DonnishJournals 2041-1180

Donnish Journal of Medicine and Medical Sciences
Vol 2(3) pp. 047-051 April, 2015.
http://www.donnishjournals.org/djmms

Original Research Article

Primary Sources of Salmonella Species in Poultry Production Settings in Calabar, Cross River State, Nigeria

[1]Nchawa Yangkam Yhiler and [1,2]Bassey Enya Bassey*

[1]Department of Medical Laboratory Science, Faculty of Allied Medical Science, University of Calabar, Calabar, Nigeria.
[2]World Health Organization, UN House, Plot 617/618, Central Area District, FCT, Abuja, Nigeria.

Accepted 7th April, 2015.

Background: **The poultry production settings have been frequently revealed as a major reservoir for Salmonella species. Salmonellosis is endemic in many countries, including Nigeria, where the poultry production industry is rapidly increasing. This study was designed to determine the gateways of entry of Salmonella into the poultry production setting so as to suggest measures to curb the introduction and maintenance of these organisms into the poultry production systems.** ***Methods:*** **Between the period of August 2013 and May 2014, 120 poultry feed samples were collected from commercial feed outlets, 120 water samples from poultry water storage tanks, 90 water samples from primary water sources (bore hole and pipe borne), 120 samples from both eggshell and the internal egg contents and 180 samples from day-old chicks within the poultry production settings in Calabar. Samples were collected aseptically and bacteriological analysis for the presence of Salmonella was performed according to the ISO 6579:2002 including standard bacteriological, biochemical and serologic techniques. The Chi-square parameter was used to determine the level of significance at 95% confidence level.** ***Results:*** **The rate of recovery of Salmonella species from the sources of poultry feed from commercial outlets, poultry water storage tanks and water from Primary water sources (bore hole and pipe borne) were respectively 7.5%, 11.7% and 3.3%. However, there was no statistical significant difference (*P-value = 0.084*) in the rate of recovery of Salmonella species from these sources, hence implicating these sources as primary routes for the entry of Salmonella species into the poultry production setting. There was also no statistical significant difference in the rate of recovery of Salmonella species from eggs and day-old chicks suggesting that the source of infection of day-old chick is from the incubation of contaminated eggs.** ***Conclusion:*** **The results of this study also suggest a relationship between eggshell contamination and internal egg content contamination as well as the contamination of the egg contents even before egg formation.**

Keywords: Primary-sources, Salmonellosis, Eggshell-contamination, Internal-egg-content contamination

INTRODUCTION

The poultry production setting is a major reservoir for Salmonella species, the agent of Salmonellosis. Salmonellosis is still a major problematic zoonosis responsible for significant morbidity in humans and animals worldwide. In spite of the several attempts to minimize the causative agents to near zero tolerance, the disease remains a major threat to the commercial production of poultry and is endemic in Nigeria [1, 2]. Several outbreaks of salmonellosis in humans have been linked to the consumption of eggs, egg-containing foods, poultry meats and other poultry products contaminated with the Salmonella organisms [3].

The increased outbreaks of human salmonellosis have been attributed to infection of breeder and layer flocks, the ability of some Salmonella serovars to cause infection of the ovaries and oviduct of birds and subsequently their eggs, the increased consumption of poultry and poultry products, and the abnormal storage temperature of eggs which allow the Salmonella organisms to increase in numbers [4,5,6]. The

Corresponding Author: email:bassey69@yahoo.com

vertical transmission of the infection from the ovaries to the eggs is termed trans-ovarian transmission. Apart from the host-adapted Salmonella serovars (*S. pullorum* and *S. gallinarum*), *S. enteritidis, S. typhimurium* and *S. heidelberg*, have been frequently implicated to infect eggs by trans-ovarian transmission [7,6].

However, this is not the only route by which eggs may become contaminated. In the poultry production setting, day-old chicks from hatcheries may become infected with Salmonella via contacts with formites and eggshells contaminated with faecal matter or dust and other debris present in poultry houses. Salmonella organisms belonging to a variety of serovars can contaminate eggs by penetration of the egg shell into the internal egg contents [8,9] or by trans-ovarian transmission [6]. The surpluses of hatching eggs may be diverted from incubation to be used as table eggs. When such eggs are contaminated with the Salmonella organisms they become a source of human infections when consumed [10].

The growth and survival of Salmonella species in water can be maintained given the right conditions. Water reservoirs for poultry use can readily favour the growth and maintenance of *Salmonella* species consequently leading to the infection of birds in the flock. Feeds and feed ingredients amongst others are important sources whereby the Salmonella organisms can be introduced in the poultry production setting with the consequent infection of the entire flock as well as humans and other animals [11, 12].

In order therefore to control Salmonella and other pathogenic microorganisms to near zero tolerance in the poultry production setting, strict hygiene and sanitation measures should be taken into account at every stage involved in the production of poultry. This study was therefore aimed at investigating the major gateways of introduction and maintenance of the Salmonella organisms in the poultry production setting by determining the rate of contamination of feeds from commercial outlets, poultry water reservoirs and water from primary sources as well as eggs and day-old chicks as primary sources of Salmonella species.

MATERIALS AND METHODS

This study was conducted in Calabar, Cross River State, Nigeria between August 2013 and May 2014. A total of 630 specimens consisting of 180 from day-old chicks, 120 egg pools, 120 from poultry feed in commercial outlets, 120 from poultry water reservoirs and 90 from primary water sources (Pipe-born and bore-hole) located within the Calabar Metropolis.

Sample collection

The day-old chicks were collected from 6 hatcheries and were immediately analyzed upon arrival in the Pentamed Scientifics Laboratory (No. 50 Atamunu, Calabar, Nigeria). Cloacal swabs and organs (liver and yolk sac) were collected from each day-old chick. 10 g of poultry feed was collected from commercial feed outlets into dry sterile polythene bags. 100 ml of water was collected aseptically directly from the sources and transported to the Laboratory within 4 h. Eggshell samples were collected by placing the intact egg in a sterile polythene bag containing 10 ml modified Buffered Peptone Water (10%) (HARDY Diagnostics, 1430 West McCoy Lane, Santa Maria, CA 93455, USA) and the egg was thoroughly rubbed through the bag for 1 minute without breaking it [13]. The diluents were then used as the eggshell sample and incubated at 37°C for 18 hours the internal egg contents were collected aseptically by first disinfecting the eggshell with 30% hydrogen peroxide (H_2O_2) solution, followed by 70% ethanol and burning off the alcohol briefly. The disinfected egg was broken using a sterile blade and sanitized plastic gloves. The egg content was enriched in modified Buffered Peptone Water and incubated at 37°C for 18h [13]

Isolation and identification of Salmonella species

The isolation of Salmonella species from poultry feeds, poultry water reserves and day-old chicks and eggs was carried out in accordance with the ISO 6579:2002. The reagents were obtained from HARDY Diagnostics, 1430 West McCoy Lane, Santa Maria, CA 93455, USA. By means of a sterile pipette, 1 ml and 0.1 ml of the non-selective enrichment (modified Buffered Peptone Water) was inoculated into 9 ml of Muller-Kauffmann Tetrathionate-novobiocin (MKTTn) and Modified Semi-solid Rapapport-Vassiliadis (MSRV) selective enrichments respectively. These were respectively incubated at 37^{o}C and 42^{o}C in separate incubators for 24 h.

After incubation, a loop full from each of the selective enrichments was streaked onto both Brilliant Green Agar (BGA) and Xylose Lysine Desoxycholate Agar (XLDA) selective plates in order to obtain distinct Salmonella-like colonies. On BGA, typical Salmonella-like colonies appear as 1-2 mm pink colonies which convert the agar from green to red, whereas on XLDA, they appear as pink colonies with or without the presence of black centres (indicating the production of H_2S).

The typical Salmonella-like colonies on BGA and XLDA were then picked by means of a sterile wire needle in order to make smears on microscope slides for gram staining as well as to stab and streak on pre-prepared Triple Sugar Iron Agar (TSIA) and Christensen agar (CA) slants. Those Salmonella-like colonies that showed Gram-negative small rods by microscopy, produced alkaline slope/acid butt with or without the production of H_2S and gas, on TSIA slant and Urease negative on Christensen agar slant were considered suggestive of Salmonella. They were then sub-cultured on nutrient agar slants overlaid with sterile paraffin oil and kept in a cool, dark corner prior to further biochemical reactions [14].

Confirmation of Salmonella species was conducted based on the standard biochemical techniques in order to identify the isolates which belong to the genus *Salmonella*. This involved the use of Lycine Decarboxilation (LCD) test, β-galactosidase test, Acetone production test and Indole production test. Serotyping of obtained Salmonella isolates was further supplemented by means of the commercially available polyvalent Salmonella antisera kit (Denka Seiken Co. Ltd. Tokyo, Japan) specific for all group and type-factor Salmonella antigens. A loop full from Salmonella isolates that satisfy all the confirmation procedures were then emulsified with a drop of normal saline (0.85% NaCl) on a microscopic glass slide [14]. The preparation was gently stirred and observed for auto-agglutination. If there was no self-agglutination, a drop of the polyvalent antisera was added and gently agitated by rocking back and forth for about three minutes and observed for agglutination. Those that showed agglutination were considered to belong to the genus Salmonella.

Statistical Analysis

The data obtained in this study were analyzed by means of the Predictive Analytical Software (PASW) 18.0. The simple descriptive statistics was used to analyse the prevalence rate

of *Salmonella* species in the samples obtained from the production of poultry while the level of significance in the rate of recovery of the *Salmonella* species was determined using the Chi-square parameter. *P-values* of less than *0.05* were considered statistically significant.

RESULTS

Out of 330 samples consisting of poultry feed from commercial outlets (120), poultry drinking water from poultry water storage tanks (120) and water from primary sources (pipe-borne and bore-hole) (90), Salmonella isolates were respectively recovered thus: 7.5%, 11.7% and 3.3% (Table 1). There was no statistical significant difference in the rate of recovery of Salmonella isolates from these sources (*P-Value = 0.084*).

According to table 2, the rate of recovery of Salmonella isolates from egg samples and day-old chicks from hatcheries were 10.0% and 7.2% respectively. However, there was no statistical significant difference in the rate of Salmonella species from eggs and day-old chick samples (*P-value = 0.402*).

The Venn diagram shows that Salmonella species were recovered from 27 + 12 = 39 (32.5%) of eggshells and 2 + 12 = 14 (11.7%) of egg contents. 2 (1.7%) of the egg contents alone were contaminated with Salmonella species, while 27 (22.5%) of the egg shells alone were contaminated with Salmonella species. Furthermore, Salmonella species were recovered from 12 (10.0%) of both egg shells and egg contents. The differences in the rate of recovery of Salmonella isolates from the egg shells and the egg contents was statistically significant (*P-Value = 0.000*).

DISCUSSION

Salmonellosis is a significant problematic zoonosis worldwide from the point of view of public health and it accounts significantly to the drawbacks encountered by food production systems especially in poultry and poultry products [15]. The bacteriological analysis of the samples from commercial feeds, poultry water reservoirs and primary water sources (pipe-borne and bore-hole) revealed low rates of recovery of Salmonella species and this was in conformity with the results of the studies conducted in Zaria, Nigeria and Jamaica [12,16].

According to the results of this present study, in spite of the least rate of recovery of Salmonella isolates from the primary water sources (borehole and pipe-borne), there was no statistical significant difference in the rate of recovery of Salmonella species (*P-Value = 0.084*). This suggests that these sample sources (poultry feed from commercial outlets, poultry drinking water from reservoirs and water from primary sources) serve as important portals of entry of the Salmonella organisms into the poultry production system.

Therefore, it would be strongly recommended that water for poultry use should be treated. This is in conformity with the work of other researchers who demonstrated a significant difference in the Salmonella contamination rate of treated (1.0%) and untreated (7.5%) water for poultry use [12]. Moreover, the feed production industries and poultry feed vendors should observe strict hygiene and sanitation measures during feed processing, transportation and storage in order to minimise bacteriological hazards. Trans-ovarian contamination occurs when the Salmonella organisms reside in the reproductive tissue of an infected bird and are transferred to the internal contents of the egg during egg formation [17]. The microbiological analysis of Salmonella from eggs and day-old chicks as revealed by this study correspond with the studies carried out by other workers [17] and in Brazil who isolated Salmonella species from day-old chicks and table eggs [18]. However, other workers showed considerably higher rates (37.5% and 24.3%, respectively) of recovery of Salmonella species from day-old chicks [19, 20]. Under normal circumstances, eggs are sterile and hence day old chicks. However, this current study revealed 7.2% rate of recovery of Salmonella species from day-old chicks. The results of this study, therefore, suggest two possible sources of the Salmonella infection, which is in agreement with literatures that eggs may become contaminated with the Salmonella organisms via vertical transmission (trans-ovarian transmission) and/or the contamination of the egg, post egg formation with the subsequent penetration of the Salmonella organisms into the internal egg contents [17].

The results of this study showed that Salmonella species were recovered from 10.0% of eggs destined for incubation and there was no statistical significant difference in the rate of recovery of Salmonella species from eggs destined for incubation and from day-old chicks (*P-value = 0.402*). This invariably suggests that, infection of day-old chicks is dependent upon the contamination of eggs destined for incubation and as a consequence serves as an important portal for the entry of the Salmonella species into the poultry production systems. On the other hand, the difference in the rate of recovery of Salmonella isolates from egg shells (32.5%) and egg contents (11.7%) in this current study was statistically significant (*P-value* of *0.000*), implying that the contamination of eggshell is independent from the of contamination of egg contents.

This is in conformity with the works of Albuquerque *et al* and Gantois *et al* who distinguished between the trans-ovarian contamination of eggs from the contamination of egg post egg formation [8,17]. Trans-ovarian contamination usually occurs before or during egg formation in the oviduct while the contamination of eggshell after egg formation usually occurs when the formed egg makes contact with contaminated faeces in the cloaca during oviposition or when in contact with contaminated debris or matter found in the poultry setting post oviposition [8, 17]. However, Salmonella species were recovered from both the eggshell and the egg content, in 12 (10%) of the egg samples used in this study, hence suggesting the source of the contamination of the internal egg contents could be traced from the contaminated eggshell.

Several studies have demonstrated the penetration of Salmonella serovars from the eggshell into the egg contents [6, 17]. This implies that a contaminated environment can lead to the contamination of eggshell with the subsequent contamination of the internal egg contents. The contamination of eggs destined for incubation consequently leads to the production of infection of day-old chicks and hence the entry of Salmonella species in the poultry production setting. In like manner, when such eggs are diverted for food, this may lead to the infection of the consumers.

Finally, the results of this study revealed that only 2 (1.7%) of the egg content samples were contaminated with Salmonella species without eggshell contamination. Such contamination of internal egg contents without eggshell contamination can most likely be implicated to have occurred at the level of the oviduct rather than post egg formation, hence trans-ovarian transmission.

Table 1: Distribution of Salmonella species by poultry feed and water sources

Source of sample	Number of sample tested	Number of sample positive	Percentage (%) of sample positive
Poultry feed from commercial outlets	120	9	7.5
Poultry water reservoirs	120	14	11.7
Primary water source	90	3	3.3
Total	**330**	**26**	**7.9**

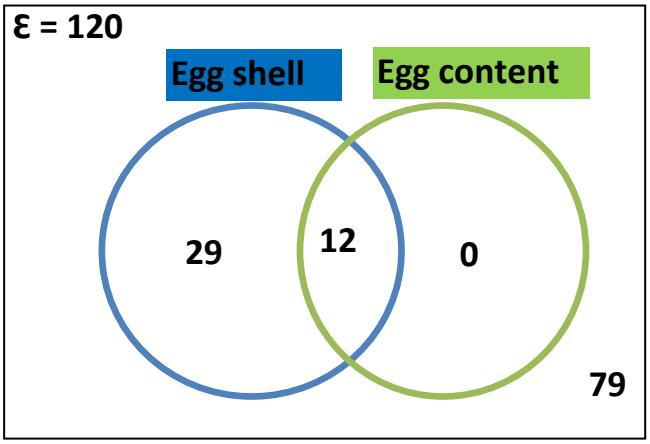
$\chi^2 = 4.958$, $df = 2$, $P\text{-value} = 0.084$ (two-tailed).

Table 2: Distribution of Salmonella species by eggs and day-old chicks from hatcheries

Type of sample	Number examined	Number infected (%)
Eggs	120	12 (10.0)
Day-old chicks	180	13 (7.2)
Total	300	25 (8.3)

$\chi^2 = 0.727$, $df = 1$, $P\text{-value} = 0.402$ (two-tailed) (Fisher's Exact Test for 2 X 2 tables)

Venn diagram of the distribution of Salmonella species in egg contents and egg shell



$\chi^2 = 20.458$, $df = 1$, $P\text{-value} = 0.000$ (two-tailed) (Fisher's Exact Test for 2 X 2 tables)

CONCLUSION

The results of this study have demonstrated that in spite of the major advances in technology in the appropriation of adequate sanitation, there is yet a dearth in the implementation of these strategies. The water and feed given to the poultry birds are frequently contaminated which poses both the birds and the consumers at risk of the infection. Eggs may become contaminated prior or post oviposition and consequently infection of chicks from hatcheries as well as infection to the consumers of egg and egg products.

It is therefore necessary to implement standardized bacteriological hazard control points at the level of feed production, transportation and storage. It would also be necessary to appropriately treat water for poultry use. Chicks and layer flocks should be screened for bacteriological hazards before introduction into the poultry production setting. There is also a need for a surveillance control mechanism to monitor and check these control strategies in order to ultimately minimize or eliminate the hazard of *Salmonella* contaminations and hence curb human salmonellosis.

AUTHORS' CONTRIBUTIONS

NYY and BEB conceived the study; carried out the statistical analyses; field interviews; and drafted the manuscript. Authors read and approved the final manuscript.

ACKNOWLEDGEMENTS

The contributions of Dr Godwin Iwatt.

Funding

No funding support

Competing Interests

None declared.

Ethical Approval

Not required

REFERENCES

1. Akond, M. A., Shirin, M., Alam, S., Hassan, S. M. R., Rahman, M., & Hoq, M. (2012). Frequency of drug resistant Salmonella spp . isolated from poultry samples in Bangladesh, *2*(1).
2. Yhiler, N. Y., & Bassey, B. E. (2015). Critical segments in the dissemination and transmission of Salmonella species from poultry production in Calabar , Nigeria, *3*(2), 168–174. doi:10.11648/j.sjph.20150302.13
3. De Buck, J., Van Immerseel, F., Haesebrouck, F., & Ducatelle, R. (2004). Colonization of the chicken reproductive tract and egg contamination by Salmonella. *Journal of Applied Microbiology*, *97*, 233–245. doi:10.1111/j.1365-2672.2004.02294.x
4. Assefa, M., Teklu, A., & Negussie, H. (2011). The Prevalence and Public Health Importance of Salmonella from Chicken Table Eggs , Ethiopia, *11*(4), 512–518.
5. Hedican, E., Miller, B., Ziemer, B., Lemaster, P., Jawahir, S., Leano, F., & Smith, K. (2010). Salmonellosis Outbreak Due to Chicken Contact Leading to a Foodborne Outbreak Associated, *7*(8).
6. Poppe, C., Duncan, C. L., & Mazzocco, A. (1998). Salmonella Contamination of Hatching and Table Eggs : A Comparison, 191–198.
7. Aly, M. M., & Radwan, M. I. (2010). Salmonella spp. Infection in Imported 1-Day-Old Chicks, Ducklings, and Turkey Poults: A Public Health Risk 1 1, *7*(4).
8. Albuquerque, Á. H. De, Maciel, W. C., & Lopes, E. D. S. (2014). Presence of Salmonella spp . in One-day-old Chicks from Hatcheries in the Metropolitan Region of Fortaleza , Brazil, *55*(November), 1–7.
9. Braden, C. R. (2006). Salmonella enterica serotype Enteritidis and eggs: a national epidemic in the United States. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, *43*, 512–517. doi:10.1086/505973
10. Hidayah, N. (2011). Review Article Salmonella : A foodborne pathogen, *473*, 465–473.
11. Ocholi, R. A., Oyetunde, I. L., Kumbish, P. R., Odugbo, M. O., Ta'ama L., Usman, M. G., Ogbe, A., Waziri, E., Ahmed, J., Joannis, T. M., Ularamu, H. G., Shittu, I. A., Egbuji, A. N., Suleiman, L. K., Makinde, A. A. & Lombin, L. H. (2006). Epidemiology of an outbreak of highly pathogenic avian influenza caused by the virus sub -type H1N5 in Nigeria in 2006. *Vom Journal of Veterinary Science,* special edition: 1-11
12. Musa, I. W., Mansur, M. S., Sa'idu, L., Mohammed, B., Kaltungo, B. Y., Lawan, M. K., Talba, A. M.. Isolation and Antibiogram of *Salmonella* Species from Water and Poultry Feed In Selected Commercial Farms In Zaria, Nigeria. *Time Journals of Agriculture and Veterinary Sciences,* 2014; 2(2): 75-80.
13. Reu, K. De, & Heyndrickx, M. (2008). Estimation of the vertical and horizontal bacterial infection of hen's table eggs. *World's Poultry Science* …, (2), 4–6. Retrieved from http://www.cabi.org/AnimalScience/Uploads/File/AnimalScience/additionalFiles/WPSA2007/7_De Reu Koen.pdf.
14. OIE Terrestrial Manual 2008. World organisation for animal health manual of diagnostic tests and vaccines for terrestrial animals (mammals, birds and bees), Sixth Edition, Volume 2, 2008.
15. Okonko, I. O., Nkang, A. O., Fajobi, E. A., Mejeha, O. K., Udeze, A. O., Motayo, B. O., Ogun, A. A., Ogunnusi, T. A. &Babalola, T. A. Incidence of multi-drug resistant (MDR) organisms in some poultry feeds sold in Calabar metropolis, Nigeria. EJEAFChe, 2010; 9 (3): 514-532.
16. Curtello, S., Justiz, A. A., Vaillant, H., Asemota, P. E. A. & Monica, P. S. Prevalence of Salmonella Organisms in Poultry and Poultry Environments in Jamaica. British Microbiology Research Journal, 2013; 3(4): 461-469.
17. Gantois, I., Ducatelle, R., Pasmans, F., Haesebrouck, F., Gast, R., Humphrey, T. J., & Van Immerseel, F. (2009). Mechanisms of egg contamination by Salmonella Enteritidis: Review article. *FEMS Microbiology Reviews*, *33*, 718–738. doi:10.1111/j.1574-6976.2008.00161.
18. Átilla Holanda de Albuquerque, William Cardoso Maciel, Elisângela de Souza Lopes, Régis Siqueira de Castro Teixeira, Rosa Patrícia Ramos Salles, Debora Nishi Machado, Windleyanne Gonçalves Amorim Bezerra, Ruben Horn Vasconcelos, Sanjay Veiga Mendonça1 & Carlos Buxadé Carbó. Presence of *Salmonella* spp. in One-day-old Chicks from Hatcheries in the Metropolitan Region of Fortaleza, Brazil. Acta Scientiae Veterinariae, 2014. 42: 1222.
19. Dougherty T.J. 1976. A study of Salmonella contamination in broiler flocks. Poultry Science. 55(5): 1811-1815.
20. Tessari E.N.C, Cardoso A.L.S.P., Castro A.G.M., Zanatta G.F. & Kanashiro A.M.I. 2003. Incidência de Salmonella spp. em pintos de corte recém-nascidos. Arquivos Instituto Biológico. 70(3): 279-281.