SHORT COMMUNICATION

Salmonella organism transmission in hatching broiler eggs¹ Olorunsola, R. A., ² Erubetina, D., ² O.M O Idowu ³ Oyekunle, and ² Ogunade, I.M.¹ Biological Sciences Department, Ondo State University of Science and Technology, Okitipupa.² Animal Nutrition Dept., Federal University of Agriculture, Abeokuta. ³ Veterinary Micro Biology

Dept., Federal University of Agriculture, Abeokuta,

Ogun State, Nigeria.

Correspondent author ajiwale2002@yahoo.com

Abstract

The vertical transmission of *Salmonella* organism in hatching broiler eggs were investigated in selected states in South-Western Nigeria. Hatching eggs were obtained from fifteen major hatcheries, five (hatchery A, B, C, D and E) from each State [Lagos (LA), Oyo (OY) and Ogun (OG)]. A total of 300 hatchable eggs of 20 eggs from each hatchery were collected. Individual egg was broken and separated into Shell, albumin and yolk. The samples were incubated into buffer peptone water and incubated at 37°C for 24 hours. Typical colonies of salmonella grown on Xylose lysine deoxycholate agar were seen as lining black center and lightly transparent zone of reddish colour which were further cultured on nutrient agar plates for confirmatory test. The biochemical characterization was carried out using rapid kit for the identification of members of Enterobacteria family. The study revealed that *Salmonella* pullorum was isolated from the albumin in hatchery (OG-A) and hatchery (LA-A), *Salmonella* arizonae was also isolated in the Yolk from the hatchery (OG-C), *Salmonella* gallinarum was found in the albumin in Ogun hatchery (OG-A), however, *Salmonella* paratyphi A was isolated in the albumin in Oyo hatchery B (OY-B). It is evident from the study that salmonella organism reside in various fractions of the egg like albumin, shell and yolk.

Keywords: *Salmonella* and hatching broiler eggs

Introduction

Salmonella infection in eggs is a worldwide problem leading to millions of avoidable cases of food poisoning, (Gast *et al.*, 2006). However, much of the poisoning has been linked to consumption of raw uncooked eggs, broilers and hatching eggs have also been a reservoir of the infection of day-old chicks. In the European Union, two species of bacteria viz. *Salmonella* and *Campylobacter* caused the major part of the reported cases of food borne outbreaks in 2001 (Cavite, 2003). Foods from animal origin, especially poultry, poultry products, eggs and egg products are often implicated in sporadic cases and outbreaks of human

salmonellosis (Bryan and Doyle, 1995). Food associated with *Salmonella enteritidis* outbreaks includes eggs and egg products in 68.2% of the cases (W.H.O., 2001)

There are two possible routes of salmonella contamination of intact eggs viz the transovarian route (vertical transmission) or the trans-shell route (horizontal transmission). In the Transovarian route the yolk membrane (very infrequently the yolk itself) or the albumin surroundings is directly contaminated as a result of salmonella infection of the reproductive organs before the egg is covered by the shell. In the trans-shell route (horizontal

transmission), salmonella penetrates through the egg shell after the egg is laid (Miyamoto *et al.*, 1998, Gast and Beard, 1990).

This study was carried out to investigate the vertical transmission of salmonella organism from hatching broiler eggs to the day old chicks in hatcheries in three selected States in South-Western Nigeria.

Materials and Methods

The study was carried out with hatchable eggs from 15 major hatcheries located within three South Western States namely Ogun, Oyo and Lagos. For ease of effective coverage, the sample size in each state was divided into area where livestock farming and the major poultry products like eggs and chickens are produced and distributed to other parts of the country. Five hatcheries each from Oyo, Ogun and Lagos (coded as A, B, C,D and E for each of the States) were randomly selected. Twenty-hatching eggs were obtained from each hatchery for salmonella isolation/and characterization making a total of 300 eggs. The eggs from each hatchery were grouped into four replicates of five eggs each. The five eggs were broken and separated into shell, albumin and yolk. The separated components of the eggs were pooled together for each hatchery on a replicate bases. The samples were inoculated into buffer peptone water (BPW Ager) in bijou bottle for pre enrichments and incubated at 37°C for 24 hours. A volume of 0.1ml of each of the pre-enriched samples (shell, albumin and yolk) was transferred into 10 ml of *Rappaport Vassiliadies* Broth with Soya (RVS) medium in bijou bottle and incubated at $41.5 \pm 1^\circ\text{C}$ for 24 ± 3 hours. The samples were thereafter inoculated by mean of sterile loop into plates containing Xylose lysine deoxycholate agar (XLDA) plates and incubated at 37°C for 24 hours.

Colonies resembling salmonella were sub cultured into brilliant green agar (BGA) plates and incubated at 37°C for 24 hours. The inoculated plates were inverted and transferred to an incubator at 37°C for 42 hours. Typical colonies of salmonella grown on XLDA were seen as lining a black centre and lightly transparent zone of reddish colour. When sub cultured in BGA the colonies appeared as pink colour of 1mm to 2mm diameter. Suspended colonies were seeded into pre-dried nutrient agar plates and incubated at 37°C for 24 hours to obtain pure cultures. The cultures on nutrient agar plates were further used for confirmatory test. The biochemical characterization was carried out using rapid kit (Oxoid microbact 24E) for the identification of members of the Enterobacteriaceae family.

Results

Table 1 showed the occurrence of salmonella organism in hatching broiler eggs from different hatcheries across Lagos, Oyo and Ogun States of Nigeria. The result for salmonella organism as evident in the table revealed that the yolk and albumin were positive for salmonella organism in the three States. In Oyo State, only albumin was positive for salmonella organism in hatchery B while yolk and albumin were positive for salmonella organisms in Lagos State (Hatchery A). Salmonella organisms were also found in hatchery A and C in Ogun State.

Table 2 showed the biochemical characterization of isolated salmonella organism from hatching broiler eggs from different hatcheries in the three selected states in South Western Nigeria. The result revealed two hatcheries in Ogun State (A and C) that are positive for salmonella organism. Hatchery A was identified to contain the serotype *Salmonella arizorae* in

Table 1: Presence of Salmonella in Hatching broiler eggs from different Hatcheries in Ogun State, Oyo and Lagos state Nigeria.

Hatcheries		A	B	C	D	E
Ogun State						
Yolk		-	-	+	-	-
Albumin		+	-	-	-	-
Shell		-	-	-	-	-
Oyo State						
Yolk		-	-	-	-	-
Albumin		-	+	-	-	-
Shell		-	-	-	-	-
Lagos State						
Yolk		+	-	-	-	-
Albumin		+	-	-	-	-
Shell		-	-	-	-	-

the yolk. The table also revealed *Salmonella pullorum* in the yolk of egg from hatchery A in Lagos State and *Salmonella gallinarum* from the albumin of hatching egg from hatchery B in Oyo State,

however, no salmonella isolate was detected from OG-A yolk, OG-C albumin and OY-B yolk.

Discussion

Virulence factor may influence the ability of salmonella to contaminate and survive in chickens (CDCP, 2002). Specific fimbriae, chromosome-encoded bacterial surface adhesion, hemagglutinine and epithelia cell induction of bacterial polypeptide can promote colonization and adhesion of salmonella in chickens egg (Cox *et al.*, 2000). From this study, *Salmonella gallinarum* and *S. paratyphi A* were found in the albumin of hatching eggs. *Salmonella arizonae* and *Salmonella pullorum* observed in the yolk of hatching eggs is in line with the findings of Pope (2000) who reported that vertical transmission can occur when follicles in the ovary are infected or the developing eggs become

Table 2: Biochemical Test for Salmonella Isolate

Samples	Motility	Methionine	Arginine	Sulfur	Raffinose	Adonitol	Arabinose	Sucrose	Rhamnose	Sorbitol	Inositol	Maltinose	Gelatin	IDA	Citrate	Urease	Indole	ONPG	Xylose	Mannitol	Glucose	H ₂ S	Oxidation	Lysine	Suspected Isolate
OG-A Albumin OG-A Yolk	- -	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	<i>S. gallinarum</i>
OG-C Yolk OG-C Albumin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	<i>S. arizonae</i>
L-A Yolk L-A Albumin	- -	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	<i>S. pullorum</i>
L-A Albumin L-A Yolk	- -	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	<i>S. gallinarum</i>
OY-B Albumin OY-B Yolk	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	<i>S. paratyphi A</i> No isolate

Key

OG-A Albumin- Ogun state Hatchery A

OG-C yolk- Ogun state Hatchery C

L-A yolk-Lagos state hatchery A

L-A Albumin- Lagos state hatchery A

OY-B Albumin- Oyo state Hatchery B

infected in the oviduct. Cox *et al.* (2000) also, reported the possibility of salmonella organisms reaching the yolk content through the oviduct, however, there is possibility of salmonella to get to the yolk through egg shell penetration. Davies and May (1994) also reported that salmonella bacteria are not a single entity but exist in a huge range of serotypes (serovirs) from dedicated poultry pathogens like *Salmonella pullorum* and *Salmonella gallinarum* as recorded in this present study. Migration of bacteria from outside through the porous shell matrix of undamaged egg is possible especially when eggs are freshly laid under humid conditions. Cox *et al.* (2000).

Conclusion

It can be concluded from this study that vertical transmission of *Salmonella* from the parent stock to the day-old chicks occurred, since *Salmonella* organisms (e.g. *Salmonella gallinarum*, *S. arizoreae*, *S. pullorum*, *S. paratyphi A*) were found in both the albumin and yolk of broiler eggs in three selected States.

References

- Cavitte, J.C. 2003. Present and future control of food-borne pathogens in poultry; revision of the European Community legislation on zoonoses. In: *salvat G and Protais J (ed), Proceedings of the Xvith European symposium on the quality of poultry meat and xth European symposium on the quality of Eggs and Egg product.* Saint-Brieuc, Prontragon, France, September 23-26, 2003. Volume 1 pp 46-58.
- Miyamoto, T., Horie, T. Baba, E., Sassal S. and Solomon, S. E. 1992. Relationship between egg shell structure and increment of *Salmonella enteritidis* across the egg shell associated with freshness of laid eggs and refrigeration. *Journal of Food Protection* 61:350-353.
- Bryan, F. L. And Doyle, M. P. 1995. Health risks and consequences of *Salmonella* and *Campylobacter jejuni* in raw poultry. *Journal of food protection* 58:326-344.
- Center for Disease Control and Prevention. 2002 Preliminary Food Net data on the incidence of food borne illnesses-selected sites., United State. *MMWR Morb Mortal Wkly Rep* Apr;51 (15):325-9
- Cox., N.A, Berrang., M.E, and Cason J.A. 2000. *Salmonella* penetration of egg shells and proliferation in broiler hatching eggs-a review. *Poult Sci Nov*, (79);1571-4.
- W.H.O. 2001. World Health Organisation Surveillance programme for control of food borne infection and intoxications in Europe. Seventh report, 1993 – 1998. In: Schmidt K. and Tirado E. (ed). *Federal Institute for Health Protection of Consumers and Veterinary Medicine (Bg vv).* Berlin, Germany, pp 415, 422-423.
- Gast, R.K., Bearsd C.W 1990. Production of salmonella Enteritidis-contaminated eggs by experimentaly infected hens. *Avian DiS* April-June;34(2)438
- Gast, R. K., Guraya, R., Bowldin, J. G., Holt, P. S. and Moore, R. W. 2006.

Poppe, C., Wray, C and Wray, A. 2000. Salmonella infections in the domestic fowl. In: Salmonella in domestic animals, CAB International, p. 107-124.

Received: 19/07/12
Accepted: 20/02/14