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Isolation of *Escherichia coli* and *Salmonella* Spp from Dead in Shell Embryos of Chicken

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Original Research Article

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Abstract: The study was aimed to identify causes and isolate *Escherichia coli* and *Salmonella* spp. from dead in shell embryos of chicken. The etiology and epidemiology of dead-in-shell embryos from two hatcheries (Government and Commercial) at Chittagong division were studied by using established bacteriological techniques alone with epidemiological study of farm biosecurity. Percentage of the condition in the Commercial hatchery and in Government hatchery was found to be 8.57% and 12.07%; respectively. The prevalence of isolated *Escherichia coli* and *Salmonella* spp from dead in shell embryos were 48% and 1%; respectively. But the prevalence of *Escherichia coli* was higher in Government farm (68%) compared to Commercial farm (28%). *Salmonella* spp was negative in the sample collected from Government farm but positive in Commercial hatchery (2%). Epidemiological investigation of both farms reveals that biosecurity and location of the farm might be a cause of higher embryonic death in Government farm in compare to commercial one. The study will help researcher to know the prevalence of *Escherichia coli* and *Salmonella spp*. from dead in shell embryos of chicken alone with epidemiology of farm biosecurity at Chittagong division.
Keywords: Dead-in-shell embryo, *Escherichia coli*, *Salmonella spp*, Biosecurity.

INTRODUCTION

Bacterial infection of poultry is representing a worldwide important factor in term of their economic losses and public health. Some organism decrease egg production and lead to high embryonic mortalities, others are widely distributed in hatcheries eggs may be a source of spreading the infection [4, 5].

Hygiene is an important factor to maintain production performance and food safety [21].Though numerous step in production, hatchery can be an important source of spread of a variety of pathogenic microorganisms that can cause diseases problems in poultry farm and human as well [6, 20]. Hatchery waste like egg shell debris, infertile eggs, dead in shell embryos etc acts as source of infection. Salmonellosis and colibacillosis are two most frequent zoonotic illnesses in chicken [22]. Fecal contamination of eggs may result in the penetration of *Escherichia coli* (*E. coli*) through the shell and may spread to the chicks during hatching and is often associated with high mortality rates, or it may give rise to yolk sac infection. In association with various disease conditions, *E. coli* results in heavy economic losses either as primary or as a secondary pathogen [8]. A large proportion of the embryos die at different stages of incubation because of bacterial contaminations. Many bacterial agents isolated from dead-in-shell embryos in *E. coli*, *Salmonella* spp [2]. Different bacterial pathogens that contaminate hatcheries have been isolated from egg shell, egg content as well as from dead in shell chicken embryos.They included *Salmonella spp., Escherichia coli spp., Klebsiella spp., Proteus spp.* and *Pseudomonas spp* [1, 13, 15]. Salmonella infections acquired vertically from parents or horizontally in the hatchery caused significant growth depression and mortality in young chicks [10]. Therefore, the present study was aimed to identify the causes and isolate *E. coli* and *Salmonella* from dead-in-shell chick embryos within hatcheries at Government poultry farm and a commercial hatchery at Chittagong district in Bangladesh.

MATERIALS AND METHODS

Ethical statement

The study was approved by the ethical committee of Chittagong Veterinary and Animal Sciences University (CVASU), Khulshi, Chittagong, Bangladesh.

Study population and sample collection

A total of 100 dead-in-shell embryos were collected from two hatcheries one government hatchery and another one commercial hatchery in Chittagong division. Selected eggs for hatching were candled on the 6th day of incubation to eliminate infertile eggs. Eggs were candled again on the 18th day of incubation. The embryonated eggs that died between the 6th and 18th day of incubation were used for this study. All samples were macroscopically examined. Eggs with cracks and those embryos that piped the shell but failed to hatch were discarded to minimize the incidence of external contamination.

Isolation of *Escherichia coli* and *Salmonella* spp

The surface of un-hatched eggs was disinfected using 70% ethyl alcohol and flamed. The egg shell was broken and the un-hatched embryo was removed with sterile forceps and putted in sterile Petridish and opened to expose the internal organs. With sterile dry swabs, impression smears were made from the yolk, liver and heart and put in sterile test tubes containing nutrient broth followed by Sub-culturing done in Eosin methylene Blue agar, XLD agar for *E. coli* and *Salmonella* Spp.; respectively. Isolated bacteria was identified by visual examination; greenish metallic sheen which is identical for *E. coli* in EMB agar and red centered with white surroundings colonies for *Salmonella* spp. in XLD agar. Biochemical tests (Indole and TSI - Triple Sugar Iron test) and microscopic examination after gram staining were done for identifying bacterial agent (Figure 1).

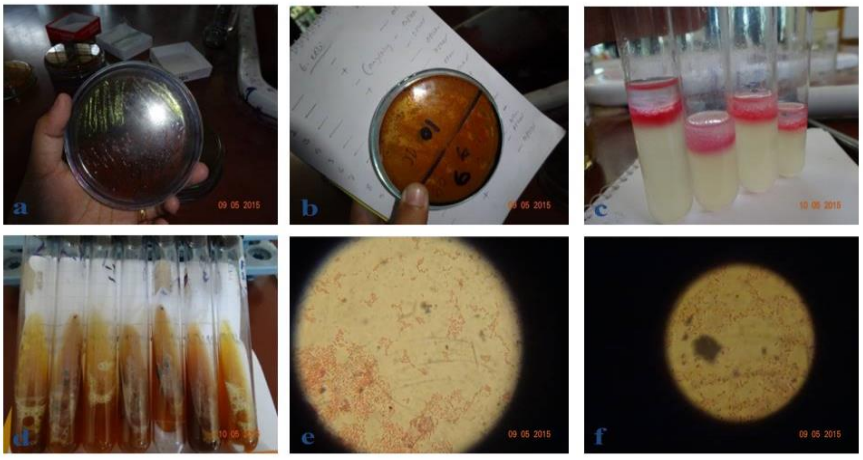

Fig-1: a. Metallic sheen in EMB agar, b. Red centered with white surrounding colony in XLD agar, c. Pink color ring formation (Indole test positive), d. TSI test for *E.coli*, formation of gas bubble and decolorization of media, e. *Salmonella* spp under microscope and f. *Escherichia coli* under microscope

RESULTS

Isolation of *Escherichia coli* and *Salmonella* spp. from overall samples

Isolated *Escherichia coli* and *Salmonella* spp. from overall dead in shell embryo samples of two hatcheries are given bellow (Table 1).

About 8.57% and 12.07% dead in shell embryo was found after analysis of hatchery data during the study period in commercial and government hatchery; respectively. From those dead in shell embryos, about 48 % samples were positive for *E. coli* while *Salmonella* spp. was 1%.

Table-1: Isolated *E.coli* and *Salmonella* spp. from overall samples

Type of Bacteria	Positive	Negative	Total
E. coli	48 (48%)	52	100
Salmonella spp	1(1%)	99	100
Total	49	151	200

Prevalence of *Escherichia coli* in Government and Commercial hatchery

The prevalence of *Escherichia coli* in Government and Commercial hatchery are given in (Table 2).

Here, 68% samples of government hatchery were positive for *E. coli* while only 28% was in commercial one. About 2% sample of commercial hatchery was positive for *Salmonella* spp while no *Salmonella* was found in government hatchery.

Table-2: Percentage of *E.coli* in commercial and government hatchery

Farm	*E. coli* positive	*E. coli* negative	Total	P value
Commercial farm	14(28%)	36(72%)	50	
Government farm	34(68%)	16(32%)	50	0.00

Prevalence of *Salmonella* spp in Government and Commercial hatchery

The prevalence of *Salmonella* spp. in Government and Commercial hatchery are given in (Table 3).

Here, *Salmonella* spp was negative in the sample collected from Government hatchery but positive in Commercial hatchery (1%).

Table-3: Percentage of *Salmonella* sp. in commercial and government hatchery

Farm	*Salmonella* spp positive	*Salmonella* sp negative	Total	P value
Commercial farm	1(2%)	49(98%)	50	
Government Farm	0	50	50	0.315

DISCUSSION

Hatchery industry is considered as one of the major sector for poultry production. Good sanitation and lowering bacterial contamination plays an important role to increase hatchability and decrease embryonic death as well. In this study the overall embryo mortality rate from analysis hatchery data during the study period was 10.32% which was disagreed with the work of Bungo *et al*. [7] and Mazengia *et al*. [14], who reported 26.7% and 27.23 % embryo mortality respectively during incubation period. This variation may due to sterilization of egg and hatcheries as well. Improper sterilization helps to grow different bacteria in vitro of egg and hamper embryonic growth. Bacteria that are contaminated through shell or transovarilly may be the potential source of embryonic death during incubation. Various bacteria may be considered as etiology of dead in shell embryos like *E. coli, Staphylococcus sp., Salmonella sp. Klebseilla sp.* etc. Iqbal *et al*. [11] and Saif *et al*. [19], which support our findings that *E. coli* and *Salmonella* spp found in present study. *Escherichia coli* predominantly isolated from dead-in-shell embryos by Raji *et al.* [16] as here 48% samples were positive for *E. coli*. The percentage of *Escherichia coli* (48%) isolated from dead in shell embryos in this study was similar to the work of Cortes *et al*. [9], who had isolated *Escherichia coli* about 45.50%. This study findings is close to the level of *E. coli* in dead-in-shell embryos as reported by Khan *et al*. [12] reported that 52.54% *E. coli* and 5.93% *Salmonella* spp isolated from dead in shell from local hatchery of Faisalabad, Pakistan. In Mexico Rosario *et al*., [18] isolate *E. coli* from dead-in-shell embryo and chicken with yolk sac infection which is agreed with these findings Amer *et al*. [3] isolate 7.78% *E. coli* and 0.625% from dead in shell embryos which is higher for *E. coli* (48%) but similar to *Salmonella* spp (1%). Mazengia *et al*. [14], reported that 85.71% *Escherichia coli* in dead-in-shell chick embryos while this study reveal 48 %. Rezk [17], reported *Salmonella* spp. (4.4%) in dead in shell embryos which agreed with present study.

CONCLUSION

Different bacteria (basically *Escherichia coli* and *Salmonella* spp.) associated with dead- in-shell embryos of chicken in two hatcheries (Government and Commercial) of Chittagong division were isolated and identified. Variation in bacterial infection depends on biosecurity and management provided in two hatcheries. The study will help researcher to know the prevalence of *Escherichia coli* and *Salmonella* spp. from dead in shell embryos of chicken alone with epidemiology of farm biosecurity at Chittagong division.

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CONFLICT OF INTEREST

All authors declared that they have no conflict of interests.

AUTHOR CONTRIBUTIONS

Jabin Sultana planned the study, performed the experimental works and wrote the manuscript. Md. Forhad Uddin helped during the laboratory works and manuscript preparation. Tuli Dey helped to write manuscript and Sonnet Poddar helped in formatting the manuscript

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