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Sources of *Salmonella* Infections in Selected Poultry Farms in Jos, Northern Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors NMS and PEE performed the statistical analysis and wrote the first draft of the manuscript. Authors ECO and JK designed the study. Authors HMK and MM wrote the protocol. Author AAL serotyped the isolates. Authors IOF and LUE managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Salmonella infections are not new in Nigerian farms and most times also, it is not clear whether the infections are coming from the hatcheries or they are acquired on the farms. The aim of this study was to determine the sources of salmonella infections in nine selected farms prior to fowl typhoid vaccine administration in order to suggest preventive measures towards minimizing or eradicating its occurrence in the farms and hatcheries. Samples taken at the hatcheries were dead chicks and

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faeces from chick boxes. These samples were processed and analysed for Salmonella species using standard microbiology methods. Four (44.4%) of the nine farms had *Salmonella* in the tissues of dead birds and/or in their faeces before the birds were introduced to the farms. *Salmonella* specie was also found two weeks later in faeces and feed in one out of the four farms. Subsequent visits yielded no *Salmonella* species in the previously infected farms. One of the selected farms whose chicks were *Salmonella*–free before reaching the farm eventually had *Salmonella* species isolated from the litter a few weeks later. A total of eleven isolates comprising four different serotypes (*Salmonella oakland, S. enterica* subsp *enterica, S. bonariensis and S. kentucky)* were encountered in this study that demonstrates the need for routine screening of breeder farms against infectious diseases, the regulation of activities in hatcheries and the practice of biosecurity on farms to reduce disease transmission to the barest minimum.

Keywords: Salmonella; sources; infection; poultry farm.

1. INTRODUCTION

There are several potential sources of Salmonella contamination in an integrated poultry operation. Environmental factors such as air, litter and unclean facilities, and vectors, such as insects, humans, and rodents, are responsible for Salmonella contamination in poultry farms [1]. Chickens can be infected with many different serovars of Salmonella. Some serovars, such as *S. Pullorum* and *S. Gallinarum*, are host specific for chickens, whereas other serovars, such as *S. typhimurium*, *S. enteritidis*, and *S. heidelberg*, are able to infect a wide range of hosts. There are a number of commonly identified Salmonella serotypes that are associated with chickens; the most common being *S. enteritidis, S. kentucky, S. heidelberg* and *S. typhimurium* for clinical isolates and *S. heidelberg, S. kentucky, S. typhimurium, S. senftenberg,* and *S. enteritidis* for non-clinical isolates [2]. *S. heidelberg* has been the most prevalent serovar reported since 1997, with a peak in 2000 of just over 50% of all isolates reported being *S. heidelberg*. In the early to mid-1990s, *S. enteritidis* was the most frequently reported serotype in the United States, as well as in Europe [1]. Salmonella contamination of poultry in pre-harvest environments can usually be traced to production issues that include contaminated poultry feed or pathogen introduction to the facilities via a wide range of carriers including house pets, wild animals as well as insects [3].

Many of these environmental sources have been reviewed extensively elsewhere but poultry feed has been discussed in more detail than most other sources [4,5]. There are several reasons for the extensive focus on poultry feeds as a source of Salmonella contamination. First, since one Salmonella organism per gram of feed can colonize young chicks, low or undetectable numbers of Salmonella represent a high risk for infection in these birds that is further enhanced by the increased feed mixing and incorporation of individual feed ingredients from a multitude of sources. This becomes of particular concern if breeder flock hatchlings are exposed since they represent the starting point for all commercial flocks. Secondly, Salmonella can linger in feed for extended periods with reports of bacterial cells remaining viable for several weeks up to 16 months in dry feed stored at 25ºC. This is further confounded when feeds are treated with antimicrobials such as organic acids where Salmonella either can become acid tolerant or their recovery and/or subsequent enumeration accuracy using conventional plating methods is influenced by carryover of antimicrobial compounds into the media [6]. Contaminated feed is also regarded as a source of infectious transmission of Salmonella among flocks. This is further accentuated by the larger numbers of birds housed in confinement resulting in an increase in more birds being infected simultaneously via aerosols and other routes [3].

In Nigeria, poultry farming is an activity that is popular both in rural and urban settings irrespective of the practice being for large commercial purposes or for peasant farming for households. In livestock production, poultry occupies a prominent position in the provision of animal protein and this account for about 25% of local meat production in Nigeria [7]. Small- scale poultry farmers in Nigeria loose up to 18% of chicks in the first two weeks of rearing, and mortality is often associated with salmonellosis and this exerts negative socio economic and food security effects on farmers [8]. This study set out to determine whether the *Salmonella* infections commonly found in poultry farms were coming from the hatcheries or acquired on the farms. The source of infection in this study is discussed.

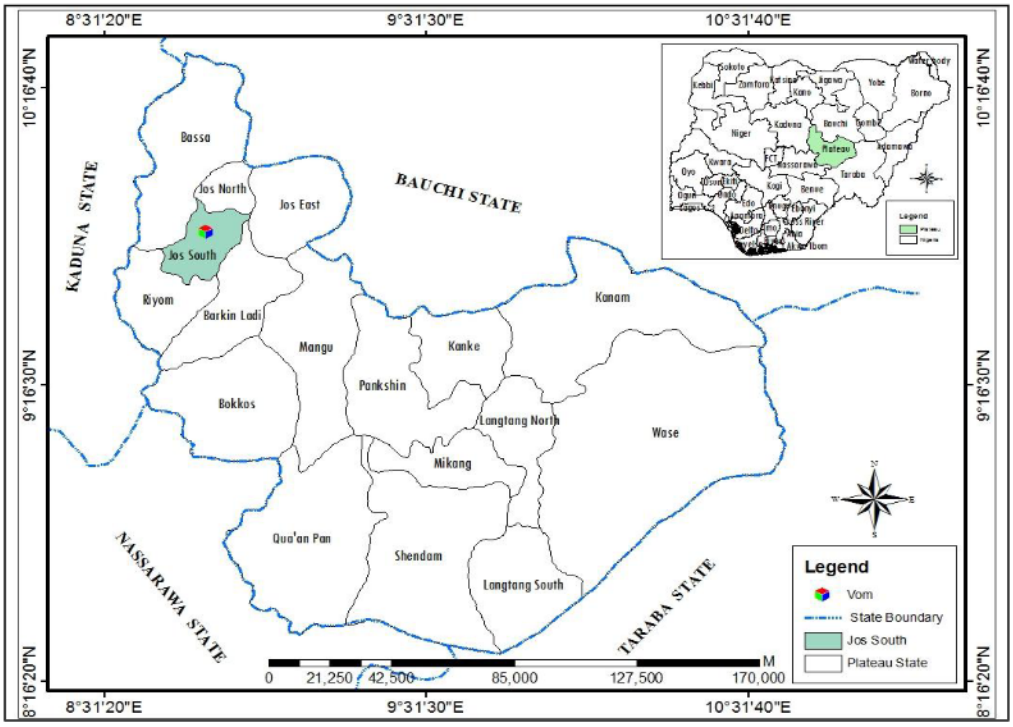

Fig. 1. Map of plateau state showing sample location. (Source: Modified from Administrative Map of Plateau State Using ArcGIS 10.3 Software)

2. MATERIALS AND METHODS

2.1 Sampling Methods

Three hatcheries were selected at random in a city in northern Nigeria and three farms were again selected from each of the three hatcheries using simple random selection methods.

2.2 Evaluation of Day Old Chicks (DOC) for *Salmonella* at the Hatcheries

Samples (n=18) per hatchery (including two carcasses and four faecal samples were taken from each of the three farms selected /hatchery from the three hatcheries). A total of 54 samples collected at the hatcheries before the chicks were taken to their respective farms.

2.3 Evaluation of the Chicks for *Salmonella* at the Farms

Six samples consisting of faecal, feed, water and litter were collected from the nine different farms every two weeks for eight weeks. Proper disinfection as well as change of laboratory wears were carried out from farm to farm to minimize or eliminate cross contamination.

2.4 Laboratory Procedures

2.4.1 Faecal, feed, litter, water and tissue samples

The samples collected were processed using standard microbiology methods [9]. One gram or litre (1 g or 1l) of the sample was pre-enriched in buffered peptone water (BPW) in the ratio of 1:10 sample to BPW and incubated at 37°C for 24hours. Samples were then enriched on Rappaport Vassiliadis (RV) broth (0.1 ml of sample from BPW into 10 ml of RV broth) and incubated at 42°C for 24hours. Tissues from dead birds consisting of lungs, liver, spleen and caeca and heart were also processed according to standard microbiology methods of isolation by [9]. Following enrichment process, broth cultures were inoculated onto two selective media Xylose Lysine Tergitol 4 (XLT4) and Brilliant Green Novobiocin Agar (BGN). Suspect colonies were then inoculated into Triple Sugar Iron (TSI) agar for 24hours at 37°C. These isolates were sent to the *Salmonella* reference laboratory in Padova, Italy for serotyping following specific pattern of agglutination reactions using the Kauffmann-White classification scheme [10].

3. RESULTS

From the three hatcheries (A, B and C) selected, two hatcheries (B and C) had *Salmonella* isolated either from the tissues or from the faeces before birds were introduced to the different farms where they were to be raised (Table 1).

By the 14th day when the first farm visit was made, all the three farms from hatchery A maintained their *Salmonella*-free status. There was no trace of *Salmonella* in farm B3 where there was previous infection. Only farm C1 had *Salmonella* and it was found in the feed, faeces and drinking water of the chicks. All the nine farms yielded no *Salmonella* on the three more farm visits except farm A3 where *Salmonella* was isolated during the third farm visit. Table 2 shows the distribution of *Salmonella* from the hatcheries to the farms during the course of this study.

A total of 918 samples (Faecal = 252, water = 216, feed = 216, litter = 216 and tissues =18) Table 4 were taken after the chicks had been introduced to the nine farms. Six (0.7%) of these were positive for Salmonella species including *Salmonella oakland, Salmonella bonariensis and Salmonella Kentucky* (Table 3). Eighteen tissue samples were analysed and four (22.2%) were positive for *Salmonella* (*S. oakland, S. enterica subsp enterica).* A total of 252 faecal samples were collected and three (1.2%) were *Salmonella* positive *(S. oakland)*, while two (0.9%) out of the feed samples were positive (*S. oakland and S. bonariensis)* One water (*S. bonariensis*) out of 216 (0.5%) and one litter sample out of 216 (0.5%) were *Salmonella* positive (*S. kentucky*) as depicted on Tables 4 and 5.

4. DISCUSSION

Epidemiological studies have demonstrated a variety of routes through which *Salmonella* can be disseminated within a poultry enterprise [11]. Young chicks may be colonized by *Salmonella species* directly through ovarian transmission or penetration into the egg shell after the egg has been laid [12]. Newly hatched chicks are at their peak of susceptibility to *Salmonella* colonization [13]. As can be seen in this current study, hatcheries B and C were already observed to have Salmonella contamination (in tissues or faeces) before birds from there were introduced and raised in the farms. The presence of salmonella in hatcheries B and C was as a result of non-adherence to good Agricultural best Practices and Hazard Analysis Critical Control Point (HACCP) principles which compliments the Code of Hygienic Practice for Eggs and Egg Products (CAC/RCP 15-1976) and Guidelines for the control of *Salmonella* in chicken meat (CAC/GL 78- 2011). Hatchery A in this study maintained its Salmonella-free status while farms B3, C1, C2,and C3 initially had salmonella contamination but were salmonella free after eight weeks due to strict adherence of the above guidelines. Salmonella-infected poultry may have high number of organisms in their intestines and are therefore an important source of contamination [14] as seen in this study where

Table 1. Showing number of hatcheries tested and those positive for *Salmonella* in Jos, northern Nigeria

Hatchery	No of Dead birds	No of Dead birds positive	No of faecal samples	No of faecal samples positive	Total no of samples	*Salmonella* isolation (%)
A	6	0	12	0	18	0 (0%)
B	6	1	12	0	18	1 (5.6%)
C	6	3	12	1	18	4 (22%)
Total	18	4	36	1	54	5 (27.8%)

Table 2. Shows the distribution of *Salmonella* from the hatcheries to the farms during the course of this study

Farm visits	A1	A2	A3	B1	B2	B3	C1	C2	C3
Hatchery status	-	-	-	-	+	+	+	+	-
2 weeks	-	-	-	-	-	-	+	-	-
4 weeks	-	-	-	-	-	-	-	-	-
6 weeks	-	-	+	-	-	-	-	-	-
8 weeks	-	-	-	-	-	-	-	-	-

Key: + = Positive for Salmonella; - : Negative for Salmonella

most samples analyzed were from faecal materials and the least were from tissues, yet the tissues had the highest amount of Salmonella species contamination. Of all the four different serotypes of Salmonella species that were encountered in this study *Salmonella oakland* were the most frequent and no previous studies from this part of the country have reported the isolation of *Salmonella oakland* to the best of the author's knowledge.

Poultry can become infected by horizontal transmission through infected litter, faeces, feed, water, dust, fluff insects, equipment, fomites, diseased chicks and rodents contaminated with *Salmonella* [14], and some of these have been seen during the course of this study. These findings are in agreement with the report of Muhammad M et al. [15], who isolated *Salmonella* from day old chicks from hatcheries in Jos, Nigeria.

Environmental sources are some other ways *Salmonella* gets into poultry farms. Numerous environmental factors can influence the likelihood and outcome of infections of poultry with *Salmonella*. Lengthy environmental persistence of pathogens can generate extended opportunities for horizontal transmission within and between flocks [13]. In this study, *Salmonella* from environmental sources accounted for 63.6% (7/11) of the isolates obtained. Isolating the organism from the environment is difficult because of the few Salmonellae in these sources [9] and the fragility of the organism in these samples.

Table 3. Serotypes of *Salmonella* isolated from the different farms sampled in Jos, northern Nigeria

Farm	# no of samples	Positive samples	Serotype isolated
A1	96	0	0
A2	96	0	0
A3	96	1	1*
B1	96	0	0
B2	96	0	0
B3	96	0	0
C1	96	5	4**
C2	96	0	0
C3	96	0	0
Total	864	6	6

- *S. Kentucky; ** . S. Oakland and S. bonariensis*

It has been reported that bacteriological sampling does not always provide an accurate indication of infection within a flock because of low incidence of infection and the intermittent excretion of *Salmonella* organisms [16]. This might also explain why the rate of isolation of the organism was low in this study besides the isolation conditions.

Table 4. Isolation of *Salmonella* from the different samples collected

Type of sample	Total number of samples	Number positive for *Salmonella* (%)
Tissues	18	4 (22.2)
Faeces	252	3 (1.2)
Feed	216	2 (0.9)
Water	216	1 (0.5)
Litter	216	1 (0.5)
Total	918	11 (1.2)

Table 5. *Salmonella* serotypes isolated from different samples and their percentages

Isolate	Sample type	Number isolated (%)
S. kentucky	Litter	1 (9.1)
S. enterica subsp enterica	Tissue	1 (9.1)
S. oakland	Faeces, Feed, Tissues*	7 (63.6)
S. bonariensis	Feed, Water**	2 (18.2)

- *Faeces (n = 3), Feed (n =1) , Tissues (n = 3); ** Feed (n = 1), Water (n = 1)*

5. CONCLUSION AND RECOMMENDATION

Salmonella infections are not just acquired on farms, but sometimes the hatcheries that supply chicks are responsible for disseminating the organisms as was seen in this study, resulting in high chick mortality, poor feed conversion and unnecessary exposure of farmers/consumers to infections.

The authors are of the opinion that, enforcements of existing laws that prohibit establishment of poultry farms and hatcheries without adequate training for farmers should be implemented. Also, there should deployment of veterinary and animal health extension services in all the rural areas of the communities to ensure adequate records, proper monitoring, and effective management of *Salmonella* infections. Finally, surveillance is needed to help prevent food-borne disease outbreaks and raise awareness among health authorities, food producers, food regulators, and consumers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. CDC. Salmonella Surveillance: Annual Summary. Centers for disease control and prevention. Atlanta, GA; 2005.
2. Cogan TA, Humphrey TJ. The rise and fall of *Salmonella enteritidis*in the UK. J. Appl. Microbiol. 2003;94:114S–119S.
3. Park SY, Woodward CL, Kubena LF, Nisbet DJ, Birkhold SG, Ricke SC. Environmental dissemination of foodborne Salmonella in preharvest poultry production: reservoirs, critical factors and research strategies. Crit. Rev. Environ. Sci. Technol. 2008;38:73-111.
4. Jones FT. A review of practical Salmonellacontrol measures in animal feed. J. Appl. Poult. Res. 2011;20:102–13.
5. Ricke SC, Dunkley CS, Durant JA. A review on development of novel strategies for controlling *Salmonella enteritidis* colonization in laying hens: Fiber-based molt diets. Poult. Sci. 2013a;92:502-525.
6. Carrique-Mas JJ, Bedford S, Davies RH. Organic acid and formaldehyde treatment of animal feeds to control Salmonella: efficacy and masking during culture. J. Appl. Microbiol. 2007;103:88-96.
7. Agbaje M, Davies R, Oyekunle MA, Ojo OE, Fasina FO, Akinduti PA. Observation on the occurrence and transmission of *Salmonella gallinarum* in commercial poultry farms in Ogun State, South Western Nigeria. African Journal of Microbiology Research. 2010;4(9):796-800.
8. Chao MR, Hsien CH, Yeh CM. Chou SJ, Chu C, Su CY, Yu CY. Assessing the prevalence of *Salmonella enterica* in poultry hatcheries by using hatched eggshell membranes. Poultry Science 2007;86:1651-1655.
9. Muhammad M. The role of *Salmonella sp* in early chick mortality and the application of hazard analysis critical control point (HACCP) for control. Ph.D Thesis University of Maiduguri; 2008.
10. Waltman WD, Gast RK, Mallinson ET. Salmonellosis. A laboratory manual for the isolation and identification of avian pathogens. In: Swayne DE, Glisson JR, Jackwood MW, Pearson J.E and Reed W.M.(Eds) American Association of avian pathologists 4th edition. University of Pennsylvania, USA. 1998;4-13.
11. Popoff MY, Bockemühl J, Brenner FW, Gheesling LL. Supplement 2000 (no. 44) to the Kauffmann-White scheme. Research in Microbiology. 2001;152:907–909.
12. Nayak R, Stewart T, Wand RF. Lin J, Cerniglia CE, Kenney PB. Genetic diversity and virulence gene determinants of antibiotic resistant *Salmonella* isolated from preharvest turkey production sources. International Journal of Food Microbiology. 2004;91:51–62.
13. Cox NA, Berrang ME, Cason JE. *Salmonella* penetration of egg shells and proliferation in broiler hatching eggs: A review. Poultry Science. 2000;79:1571–1574.
14. Gast RK. Serotype-specific and serotype-independent strategies for preharvest control of food borne *Salmonella* in Poultry. Avian Diseases 2007;51:817-828.
15. Poppe C. *Salmonella* infections in the domestic fowl, in Wray, C. & Wray, A. (Eds) *Salmonella* in domestic animals, (Wallingford CABI Publishing).2000;107-132.
16. Muhammad M, Muhammad LU, Ambali A, Mani AU, Azard S, Barco L. Prevalence of *Salmonella* associated with chick mortality at hatching and their susceptibility to antimicrobial agents. Veterinary Microbiology. 2010;140:131-135.

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