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PREVALENCE OF *SALMONELLA* TYPHIMURIUM FROM COMMERCIAL POULTRY AND HANDLERS IN NASARAWA STATE, NIGERIA

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ABSTRACT
This study was designed to investigate the prevalence of *Salmonella* Typhimurium from commercial poultry and handlers in Nasarawa State, Nigeria. This was conducted in the Microbiology Laboratory, Nasarawa State University, Keffi, Nigeria, from 1st November 2017 to 31th April 2018. A total of 1500 samples (poultry droppings, flesh feed, handlers' faeces and hand swabs) were screened for the presence of *Salmonella* Typhimurium using standard bacteriological methods. Presumptive *Salmonella* colonies were confirmed as serovar Typhimurium using both the conventional biochemical screening tests and Microgen Bioproduct GN identification system, and serotyping by the slide agglutination test using polyvalent antiserum according to Kauffman White's scheme. The prevalence of *S.* Typhiumurium was 7.1% (106/1500), with the highest sample-related prevalence in droppings (16.7%, 50/300), the highest location-related prevalence in NW (11.8%, 59/500) and zero prevalence in hand swabs across all locations. The differences between the prevalence rates from the various sample types were insignificant (p = 0.10).The results would bridge the gaps in data of prevalence of *S.*Typhimurium in poultry and handlers in Nasarawa State. The Findings will be beneficial to individuals, public health officials, regulatory agencies and poultry handlers on the need for observing strict sanitation and hygienic practices in poultry rearing and processing. *Salmonella* Typhimurium contamination is prevalent in poultry, and handlers' infection is possible via direct and/or indirect contact with colonized poultry.

Keywords: *Salmonella* Typhimurium; Poultry; Prevalence; Contamination; Nasarawa

INTRODUCTION

Poultry is one of the common carriers of non-typhoidal *Salmonella* (Tadesse, 2017) and *Salmonella* in healthy poultry is the main risk factor for the possible outbreak of human salmonellosis as evidenced from epidemiological studies (Antunes *et al*., 2015; Hugas *et al*., 2014). *Salmonella* Enteriditis and *Salmonella* Typhimurium are known to be the serovars most commonly associated with human disease for which poultry are a major source of infection (WHO, 2018). They are known to be prevalent in poultry, livestock and reptiles (Dar *et al*., 2017). *Salmonella* Typhimurium, being a zoonotic pathogen, can readily pass from animal to man, through consumption of contaminated food (Cosby *et al*., 2015). The occurrence of *Salmonella* in poultry droppings, feeds, eggs, and chicken meat continues to be on the increase with high morbidity and in some cases death (Adeyanju *et al.,* 2014). The expansion of poultry rearing and farming has made salmonellosis to become an important public health problem in Nigeria and other parts of the world causing heavy economic loss (Mohammed *et al*., as cited in Agada *et al*., 2014). In Nasarawa State, Nigeria, no documented evidence is known to the authors on the prevalence of *S.* Typhimurium from poultry and handlers from these sources. This study thus investigated the prevalence of *S.* Typhimurium from commercial poultry and handlers in Nasarawa State, Nigeria. The outcome of this study can have an overwhelming impact on public health and the economy considering the booming poultry sector in Nigeria.

MATERIALS AND METHODS

Study Area

The study was conducted in Nasarawa State, north central Nigeria. The State lies between latitude 7^0 45' and 9^0 25'N of the equator and between longitude 7^0 and 9^0 37'E of the Greenwich meridian. It occupies an area of 27,117 km^2 and a population of 1,869,377 as at 2006 census. Agriculture is the mainstay of the economy. The poultry population is unknown in Nasarawa State but as at 2017, Nigeria's production of poultry meat was estimated at 201,493 tonnes (Knoema, 2019). The State has three Senatorial Districts namely: Nasarawa North (NN), Nasarawa South (NS), and Nasarawa West (NW) and the average rain fall is 104.75 cm (Saliu *et al*., 2014). The study areas were selected using stratified random sampling and a total eighteen farms were sampled which comprised of; Akwanga and Nasarawa Eggon (in Nasarawa North), Lafia and Keana (in Nasarawa South) and Keffi and Karu (in Nasarawa West).

Sample Size Determination and Collection

The sample size was determined using the formular of Daniel (1999):

$$n = \frac{Z^2 P(1-q)}{d^2}$$

where n = sample size
Z = Z statistic for a level of significance, 1.96 at 95% confidence interval
P = expected prevalence or proportion which was found to be 26%. Hence, P= 0.26 from previous prevalence.
q = (1-p) = 1-0.26 = 0.74.
d = precision which is taken at 5% = 0.05

$$n = \frac{(1.96)^2 0.26(1-0.74)}{(0.05)^2} = 295$$

The total minimum number of each sample required was 295. However, 300 of each sample type, making a total of 1500, were collected across the senatorial districts using simple random sampling Technique. Samples include poultry droppings (diarrhoeic and non-diarrhoeic), poultry flesh, poultry feeds, faeces (diarrhoeic and non-diarrhoeic), and hand swabs from handlers. Informed consent, voluntary participation was obtained from farm owners and workers. Ethical approval was obtained. Stool samples were collected in sterile stool containers whereas hand swabs were obtained using sterile cotton swabs immersed in sterile 0.85% buffered peptone water (BPW: Oxoid Ltd (Hampshire, UK). The poultry flesh, droppings and feeds were also aseptically collected in sterile leak-proof wide mouthed plastic bags. Additional data was also collected using questionnaires administered on 300 respondents i.e. poultry handlers in the study area to investigate socio economic characteristics, Farm house infrastructure, hygienic practices, knowledge on *Salmonella* contamination control plan and symptoms relating to salmonellosis.

Isolation of Presumptive *Salmonella*

Isolation of *Salmonella* was carried out according to ISO 6579-1 (2017) as follows; using pre-enrichment of samples in buffered peptone water (1:10) dilution with subsequent aerobic incubation at 37 ^{0}C for 18 hours. This was followed by selective enrichment in Rappaport-Vassiliadis Broth (RVB). 1ml of overnight pre-enriched culture media was transferred into a tube containing 9ml of RVB with subsequent incubation at 42 ^{0}C for 24 hours. A loop full from the RVB was and then sub-cultured by streaking onto SSA and XLD media respectively with incubation at 37 ºC for 24 h. The cultured plates were examined for the presence of typical colonies of *Salmonella* based on cultural and morphological characteristics on the media.

Identification and Confirmation of *Salmonella* Typhimurium

Presumptive *S.* Typhimurium isolates were confirmed by Gram staining, use of conventional biochemical tests as described by Cheesebrough (2006) and Microgen^{TM} GnA+B-ID System Bioproducts Limited (Camberley, UK) also for biochemical identification as specified by the manufacturer. The isolates were further tested for somatic 'O' and flagella 'H' antigen using polyvalent *Salmonella* antisera (Oxoid, UK) in accordance to the Kauffmann-White Scheme using slide agglutination test.

Statistical and Data Analysis

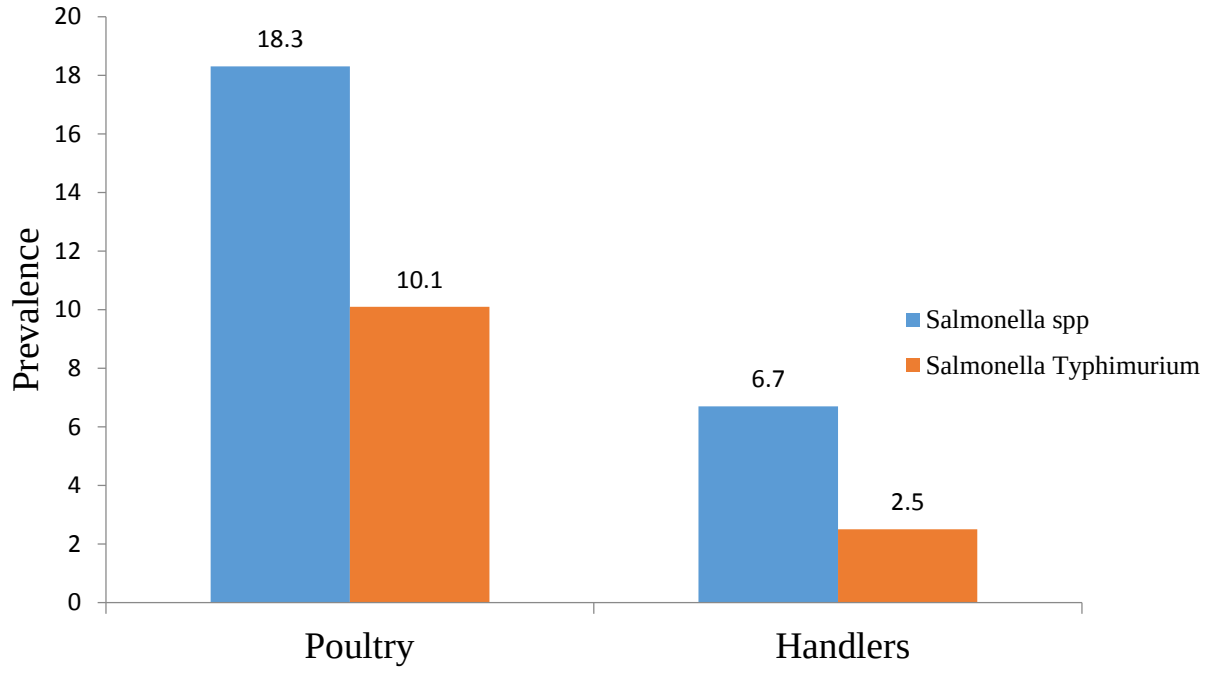
Statistical analysis was performed with SPSS version 20.0. Descriptive statistics (Frequency counts) and inferential statistics (chi-square and T-test) were used for data analysis.

RESULTS

Prevalence of *Salmonella* Typhimurium

Isolation rates of *Salmonella* in Poultry and Handlers

The isolation rates are as shown in Fig.1. Poultry yielded the highest isolation rates of *Salmonella* spp (18.3%) as well as *S.*Typhimurium (10.1%) while the rates from handlers were 6.7% *Salmonella* spp and 2.5% *S.*Typhimurium.



The isolation rates for *Salmonella* Typhimurium from poultry droppings, flesh, feeds and handlers' faeces, and hand swabs are presented in Table 1. The overall rate is 7.1% (106/1500) with droppings having the most frequency (16.7%) > flesh (11.7%) > feeds (2.0%) > faeces (5.0%). The prevalence rates of *Salmonella* spp and *Salmonella* Typhimurium from the various sample sources is significant (p = 0.00). However, for each one respectively it is not significant.

Table 1: Isolation rates of *Salmonella* Typhimurium in relation to Sources

Source	No. of Samples examined	No. (%) *Salmonella* spp	No. (%) *S.* Typhimurium
Droppings	300	80(26.7)	50(16.7)
Flesh	300	65(21.7)	35(11.7)
Feeds	300	20(6.7)	06(2.0)
Feces	300	40(13.3)	15(5.0)
Hand Swab	300	00(0.0)	00(0.0)
TOTAL	**1500**	**205(13.7)**	**106(7.1)**
T-value		0.75	-0.407
P-value		0.45	0.70
LOS		NS	NS

χ^2 - value = 318; P-value = 0.00, NS = Not significant, LOS = Level of significance

The overall distribution of *Salmonella* Typhimurium isolates in the three senatorial districts is as shown in Table 2. Isolation rates were of the order: NN (4.6%, 23/500) < N.S (4.8%, 24/500) < NW (11.8%, 59/500). The distribution of *Salmonella* Typhimurium isolates from the various sample sources within the senatorial districts was not significant. (χ^2 - value = 5.25; P-value = 0.51).

Table 2: Distribution of *Salmonella* Typhimurium isolates in poultry and handlers in Nasarawa State, Nigeria

Locations	No. (%) *Salmonella* Typhimurium isolated					No. of samples examined	Total (%)
	Droppings (n = 100)	Flesh (n = 100)	Feeds (n = 100)	Feces (n = 100)	Hand Swabs (n = 100)		
NN	13(13.0)	07(7.0)	00(0.0)	03(3.0)	00(0.0)	500	**23(4.6)**
NS	10(10.0)	11(11.0)	01(1.0)	02(2.0)	00(0.0)	500	**24(4.8)**
NW	27(27.0)	17(17.0)	05(5.0)	10(10.0)	00(0.0)	500	**59(11.8)**
TOTAL	**50(16.7)**	**35(11.7)**	**06(2.0)**	**15(5.0)**	**00(0.0)**	1500	**106(7.1)**

χ^2 = 5.25, *P* - value = 0.51

KEY: NN = Nasarawa North: NS = Nasarawa South: NW = Nasarawa West

DISCUSSION

The observed prevalence of *Salmonella* Typhimurium in the present study is linked to the fact that diarrhoeic samples from both poultry and handlers were included in the study. Diarrhoea is a symptom of salmonellosis (Cosby *et al*., 2015; Yan *et al*., 2010; Yang *et al*., 2010) and 60% of respondents within the study area, had symptoms of salmonellosis which thus contributed to the frequency of isolation. Incidentally, *Salmonella* Typhimurium had been reported to be the most prevalent serovar isolated from humans, poultry and other animals with non-typhoidal salmonellosis (NTS) (Mouttotou *et al*, 2012; Dar *et al*., 2017).

The prevalence in this study is similar to the findings in South western Nigeria by Fasure *et al*. (2012) in Ogun (11.4%), Fashae *et al*. (2016) in Ibadan (11.0%) and North central Nigeria by Agada *et al*. (2014) in Jos (10.9%; 8.2% for *Salmonella* species and *S.* Typhimurium respectively). Lower prevalence of 3.5% was however reported by Babatunde *et al*. (2017) in Ilorin, Nigeria. High prevalence of up to 75% was reported by Raj *et al.* (2017) in India, and Ali *et al.* (2018) in Iraq. Other studies on non-typhoidal *Salmonella* (NTS) in Asia have reported *Salmonella* spp / *S.* Typhimurium prevalence of 20.8% / 2.5% by Thung *et al*. (2018), 27.6% / 2.3% by Shafini *et al*. (2018) in Malaysia and 1.04% / 10.5% by Tahir *et al*. (2018) in Pakistan.

The higher prevalence of *S.* Typhimurium from poultry droppings compared to faeces of handlers in the present study can be attributed to the fact that poultry is a well known reservoir for NTS, (Cosby *et al*., 2015) and it has been documented to be harboured in the intestinal tract of food producing animals (Barrow *et al*., 2012). Our findings agree with reports in Uganda (Odoch *et al.*, 2018), India (Waghamare *et al.*, 2017) and in Jos, Nigeria (Anejo-okopi *et al.*, 2016).

Salmonella Typhimurium in handler's faeces is linked to direct or indirect contact with poultry droppings. Incidentally, 80% of handlers in the study area affirmed that they did not practise regular and proper washing of hands after contact with the birds. This likely resulted in contamination of the handlers arising from the poor maintenance of proper hygienic practises after contact with poultry and subsequently, prior to handling foods.

The detection and prevalence of *S.* Typhimurium in poultry flesh for this study implies its ability to grow on warm flesh. The rinse water, the knives and chopping boards were observed to be contaminated with poultry droppings, which

were repeatedly used without being washed, disinfected or changed. This suggests that the detection of *S.* Typhimurium in poultry flesh, likely aroused from cross-contamination. This is in agreement with the findings of Thung *et al*. (2018) and cross-contamination is said to partly be as a result of residual bacteria on table surfaces and knives used for poultry processing at slaughter houses, even after cleansing and food borne outbreaks of salmonellosis are most frequently connected with *Salmonella* in chicken meat (Bhaisare *et al*., 2014; Ejo *et al*., 2016).

The isolation rates in flesh for our study is similar to the 11.5% and 7.89% reported in Ethiopia and Morocco by Tadesse *et al.* (2018) and Khallaf *et al.* (2014). Higher isolation rates than the present study were reported in Calabar (67.6%) by Yhiler *et al.* (2015), China (25.0% & 53.59%) respectively by Yang *et al.* (2010); Li *et al.* (2017), Vietnam (42.91%) by Thai *et al.* (2012) and in India (33.3%) by Balakrishnan *et al.* (2018).

The isolation rate of *S.* Typhimurium from poultry feeds in this study is low when compared to poultry droppings, poultry flesh and handlers' faeces. It is possible that the presence of *Salmonella* in the feeds may have arisen from contamination with poultry droppings whilst feeding (personal observation). The presence of antibiotics incorporated in the feeds, also likely played a role in limiting its frequency of isolation by inhibiting the multiplication of *Salmonella. Salmonella* isolation rates from poultry feeds in various studies had been attributed to content of antibiotics incorporated in feeds (Mouttotou *et al*., 2017) and hygienic standard in feed compoundment (Fagbamila *et al*., 2017).

Our prevalence findings for *S*. Typhimurium in feeds closely agrees with the 1.1% reported by Agada *et al*. (2014) in Jos and the 2.3% reported by Khalil *et al*. (2006) in Jordan. It is also in line with but slightly higher than the 3.9% recorded in Ilorin, Nigeria by Babatunde *et al*. (2017). However, it is lower when compared with the findings of Orji *et al*. (2005) in Awka, Nigeria who recorded 38.3% prevalence for *Salmonella* serotypes out of which 6.7% were *Salmonella* Typhimurium. Ifeanyichukwu *et al*. (2016) in Ebonyi, Nigeria recorded 8.2% prevalence in poultry feeds while Mdemu *et al*. (2016) reported 29.4% for poultry feeds in Tanzania, which are higher than this study. The present study was found to be in discordant with the findings of Samanta *et al*. (2014) in India and Fagbamila *et al*. (2017) who sampled across six geo-political zones of Nigeria. Both recorded a prevalence of 10.0% and 13.9% respectively, of *Salmonella* without encountering *S*. Typhimurium.

Circulation of zoonotic *Salmonella* in Nigeria, as in other developing countries, may have a global impact in terms of public health because of movements beyond the area of origin, as a consequence of trade and travel (Barua *et al*., 2012). The isolation rates in the three senatorial zones of Nasarawa State were not significant but higher in Nasarawa West. This may be as a result of some unknown variations related to study areas or the ability of *S*. Typhimurium to thrive under harsh conditions of the sampling season as at the time of study. A significant difference was observed in the isolation rates of *Salmonella* Typhimurium from the various sources across the three senatorial zones and recovery of isolates was abundant from poultry source compared to poultry handlers. This was not surprising seeing that *Salmonella* Typhimurium is known to have a broad host range affecting humans and animals (Shah *et al*., cited in Agada *et al*., 2014) and is still the most prevalent serotype in Europe and America with growing importance in Africa (Smith *et al*., 2016). According to Dar *et al*. (2017), foods of animal origin, especially poultry derived items are the main source of infection by *Salmonella* spp. Overall, distribution of isolates is attributed to poor hygienic standard among the poultry handlers, such as inadequate hand washing after contact with poultry, poultry products and the observed re-using of rinse water after de-feathering although no isolates were obtained from hand swabs throughout the study. Moreover, constant contamination of feeds by droppings, in addition to poor sanitation of the poultry pens as well as exposure of the poultry birds and their feeds to rodents and locally raised fowls within the farms and markets are equally important factors. Geographical location, sampling seasons, preservation method and time taken before sample was processed in the laboratory as well as isolation methods are also known to affect prevalence results (Balakrishnan *et al*., 2018; Thung *et al*., 2018). These Observations are in agreement with the findings of Agada *et al*., 2014; Fasure *et al*., 2012 ; Fagbamila *et al*., 2017.

In conclusion, *Salmonella* Typhimurium contamination is prevalent in poultry and can affect handlers. Contributing factors include but not limited to the handlers' lack of knowledge on *Salmonella* contamination and control plan, climatic condition in the State during the period of the study, which is favourable for *Salmonella* to thrive, being a known hardy bacterium. In addition, poor sanitation and hygienic standards at the farms, slaughter house, the slaughtering process and processing of the carcass, improper washing of hands after handling poultry and poultry products prior to handling foods are among factors observed to be responsible for carcass contamination and infection of the handlers.

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