European Journal of Academic Essays 2(7): 18-22, 2015
ISSN (online): 2183-1904
ISSN (print): 2183-3818
www.euroessays.org

Isolation of *Salmonella enterica var* Gallinarum in Day Old Broiler Chicks Obtained from Some Hatcheries around Jos, Plateau State, Nigeria

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Abstract: Due to the high economic losses experienced by poultry farmers from fowl typhoid disease even at first week of live, the significance of hatcheries in the transmission of fowl typhoid was studied, the common serovars and the prevalence of the disease were also determined in the study area. Cloacae and conjunctivae swabs were collected from each apparently healthy broiler day old chick of four different hatcheries (100 from each hatchery) and pooled per bird in a sterile commercial swab container. Also, 20 moribund/dead chicks and 20 dead-in-shell embryos were examined from each hatchery totaling 80 each by harvesting the liver, heart, spleen and retained yolk sacs post-mortem aseptically. Organs from each bird were pooled together in sterile polythene bags and placed in a crucible and mashed to avoid mixing the organs of another bird from the other thus minimizing contamination. The processed tissues were then inoculated into selenite-F broth and incubated at 37^{o}C for 24 hours. These were further sub-cultured unto solid media (MacConkey and *Salmonella-Shigella* agars) for 24 hours at 37^{o}C aerobically. The samples from each hatchery were collected two weeks apart. The overall isolation rate of *Salmonella* Gallinarum was 7.14%, with highest percentage in the dead/moribund (17.5%), followed by the dead-in-shell embryos (13.75%) and the apparently healthy broiler day old chicks (3.75%) respectively. The serovar of *Salmonella* Gallinarum isolated (sero-typed) is the Gr. D1, 1. 9. 12 strain. The results were analyzed and found that hatcheries play a significant role in the transmission of *Salmonella* Gallinarum.

Key words: *Salmonella* Gallinarum, Isolation, Hatcheries and Day old broiler chicks

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1. Introduction

Salmonella is a genus of rod shaped, Gram-negative bacteria. There are only two species of *Salmonella*, *Salmonella bongori* and *Salmonella enterica*, of which there are around six subspecies and innumerable serovars of the later. The genus belongs to the same family as *Escherichia*, which includes the species *Escherichia coli* as in [1], [2].

Salmonellae are found worldwide in both cold-blooded and warm-blooded animals, and in the environment. *Salmonella* species are facultative intracellular pathogens. Many infections are due to ingestion of contaminated food. They cause illnesses such as typhoid fever, paratyphoid fever, and food poisoning. They can infect a range of animals, and are zoonotic [3].

Fowl typhoid, caused by *Salmonella* Gallinarum is a septicemic contagious bacterial infection in poultry, causing

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economic losses, due to performance moderation, and mortality in chicks of all ages [4]. The eradication of this disease is extremely problematic in countries where the ambient temperature necessitates the use of open-sided housing [5]. Where treatment is necessary a variety of drugs may be effective, including amoxicillin, tetracyclines, sulphonamides, spectinomycin, enrofloxacin and other fluoroquinolones. None, however, is capable of totally eliminating infection from a flock. If treatment of the organism is required, this should always be on the basis of pretreatment sensitivity testing of the organism isolated. Although treatment might seem to be effective, a number of birds may become carriers and further antibiotic-resistant *Salmonella* strains or *Escherichia coli* might emerge [6].

The rough mutant strain *Salmonella* Gallinarum 9R developed 50 years ago has been examined due to its protective efficacy against fowl typhoid (FT). However, the use of live *Salmonella* Gallinarum 9R vaccine is limited to layer breeds 6-weeks and above is associated with several disadvantages such as insufficient protection, low growth rate, and residual virulence that can cause hepatitis and splenic lesions in chicks. Therefore, a vaccine that can be safely administered to chickens (especially at a young age) to obtain desired immune responses and offer sufficient protection from FT is needed [5].

This study was carried out to determine the presence of the disease causative agent *Salmonella* Gallinarum in our local hatcheries.

2. Materials and Method

Sampling was carried out from four different hatcheries. Two of the hatcheries are located in the eastern part of Jos East Local Government Area, another is located in the northern part of Jos South Local Government Area and the forth is in the northern part of Barkin Ladi local Government Area of Plateau State, Nigeria. Barkin Ladi lies under 9°32′00″N 8°54′00″E with an area of 1,312.5sq km. Jos South lies on 9°48′00″N 8°52′00″E with an area of 510 km² and a population of 306,716 and Jos East on 9^{o} 55' N 9^{o} 06'E is having an area of 1,020 km² and a population of 85,602, [8]

A total of one hundred apparently healthy day old broiler chicks, twenty moribund/dead and twenty dead-in-shell eggs were collected and examined from each hatchery thus giving a total of five hundred and sixty samples collected. A degree of accuracy used was set at 0.05. Fou**r** hundred samples of cloacae and conjunctivae swabs each were collected from apparently healthy broiler day old chicks of four different hatcheries (100 from each hatchery). Also, 20 moribund/dead birds and 20 dead-in-shell eggs were examined from each hatchery totaling 80 each of moribund/dead and dead-in-shell by harvesting the liver, heart, spleen and retained yolk sacs post-mortem. Samples from each hatchery were collected two weeks apart. Organs from each bird were pooled observing asepsis. These organs were placed in separate sterile polythene bags and placed in a crucible and crushed carefully to avoid perforation in order to prevent contamination. The processed tissues were then inoculated into selenite-F broth and incubated at between 37^{o}C for 24 hours. These were further incubated on solid media for 24 hours at a temperature of 37^{o}C aerobically. The isolates were serotyped at (Padova) a reference laboratory in Italy.

3. Results

Results of the study are as presented on Tables 1-4 below. Colonies of *Salmonella* Gallinarum appeared on the media used *(Salmonella-Shigella* agar) as colorless to grey, smooth, moist and entire, as described by [7]. Biochemically, *Salmonella* Gallinarum was found to ferment Mannitol and Dulcitol. The serovar of *Salmonella* Gallinarum isolated was the Gr. D1, 1. 9. 12 strain in the area of study.

Table 1: Percentage Isolation of *Salmonella* Gallinarum from Dead/Moribund Broiler Chicks Tested.

Hatchery	Number tested	Number positive (%)	Number negative (%)
A	20	8 (40)	12 (60)
B	20	6 (30)	14 (70)
C	20	0 (0)	20 (100)
D	20	0 (0)	20 (100)
Total	**80**	**14 (17.5)**	**66 (82.5)**

X^2 of test of association between hatcheries and *Salmonella* Gallinarum = 17.66 d f= 3, P = 0.00015, **P < 0.05.** (Fisher exact test)

Table 2: Percentage Isolation of *Salmonella* Gallinarum showing Dead-in-Shell Embryo Chicks Tested From Hatcheries A-D

Hatchery	Number tested	Number positive (%)	Number negative (%)
A	**20**	**6 (30)**	**14 (70)**
B	**20**	**5 (25)**	**15 (75)**
C	**20**	**0 (0)**	**20 (100)**
D	**20**	**0 (0)**	**20 (100)**
Total	**80**	**11 (13.75)**	**69 (86.25)**

X^2 of test of association between hatcheries and *S*. Gallinarum= 12.96, d f =3, P value = 0.02497, **P < 0.05**. (Fisher exact test)

Table 3: Percentage Isolation of *Salmonella* Gallinarum from Apparently Healthy Broiler Chicks Tested.

Hatchery	Number tested	Number positive (%)	Number negative (%)
A	100	8 (8)	92 (92)
B	100	7(7)	93 (93)
C	100	0 (0)	100 (100)
D	100	0 (0)	100 (100)
Total	**400**	**15 (3.75)**	**385 (96.25)**

X^2 of test of association between hatcheries and *S*. Gallinarum= 24.84, d f = 3, P value = 0.000017, at **P < 0.05**. (Fisher exact test)

Table 4: Total Percentage Isolation of *Salmonella* Gallinarum from all Samples tested from Hatcheries A-D

Chick type	Number tested	Number positive (%)	Number negative (%)
Apparently healthy Chicks	400	15 (3.75)	385 (96.25)
Dead/Moribund Chicks	80	14 (17.3)	66 (82.5)
Dead-in-shell eggs	80	11 (13.75)	69 (86.25)
Total	**560**	**40 (7.14)**	**520 (92.86)**

X^2 of test of association between four Hatcheries and *S*. Gallinarum = 18.22, d f= 2, P value =0.0016, at **P < 0.05.** (Fisher exact test)

4. Discussion

Fourteen (14) out of eighty dead/moribund broiler chicks tested positive for *Salmonella* Gallinarum, giving a 17.5% infectivity rate. Of eighty dead-in-shell samples tested, eleven (11) were positive for *Salmonella* Gallinarum giving a 13.75% positivity rate. The apparently healthy broiler chicks tested were four hundred from the four hatcheries and fifteen (15)

tested positive for *Salmonella* Gallinarum, giving a 3.75% infectivity rate. The total infectivity rate of the four hatcheries irrespective of status stands at forty (40) birds giving 7.14% infectivity rate. The result shows that *Salmonella* Gallinarum can be transmitted by day old broiler chicks, thus proving the significance of day-old broiler chicks in the transmission of the infection (fowl typhoid) to other susceptible birds. The sero-typed result proved that Gr. DI, 1. 9. 12 strain is present in the study area. The reason behind this may be due to the vaccine strain used in that area. [9] reported that *Salmonella* Gallinarum could be transmitted trans-ovarially and the sperm of cocks could also serve as a source of the organism, and [4] and [7] also reported the organism in chicks thus; these statements justify the present study. This result demonstrates that day old broiler chicks play a significant role in the distribution of fowl typhoid other than Pullorum in chicks.

The association between the hatcheries and *Salmonella* Gallinarum could possibly be due to the following reasons; trans-ovarian transmission of the *Salmonella* organism in the eggs and or spermatozoa of cocks [9], [10] supply of dirty hatching eggs, and or lack of fumigation systems, by use of ultra-violet light or by various washing systems. Hatcher dust and fluff could be a major source of *Salmonella* contamination, if infected eggs are hatched. It is clear that shell debris, dead-in-shell and weak or dead chicks could also serve as potential sources of *Salmonella* contamination. Therefore particular attention need be paid to handling and disposal of hatchery wastes [10].

Reasons for controlled infection in poultry houses could be due to restriction of movement into the hatcheries, hence a reduction in the chances of exposure; other factors are the lack of the use of vaccines in the breeder chickens [7]. Reducing the potential of feed and feed ingredients acting as a source of *Salmonella* organisms, which can seed the environment for subsequent recycling by birds, rodents, other animals and insects, to be a necessary serious and prudent course of action must be taken seriously [10].

From personal observation, the poultry or hatcheries that proved positive were not heeding to sanitary or strict bio-security regulations. They did not have disinfectant dips at their entrances, the workers were not professionally dressed and lots of surrounding bushes were evident at their surroundings. The two hatcheries had dumping pits of waste including dead-in-shell and dead/moribund birds within the surrounding if not very close to the pens or hatcheries. The high frequency of isolation of the organism agrees with the reports of early researchers that *Salmonella* has a high incidence in many parts of Africa and that South America and Africa has reported dramatic increase in the incidence of fowl typhoid [11].

5. Conclusion

The isolation rate suggests or proves that day old broiler chicks can serve as a source of fowl typhoid, even when they are coming directly from the hatcheries as apparently healthy chicks, thus contaminating the environment as carriers, which eventually leads to the infection of other susceptible birds. This statement agrees with the report that poultry has been recognized as the commonest reservoir of *Salmonella*, [12]. The present research work has proven that Gr. D1, 1, 9, 12 serovar is the existing *Salmonella* Gallinarum strain isolated in the study area. *Salmonella* Gallinarum is prevalent in our hatcheries, so the poultry house could even acquire the organism from such infected chicks that are introduced into apparently clean farms. Effective flock monitoring at all levels of production is necessary to detect infections and to institute appropriate control measures. The industry must therefore be constantly vigilant to maintain the highest standards of management and disease security at breeding farms, hatcheries, rearing farms feed mills and processing plants in order to prevent the introduction and spread of salmonellosis both from established serovars and also from new or emerging strains [6].

Acknowledgement: We wish to acknowledge the management of the National Veterinary Research Institute (NVRI), Vom, for giving us the opportunity to embark on this work, the staffs of Microbiology Laboratory Central Diagnostic Division and Bacterial Research, NVRI, Vom for helping in laboratory isolation and identification. The staffs of Padova Italy were also of assistance in the sero-typing of the organism in all we say thank you.

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