Journal of Animal and Veterinary Advances 5(7): 598-601, 2006

Recovery of Salmonella from Incubated Eggs, Newly Hatched Chicks and Contaminated Environments

[1]Rahman, M.M., [2]Z.U.M. Khan, [3]A.N.M. Rahman and [4]M.S. Islam
[1]Department of Microbiology, Dinajpur Government Veterinary College, Dinajpur, Bangladesh
[2]Department of Botany, Jahangirnagar University;Savar, Dhaka, Bangladesh
[3]School of Agriculture and Rural Development, Bangladesh Open University
[4]Department of Physiology,Pharmacology and Biochemistry, DGVC, Dinajpur, Bangladesh

Abstract: A total of 400 hatchery samples comprised of yolk interior (100), paper pad (100), shell membrane (100) and fecal swab of newly hatched chicks (100) were tested to detect the presence of Salmonella organism by bacteriological agar plate test. Positive cases recorded in this study were 37 (37), 12 (12), 3 (3) and 19 (19%) from each sample (100) of yolk interior, paper pad, shell membrane and fecal swab of newly hatched chicks, respectively. A representative numbers of 50 isolates were used for the identification of serogoups of Salmonella prevailing in selected area by using polyvalent antisera. The result indicated that the test isolates 45(90%) were typed to a specific serogroup of "O". All 45 isolated Salmonella serogroup 'O' were then characterized by different specific biochemical media. Based on these tests, the selective isolates were identified as *Salmonella gallinarum.*

key words: Hatchery, *Salmonella gallinarum*, rapid serum plate agglutination test, fertile egg, polyvalent antisera

INTRODUCTION

Salmonella is a septicemia disease that affects primarily chickens and can spread in several ways. Oral route of infection represents the normal route of infection. The egg transmission is the most frequent way in modern poultry industry to spread the *Salmonella* infection among birds, farms or countries. Fertile eggs leaving the breeder house could carry many bacteria, both those on the shell surface and others that have penetrated beneath the shell. Dirty nesting material can contribute to egg contamination. In addition to surface contamination, a freshly laid egg that is wet and warm is susceptible to rapid penetration by microorganisms and these contaminated eggs have the potential for spreading *Salmonella* in the hatchery[1]. If chicks are hatched from infected eggs, the first sign can be observed in the hatchery. The mortality of embryos is higher than the regular one; the numbers of moribund, dead chicks are increased. The high bacteria concentration in this time makes possible that a large number of chicks born from non-infected eggs get contamination instantly after hatching even inside the machines or in the transport boxes within the first 24 h of life.With the great expansion of the poultry rearing and farming, pullorum and fowl typhoid have become wide spread problem in Bangladesh like other areas of the world[2-4]. In recent year, with the advent of mass poultry raising in this country, particularly when broilers are raised, the disease has become one of great economic importance. Heavy losses occur not only in broiler flock but also in laying birds due to morbidity, mortality, reduced production and poor chick quality. Mortality may vary from negligible to 10 to 80% or higher in severe outbreaks[5,6].

MATERIALS AND METHODS

Hatchery samples: Shell membrane and yolk interior of incubated egg, paper pad, fecal swab of hatching chick were obtained from the hatchery of CPF, Mirpur, Dhaka, Bangladesh. Randomly 5 samples were selected on each for one hatching day. 20 hatching days were considered for each sampling. So, the total number of each sample type was 5 x 20 = 100.

Shell membrane and yolk interior: On transfer to a hatchery at 18 days of incubation, 5 eggs were randomly selected for sampling each day beginning at transfer. The eggs were sanitized externally by wiping with 70% alcohol on a paper towel and then the eggs were placed individually in sterile small plastic bags. Bags were folded over once and stapled closed and shipped to the

Corresponding Author: M.M. Rahman, Department of Microbiology, Dinajpur Government Veterinary College, Dinajpur, Bangladesh

laboratory. Then the paper bags were opened with scissors. All egg handling was done using aseptic techniques. After candling to mark the position of the air cell, the shell and outer shell membrane over the air cell was rinsed with 70% ethanol from a squirt bottle and then cut open with sterile scissors. The developing chick was pulled out through the hole with for ceps and placed in a petridish along with any liquid remaining inside the egg. Chicks that had started to break through the shell were freed by carefully breaking away the shell with forceps. Embryos and hatching chicks were killed by decapitation. The yolk was separated by dissection. Yolks were dipped in 70% ethanol to sanitize the external surface and then mixed in PBS and rinsed thoroughly by hand. The shell and membranes were crushed and mixed in a plastic bag with PBS. All samples of shell and membranes and yolk interiors were enriched overnight in PBS at 37°C for further inoculation onto the bacteriological media.

Paper pad: Each paper pad of chick box was cut into pieces using sterile scissors and placed in a sterile plastic bag containing PBS. Following overnight incubation at 37°C, 1 mL of the PBS was aseptically transferred to 9 mL nutrient broth and incubated for 24 h at 37°C for further study of bacteriological characteristics on bacteriological media.

Facal samples of hatching chicks: Sterile cotton swabs were used to collect samples of freshly voided faecal material. The swabs were transferred to 9 mL of nutrient broth and incubated 24 h at 37°C and ready to streak onto the bacteriological media.

Antisera: Poyvalent "O" (A-G) and polyvalent 'H' *Salmonella* antisera manufactured by Oxoid Company Limited. Basingstoke, Hampshire, England was used for the serogrouping of *Salmonella* isolates

Inoculation of different samples inoculum onto the bacteriological media: Blood agar, MacConkey agar and S.S. agar plates were used for the detection of bacteriological characteristics of *Salmonella* from different types of samples of poultry house.. For isolation of Salmonella isolates, the isolation procedure was followed as OIE manual,[6].

Sero-grouping of *Salmonella* isolates by serum plate agglutination test using specific polyvalent antisera: A representative number of 50 positive cases of salmonella isolates identified from different sources by selective bacteriological plating media were tested for the detection of serogroup of *Salmonella* using polyvalent 'O' (A-G) and polyvalent 'H' antisera as followed by OIE,[6].

Identification of *Salmonella* field isolates by using specific biochemical media: For this study, motility, TSI (Triple Sugar Iron), dulcitol and ornithine media were selected for characterization of 45 positive isolated *Salmonella* serogroup 'O'and the method was followed by OIE,[6].

RESULTS AND DISCUSSION

An investigation was conducted on hatchery samples for the isolation and identification of *Salmonella* organism from four different kinds of samples : yolk interior, paper pad, egg shell membrane and faecal swabs of chicks. All types of samples were tested for the presence of *Salmonella* by determining cultural characteristics using different media. *Salmonella* organisms were recovered from 37 of 100 (37%), 12 of 100 (12%), 3 of 100 (3%) and 19 of 100 (19%) samples of yolk interior, paper pad, shell membrane and faecal swabs of chicks respectively (Fig. 1).

A representative numbers of 50 field isolates of poultry hatchery samples were used for the identification of the serogroups of *Salmonella* prevailing in the selected flock. 45 of the selected isolates were agglutinated and 5 isolates were not agglutinated with polyvalent 'O' antisera (A-G) within one minute and none of the selected samples were agglutinated with polyvalent 'H' antisera (Table 1). The result indicated that the tested isolates(90%) were typed to a specific serogroup of 'O'.All 45 identified serogroup 'O' isolates were inoculated into different specific media of motility, ornithine, dulcitol and TS1 for the identification of *Salmonella*. All the isolates were found to show negative reaction to motility medium and ornithine medium but all showed positive reaction to dulcitol (Table 2). The result indicated that the isolates were *Salmonella gallinarum.* Most of the scientists of the world [7-10] used this method for the detection of *Salmonella* as a diagnostic tool. The

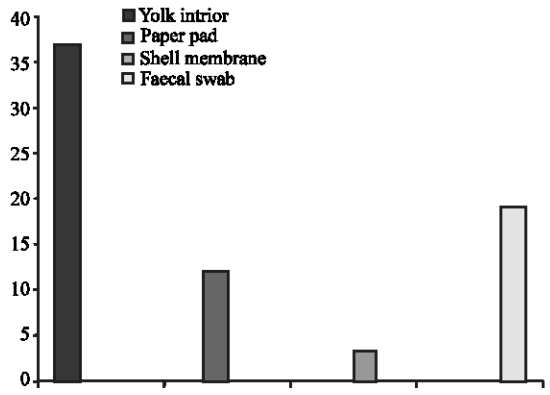

Fig.. 1: Occurrence of *Salmonella* in hatchery samples

Table 1: Serogrouping (agglutination reaction) of *Salmonella* isolates

Antisera used	Total no. of tested samples	No. positive	% positive	No. negative	% negative
I.Polyvalent 'O' antiserum	50	45	90	5	10
II.Polyvalent 'H' antiserum		0	0	50	100

Table 2: Characterization (specific) of *Salmonella* isolates

Specific tests tested	Total selected isolates tested	Response	Organisms suspected	Identification
Motility	45	negative	*Salmonella gallinarum*and *Salmonella pullorum.*	
TSI		positive	*Salmonella gallinarum*and *Salmonella pullorum.*	*Salmonella gallinarum*
Dulcitol		positive	*Salmonella gallinarum*	
Ornithine		negative	*Salmonella gallinarum*	

results of the present study for the detection *Salmonella* from the field isolates by tube agglutination test strongly supports the findings of [8,7]. Results of the present study clearly indicated that there was only *Salmonella gallinarum* prevailing in the commercial layer and breeder poultry population in Bangladesh. Results of the identification of *Salmonella gallinarum* from the field isolates of the present study by biochemical test strongly supports the results of [7,11,12].

The incidence and extent of *Salmonella* found in the present study from the different types of samples of breeder hatchery closely agree with the finding of [13-15]. There was a greater rate of *Salmonella* recovery from yolk interior than from shell membrane, paper pad and cloacal swabs of hatching eggs (Fig. 1). In fact, *Salmonella* was prevailing in the breeder flock and would presumably reflect transovarian transmission of *Salmonella gallinarum. Salmonella* status of the hatching chicks occurred at pipping with a sharp increases in positive chick rinses and yolk. The number of *Salmonella* contaminated chicks in hatchery might be reduced, if *Salmonella* are not totally eliminated from each egg, if chemical and other treatments can be found to reduce the number of bacterial cells between the shell and shell membranes. The suggestion of wilding and Baxter-Jones (1985) that some chicks acquire *Salmonella* as they hatch from their own shells and shell membranes is confirmed by this experiment. The incidence of *Salmonella* found in the present study with broiler breeder hatcheries was much lower than that reported in a previous study with broiler hatcheries[16].

There are many reasons for the lower incidence of *Salmonella* positive samples in breeder versus broiler hatcheries. Typically, primary breeder flocks are smaller in size and more remotely located with very few visitors. The hatching eggs are gathered more frequently because of the size of the flocks and the value of the eggs. The eggs are often chemically disinfected shortly after gathering. There was a greater rate *Salmonella* recovery from the shell and membrance samples than from chick rinse samples or yolk interiors. Lopez *et al.*[17] found more *Salmonella* in the shell and membranes than in the contents when the egg exterior was inoculated[18] recovered more *Salmonella* from shell and membranes than from yolk and albumen of eggs from hens infected by inoculated feed, with longer storage times increasing the recovery rate from yolk and albumen. In the present experiments, eggs went into the incubator within 30 min of inoculation, much sooner than would occur in practice if most egg contamination takes place at the breeder farm. Gast and Beard[19] found more *Salmonella* in the contents than in the shell and membranes of eggs from experimentally infected hens, but this difference would presumably reflect transovarian transmission of *Salmonella* in the infected hens, a process which is not generally accepted for other paratyphoid *Salmonella.*

The present study clearly indicates that *Salmonella* is present in significant numbers in breeders hatcheries and that breeder flocks are early critical points for preventing *Salmonella* entry into the integrated poultry operation. In this particular hatchery samples, egg interior, paper pad, faecal swab of chicks appear to be more significant sources of *Salmonella* contamination than shell membrane.

REFERENCES

1. Williams, J.E. and L.H. Dillard, 1968. *Salmonella* penetration of fertile and infertile chicken eggs at progressive stages of inoculation. Avain Dis., 12: 629-635.
2. Amin, M.M., T.L.M.F. Chowdhury and M.U.A. Chowdhury, 1969. Isolation of *Salmonella* from wild Birds, Rodents and Insects, Pak. Journ. Bet. Sci., 3: 41-47.
3. Sarker, A.J., 1976. The prevalence of avian disseases in Bangladesh Agricultural University Poultry Farm. Bang. Vet. Jour., 10: 61-66.
4. Rahman, M.M., T.I.M.F. Chowdhury, M.M. Rahman and W.I.M.A. Hossain, 1979. Surveillance of *Salmonella* and *Escherichia* organisms in Poultry Feed Ban. Vet. J., 13: 59-62.

5. William, J.E., E.T. Mallinson and G.H. Snoeyenbos, 1990. Salmonellosis and Arizonosis. Isolation and identification of Avain pathogens, Second edition. The American Association of Avian Pathologists, Printed by creative printing Company, Inc. 2011 East Manistreet, Newyork.
6. Kaura, Y.K., Jagjit, Singh, R.K. Kaushok, R.C. Kulshrestha, Minakshi and G.C. Chaturvedi, 1990. *Salmonella gallinarum* var. *duisburg*: An emerging biotype causing heavy mortality in poultry birds in northern India. Indian J. Anim. Sci., 60: 2, 127-130.
7. Hoque, M.M., H.R. Biswas and L. Rahman, 1997. Isolation, identification and production of *Salmonella pullorum* coloured antigen in Bangladesh for the rapid whole blood test. AJAS, 10: 141-146.
8. Robinson, H., Mdegela, G.S. Mmeta, Yongolo, M. Uswege, Minga and E.O. Johin, 2000. Molecular epidemiology of *Salmonella gallinarum* in chickens in Tanzania. Avian Patholo., 29: 457-463.
9. Ganapathy, K., M.H. Salamat, C.C. Lee, and M.Y. Johara, 2000. Concurrent occurrence of salmonellosis, colibaccillosis and histomoniasis in a broiler flock fed with antibiotic free commercial feed. Avian Patholo., 29: 639-642.
10. Tate, C.R., R.G. Miller, E.T. Mallinson, L.W. Douglass and R.W. Johnston, 1990. The isolation of *Salmonella* from poultry environmental samples by several enrichment procedures using plating media with and without novobiocin. Poultry Sci., 69: 721-726.
11. Ghosh, N.N. and S.N. Panda, 1988. Isolation of *Salmonella gallinarum* from poultry in Orissa. Indian Vet. J., 65: 1049-1051.
12. Smith, H.W., J.F. Tucker and M. Lovell, 1981. Furazolidone resistance in *Salmonella gallinarum*: The relationship between *in vitro* and *in vivo* determination of resistance. J. Hyg., 87: 71-81.
13. Berrang, M.E., N.A. Cox, J.S. Bailey and L.C. Blankenship, 1991. Methods for inoculation and recovery of *Salmonella* from chicken eggs. Poultry Sci., 70: 2267-2270.
14. Cox, N.A., J.S. Bailey, J.M. Mauldin, L.C. Blankenship and J.L. Wilson, 1991. Extent of *Salmonella* contamination in breeder hatcheries. Poultry Sci., 70: 416-418.
15. Cason, J.A., J.S. Bailey and N.A. Cox, 1993. Location of *Salmonella typhimurium* during incubation and hatching of inoculated eggs. Poultry Sci., 72: 2064-2068.
16. Cox, N.A., J.S. Bailey, J.M. Mauldin and L.C. Blankenship, 1990. Presence and impact of *Salmonella* contamination in commercial broiler hatcheries. Poultry Sci., 69: 1606-1609.
17. Lopez, J., E. Karpowicz and S. Becker, 1991. Penetration de *Salmonella enteritidis* en huevos clasificados intactos, sin llegar al contenido del huevo. In: Curso de Actualizacion Sobre *Salmonella enteritidis* y *Campylobacter* en las Aves Domesticas. Associacion Nacional de Especialistas en Ciencias Avicolas, mexico City, Mexico, pp: 97-121.
18. Gordon, R.F. and J.F. Tucker, 1965. The epizootiology of *Salmonella menston* infection of fowls and the effect of feeding poultry food artificially infected with *Salmonella.* Br. Poult. Sci., 6: 251-264.
19. Gast, R.K. and C.W. Beard, 1990. Current ARS research on *Salmonella enteritidis* in chickens: experimental infections in laying hens. Dairy Food and Environmental Sanitation, 10: 276-278.