Vol. 12(3), pp. 132-138, July-September 2020
DOI: 10.5897/JVMAH2020.0854
Article Number: E82583D64880
ISSN: 2141-2529

http://www.academicjournals.org/JVMAH

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Journal of Veterinary Medicine and Animal Health

Full Length Research Paper

Occurrence and characterization of Salmonella isolates in raw eggs from quail and chicken in selected poultry farms in Jos, Plateau State, Nigeria

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Received 16 June, 2020; Accepted 31 August, 2020

The study was carried out in three Local Government Areas: Jos North, Jos South and Jos East. For each egg type, twelve (12) samples each were collected from five (5) farms. A total of 360 samples were randomly collected consisting of equal number of quail and chicken eggs (180 each). A well-structured questionnaire was used to help analyze the results. Samples were examined for the presence of Salmonella isolates using standard microbiological practices. Isolates were confirmed using biochemical tests, and molecular characterization (using specific primers). Isolates were also tested for antimicrobial susceptibility by disc diffusion method. Results showed that 3(1.7%) chicken eggs were positive for Salmonella infection whereas no positive result was recorded from quail eggs. This resulted in a total prevalence of 0.9%. Bukuru and Zawan (Jos South) were the only farm locations with Salmonella positive cases with 1(8.3%) and 2(16.7%) respectively. Although the present finding has found low prevalence of salmonellosis in chicken and quail egg in the study area, there is need for constant monitoring on regular basis to avert health risks associated with consuming Salmonellae infected poultry products in endemic areas. The three (3) isolates were Salmonella Gallinarum and gave agglutination reaction with polyvalent O antisera and no reaction with polyvalent H antisera. Polymerase Chain Reaction (PCR) results confirmed all the three (3) isolates that were successfully amplified using specific primers, thus supporting phenotypic outcome. The information provided in this report is crucial to all stakeholders including the poultry farmers, consumers and regulators of chicken products.

Key Words: Salmonella; Quail and Chicken eggs; Jos; Nigeria

INTRODUCTION

Salmonella organisms are facultative anaerobic rod-shaped Gram-negative bacteria belonging to the family of Enterobacteriaceae (Douglas et al., 2015). *Salmonella*, like most Enterobacteriaceae are motile by peritrichous

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flagella except *Salmonella* Pullorum and *Salmonella* Gallinarum, which lack flagella (Bhunia, 2008). The genus *Salmonella* is divided into two species, *Salmonella* Enterica and *Salmonella* Bongori. Most pathogenic species of *Salmonella* affecting humans are within the species of *S.* Enterica*.* Over 2,500 serotypes have been reported due to differences in the somatic (O) and flagella (H) antigens (Solari et al., 2003; Barde et al., 2017). However, a recent report from the Centre for Infectious Disease Research and Policy classifies members of the *Salmonella* species into more than 2541 serotypes (serovars) according to their somatic (O) and flagellar (H) antigens (CIDRAP, 2006). The pathogen lives primarily in the intestinal tract of animals, birds, mice, farm animals and sometimes in eggs (Ellermeier and Slauch, 2006). Eggs are still considered to be an excellent source of chlorine and selenium and a good source of riboflavin. The protein found in eggs is highly digestible; the yolk contains vitamins A, D, E and K, folic acid, pantothenic acid and Zinc (Egg Nutrition Center, 2004).

World over, consumption of eggs has increased considering the nutritional importance of it to man. Eggs for their nutritional qualities are consumed more to supplement protein intake, this have led to the increase in the production through poultry farming. It is consumed due to unavailability and high cost of other protein products like beef meat. Studies have shown that eggs contain high level of cholesterol; it is still patronized by all either raw or undercooked and are part of recipes of commercial and homemade products like mayonnaise, cake, pastries, and salad (Adesiji et al., 2013; Olabode et al., 2019). While eggs are highly nutritious for humans, they are also nutritious for other living organisms. Just as the yolk provides nutrients to a growing embryo, it is also a nutritional resource for bacterial organisms when they cross egg shells and membranes. Scientific Committee on Veterinary Measures relating to public health has identified eggs and egg-based products containing raw eggs as a food group, which pose a public health hazard (European Commission, 2004).

There are two pathways for eggs to be contaminated with *Salmonella* either directly by transovarian transmission or indirectly. These pathways for contamination can be affected by the way egg is produced, processed, stored, handled and prepared (FDA, 2009). Many food handlers and populace are not educated on the necessity of good hygiene and adherence to food preparation guidelines like avoiding cross contamination of food with raw eggs, avoiding consuming raw or under cooked eggs. There has also been a shift in consumers eating habit and demand for raw, unprocessed food and fast food (Krester et al., 2014; Enas et al., 2019). Hence, this study is aimed at determining the occurrence of *Salmonella* in raw eggs from quail and chicken in selected poultry farms in Jos and to characterize the organism molecularly.

MATERIALS AND METHODS

Study area

Jos is a city in the Middle Belt of Nigeria with an area of 26,899 km^2. The city has a population of about 900,000 residents based on 2006 census (Federal Republic of Nigeria 2006 Population Census). The main occupation is agriculture with most of it populaces involved in poultry faming. Plateau is located between Latitude 08° 21' and Longitude 008°32' and 010°38' east. The weather is characterized by a near temperate climate on the upper parts of Plateau and a humid climate on its lower parts. The mean annual temperature in the state ranges between 20 and 25°C, while the mean annual rainfall figures range from131.75 cm in the southern part to 146 cm on the northern part. The study was carried out in some selected farms of three Local Government Area (LGA) of Plateau State comprising Jos North, Jos East and Jos South. Jos North has its head quarter in the state capital. Jos South having Bukuru as its head quarter, and Jos East, Angware as its head quarter.

Sample size

A total number of 370 sample size was determined using prevalence rate from previous studies (Mai et al., 2013) and the desired absolute precision with the formula (Naing et al., 2006):

$n=Z^2Pq/d^2$

Ethical clearance

Live animals were not used so there was no need for ethical approval but clearance to gain access to the farms was obtained from Department of Veterinary Service, Ministry of Agriculture, Plateau State.

Administration of structured questionnaire

Structured questionnaire with necessary information of voluntary and informed consent were administered to study participants and the poultry owners.

Statistical analysis

Data obtained were analyzed using the Chi-square test.

Collection of egg samples

A total number of 360 sampled eggs were collected, 180 samples for chicken and 180 samples for quail eggs. Each farm was sampled twice, one in the dry season and the other during the rainy season. The sampling was selected randomly from each farm.

Packaging for laboratory analysis

Chicken and quail eggs, respectively were collected in separate sterile plastic bags each, egg shell surfaces were swabbed with sterile swab stick and placed into buffered peptone water (BPW), to avoid dryness of the swab.

Transport of swab samples

All samples were placed in sterile plastic bags and then packaged in ice box and transported immediately to microbiology unit of Central Diagnostic Laboratory Department of National Veterinary Research Institute Vom, Plateau State Nigeria.

Sample processing

All samples were processed according to standard guidelines of detecting *Salmonella* both in the egg shell and internal content by International Standard Organization (ISO): 6579 (2012) and Office International des Epizooties (OIE, 2012).

Egg internal content

Eggs from the sterile plastic bag were aseptically opened with sterile scissors and the egg shell aseptically broken and the content from each egg were homogenized in a glass flask. Exactly 1 ml of the homogenized egg was transferred into 9 ml of buffered peptone water (BPW) (Pre-enrichment broth) and incubated at 42°C for 24 h.

In selective enrichment

One milliliter of the pre-enriched-culture was transferred to tubes containing 9 ml Rappaport-Vassiliadis broth (enrichment medium), then sub-cultured by streaking onto DCA and XLD agar.

Swabs from surface of egg shell

Surface swabs from egg shells collected was directly incubated in 9 ml BPW in screw capped bottles and then incubated at 42°C for 24 h for pre-enrichment. About 1 ml of the pre-enrichment broth was transferred into tubes containing 9 ml RVB, then sub-cultured by streaking onto DCA and XLD agar. The sub-cultured plates were incubated at 37°C for 24 h (Suresh et al., 2006; OIE, 2012).

Isolation and identification of S*almonella*

Salmonella was isolated and identified biochemically according to Suresh et al. (2006) and ISO-6579 (2012).

Serotyping

Cultures of organisms from a pure culture identified as *Salmonella* by biochemical tests were serotyped. The serological identification of *Salmonella* spp. was done using polyvalent *Salmonella* H antisera and *Salmonella* O antisera (Oxiod, UK).

Polymerase Chain Reaction (PCR)

Salmonella specific primers, based on the invA gene of *Salmonella* were used. Forward: 5' GTG AAA TTA TCGCCACGT TCG GGC AA3' and Reverse: 5' TCA TCG CAC CGTCAA AGG AAC C3'. After which results were viewed using a gel imaging documentation apparatus (MB Fermentase USA).

RESULTS AND DISCUSSION

The result showed the occurrence of Salmonella from the three (3) local Government Area surveyed. Jos South were higher compared to Jos North and Jos East. *Salmonella* occurrence recorded in this study was 3(0.9%), out of which chickens had 3(1.7%) while quail had 0(0.0%) (Table 1). This is in agreement with a study reported by Agada *et al.* (2014) with 10.9% occurrence of *salmonella* from human faeces/hand swab, poultry droppings, swabs from shell of intact egg and feeds. Jos South, while Jos East and Jos North recording highest and lowest respectively of *salmonella* species contamination of commercial poultry farms in Jos, plateau state (Tables 2, 3 and 4). Bata *et al.* (2016) also reported high isolation rate in Jos south compared to Jos north and Jos East. The difference in the distribution of isolates may be due to social-demography difference and other bio-security practices in the study areas. It is important to note that even though the serotype isolated in this study was *S.*Gallinarum which is host specific, were isolated from the shell of chicken egg and none isolated in egg content (Table 5).

In this study, among the different locations sampled, Bukuru and Zawan were the only locations that recorded the occurrence of *Salmonella,* with 1(8.3%) and 2(16.7%) respectively in Chicken eggs (Table 2). Quail eggs recorded 0(0.0%) among all the locations sampled. From the egg shell and egg contents sampled, chicken eggs recorded occurrence of 3 (1.7%) from the egg shell and 0(0.0%) from the egg contents (Table 5). While quail eggs recorded 0(0.0%) from both the egg content and shell (Table 5). This is far below the finding of Anejo-Okopie *et al*. (2016) who reported 28.75% (23/80) occurrence, with droppings 11/80 (13.7.5%), egg shell swabs 5/80 (6.25%) and human hand swabs 7/80 (8.75%). In a similar study conducted in Jos, Mai *et al*. (2013) reported higher percentage occurrence (32.5%) of *salmonella* in table eggs sold at different markets in Jos south. The high percentage occurrence observed in the study by Naik *et al*. (2015) and others may be due to type of samples and location of the study. These differences could also be attributed to the high level of *salmonella* species contamination in their findings compared to this study. The low percentage occurrence observed in this study may perhaps be due to increased awareness on the prevention and control of poultry diseases. This has increased the level of bio-security and may be attributed to the fact that poultry farmers practice strict bio-security practices and care in most of the poultry farms surveyed**.** Another reason for the low percentage in this study can be attributed to the fact that the eggs sampled in this study area were freshly laid eggs confined within the selected poultry houses. It is observed that the level of external contamination is minimal when compared with those eggs already in the retail shops and those already

Table 1. Occurrence of *Salmonella* isolate from quail and chicken eggs in the three Local Government Area of Plateau State, Nigeria.

LGA	Quail eggs		Chicken eggs		Total	
	Number examined	Number and % positive	Number examined	Number and % positive	Number examined	Number and % positive
Jos East	60	0 (0.0)	60	0 (0.0)	120	0 (0.0)
Jos North	60	0 (0.0)	60	0 (0.0)	120	0 (0.0)
Jos South	60	0 (0.0)	60	3 (5.0)	120	3 (2.5)
Total	180	0 (0.0)	180	3 (1.7)	360	3 (0.9)

χ^2=4.336, P< 0.05, df=2.

Table 2. Occurrence of *Salmonella* isolates from quail and chicken eggs in selected farms in Jos South Local Government Area of Plateau State, Nigeria

Locations (Jos South LGA)	Quail eggs		Chicken Eggs		Total	
	Number examined	Number and % positive	Number examined	Number and % positive	Number examined	Number and % positive
Bukuru	12	0 (0.0)	12	1 (8.3)	24	1 (4.3)
Vwang	12	0 (0.0)	12	0 (0.0)	24	0 (0.0)
Shen	12	0 (0.0)	12	0 (0.0)	24	0 (0.0)
Zawan	12	0 (0.0)	12	2 (16.7)	24	2 (8.3)
Guratopp	12	0 (0.0)	12	0 (0.0)	24	0 (0.0)
Total	60	0 (0.0)	60	3 (5.0)	120	3 (2.5)

Table 3. Occurrence of *Salmonella* isolate from quail and chicken eggs in selected farms in Jos East Local Government Area of Plateau State, Nigeria.

Locations (Jos East LGA)	Quail Eggs		Chicken eggs		Total	
	Number examined	Number and % positive	Number examined	Number and % positive	Number examined	Number and % positive
Fobor	12	0 (0.0)	12	0 (0.0)	24	0(0.0)
Angware	12	0 (0.0)	12	0 (0.0)	24	0(0.0)
Lamingo	12	0 (0.0)	12	0 (0.0)	24	0(0.0)
Shere hills	12	0 (0.0)	12	0 (0.0)	24	0(0.0)
Kwanga	12	0 (0.0)	12	0 (0.0)	24	0(0.0)
Total	60	0 (0.0)	60	0 (0.0)	120	0(0.0)

Table 4. Occurrence of *Salmonella* isolate from quail and chicken eggs in selected farms in Jos North Local Government Area of Plateau State, Nigeria.

Locations (Jos North LGA)	Quail eggs		Chicken eggs		Total	
	Number examined	Number and % positive	Number examined	Number and % positive	Number examined	Number and % positive
Faringada	12	0 (0.0)	12	0 (0.0)	24	0 (0.0)
Eto Baba	12	0 (0.0)	12	0 (0.0)	24	0 (0.0)
Mista Ali	12	0 (0.0)	12	0 (0.0)	24	0 (0.0)
AngwaRukuba	12	0 (0.0)	12	0 (0.0)	24	0 (0.0)
Naraguta	12	0 (0.0)	12	0 (0.0)	24	0 (0.0)
Total	60	0 (0.0)	60	0 (0.0)	120	0 (0.0)

Table 5. Occurrence of *Salmonella* isolates between chicken and quail eggs on egg shell and egg contents.

Type of sample	Source		Total quail and chicken (%)
	Quail number and % positive	Chicken number and % positive	
Egg shell (n=180)	0(0.0)	3(1.7)	3(1.7)
Egg Content (n=180)	0(0.0)	0(0.0)	0(0.0)
Total (n=360)	0(0.0)	3(0.8)	3(0.8)

χ^2=3.025, P< 0.005, df=1.

transported to various destination before consumption. It also confirms the report recorded by Bata *et al*. (2016) who reported occurrence of salmonella from raw beef and quail eggs from farms and retail outlets as 1.3% (3/235) of which 1.7% from egg shell and 0.8% from egg content.

Although 1.7% occurrence was recorded in this study, it is important to state that it has public health implication on human health. From this study, it showed *salmonella* contamination was from the egg shell. It was also noted that the three isolates were recovered during the rainy season. During this period, there is high moisture and high humidity, these encourages the growth and invasion of microorganisms. At this season of the year, poultry droppings, litters and laying nests are seen wet and damp. These can also encourage survival of microoganism like the *Salmonella* species and then contaminating the egg shell. Contamination perhaps could be horizontally transmitted from the feaces or housing environment of the farms. *Salmonella* in droppings can penetrate egg shell despite the multiple barriers, *salmonella* is capable of migrating to the yolk (Messens *et al.,* 2005). Temperature difference between the newly laid egg and the environment it comes into contact plays a great role. When the egg is exposed to the environment cooler that the chicken body temperature which is 42^0C, a negative pressure develops and can lead to migration easily through the egg shell and membrane to the liquid portion of the eggs. When the eggs are broken like for preparation of food, *Salmonella* from the egg surfaces could find its way into the food which could pose potential health hazards especially when it carries the resistant strains of *salmonella* thereby causing the survival of antibiotic resistance in other pathogens (Okeke *et al.,* 2005; Enas et al., 2019). Many foods particularly those of animal origin, has been identified as vehicles for transmission of microbial pathogens to humans (Uyttendaele et al., 1998). This suggest that prompt removal of chicken waste and disinfection between flocks can greatly reduce *salmonella* contamination on the shell and content.

PCR assay is a recent tool for molecular identification of micro-organisms. It is sensitive, specific, reliable and also faster when compared to the conventional cultural method of identification. The PCR result confirms the result obtained from the conventional cultural method (Plate 1). This is in agreement with several other studies. Lampel *et al.* (2000) pointed out that PCR will allow detection of salmonella serotypes within a maximum of 12hrs in clinical samples. The reports of Oliveira *et al.* (2002) and Chiu *et al* (2006) respectively demonstrated that PCR is faster in saving time to detect *salmonella*. The time required for extraction of DNA using PCR technique did not exceed one day, whereas the time required for isolation and identification of *salmonella* by bacteriological examination took 5-7 days before the results were obtained. Therefore it can be concluded that PCR is more sensitive, reliable and faster technique than bacteriological methods.

Salmonella isolates detected by PCR in this study produced bands with amplicon size of 284bp. This corroborates with the work done by Nwiyi *et al*. (2016) and that done by Anejo- Okopi *et al*.(2016).The outcome of the PCR result from the 3 isolates was 3/3 (100%) compared to the phenotypic method by culture 3/360 (0.8%) used (Plate 1). This result suggests that the detection of *invA*gene by PCR is faster, more sensitive and specific than the conventional methods, and it a good confirmatory method for the detection of *Salmonella* spp. in food and clinical samples (Mamman *et al*., 2014). The difference in amplication size and difference in primer type used could be associated with the sensitivity of the PCR result due to the PCR employed in this study compared with results of other studies which reported higher sensitivity and results (Dione *et al*., 2011; Shanmugasamy *et al*., 2011).

Conclusion

The result of this study indicated that poultry eggs were contaminated with *Salmonella* especially from horizontal route resulting from cross contamination from the poultry droppings or from the housing environment. However, no egg tested positive for *S.* Enteritidis and *Salmonella* Typhimurum which are the most frequent found in eggs. It is important to note that even though the serotype isolated in this study was *S*. Gallinarum which is host specific isolated from the shell of chicken egg and none isolated in egg content. The results showed occurrence

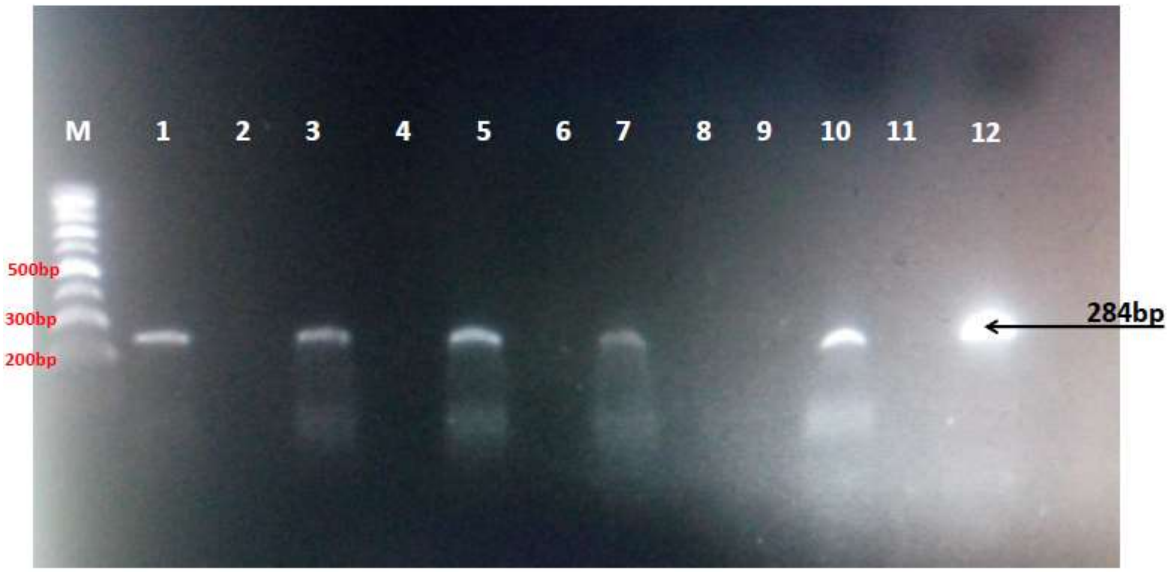

Plate 1. Electrophoresis gel image of the PCR amplicons of the *Salmonella* isolates using *Salmonella* inv A gene-specific primer pairs. Lane M: 100 base pair ladder. Lanes 1, 3 and 5 (positive samples); Lanes 2, 4 and 6 (negative samples); Lanes 7, 10, 12: positive control (*Salmonella* kenturkey vaccine strain from NVRI), Lanes 8 and 9: negative control (water)

of 3 (1.7%) chicken eggs for *Salmonella* infection whereas no positive result was recorded from quail eggs. This resulted in a total prevalence of 0.9%. Bukuru and Zawan (Jos South) were the only farm locations with *Salmonella* positive cases with 1 (8.3%) and 2 (16.7%), respectively. Quail eggs recorded occurrence of 0% *Salmonella* among all the locations sampled. Both chicken and quail egg contents had a 0% *Salmonella* occurrence.

Although this report recorded low prevalence of *Salmonella* in chicken and quail eggs in the study area, there is need for constant monitoring on regular basis to avert health risks associated with consuming *Salmonella* infected poultry products in endemic areas. Serological test for the identification of *Salmonella* isolates showed and identified all the 3 isolates as *S.* Gallinarum. The PCR result confirms the result obtained from the conventional cultural method. PCR is faster and saves time in detection and confirmation of *Salmonella*. The time required for extraction of DNA using PCR technique did not exceed one day, whereas the time required for isolation and identification of *Salmonella* by bacteriological conventional cultural examination took 5 to 7 days before the results were obtained. Therefore, it can be concluded that PCR is more sensitive, reliable and faster technique than bacteriological conventional cultural methods. This study indicated that poultry eggs were contaminated with *Salmonella* especially from horizontal route resulting from cross contamination from the poultry droppings or from the housing environment.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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