. (2020). Occurrence and Characterization of Salmonella Isolated from Table Egg Layer Farming Environments in Western Australia and Insights into Biosecurity and Egg Handling Practices. Pathogens, **9**, 56.

Trampel, D.W., Holder, T.G. and Gast, R.K. (2014). Integrated farm management to prevent *Salmonella enteritidis* contamination of eggs. *J. Appl. Poult. Res*. **23**, 353–365.

Wales, A., Breslin, M., Carter, B., Sayers, R. and Davies, R. (2007). A longitudinal study of environmental Salmonella contamination in caged and free-range layer flocks. *Avian Pathol*. **36**, 187–197.

Zhang, S., Yin, Y., Jones, M.B., Zhang, Z., Kaiser, B.L.D., Dinsmore, B.A., Fitzgerald, C., Fields, P.I. and Deng, X. (2015). Salmonella serotype determination utilizing high-throughput genome sequencing data. *J. Clin. Microbiol*. **53**, 1685–1692.

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