Bangl. J. Vet. Med. (2011). 9(1): 43 – 52

EVALUATION OF THE EFFECTIVENESS OF COMMERCIALLY AVAILABLE DISINFECTANTS AGAINST SALMONELLAE ISOLATED FROM INTERNAL ORGANS OF DEAD CHICKENS

N.A. Rumi, M.M. Rahman, M.R. Akter, M. Fakhruzzaman and M.K. Hossain

Department of Microbiology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh

ABSTRACT

The effectiveness of commercially available disinfectants was evaluated against Salmonellae isolated from different internal organs of 52 numbers of dead layer chickens in the district of Dinajpur during the period from July 2009 to June 2010. Bacterio-biochemical methods were used to isolate and identify the Salmonella organisms from 154 samples of liver, spleen, heart and lungs of birds, of which 36 (23.38%) samples had Salmonella infection. Organ-wise prevalence of Salmonella infection showed highest prevalence in liver (34.62%) , followed by spleen (23.087%) , heart (20.00%) and lowest in lungs (4.00%). Effectiveness of disinfectants was tested at different concentration on Salmonella culture on SS agar media and measured by zone of inhibition incubated for 24 hours. Highest average zone of inhibition was recorded with Desinkap® (12.50 ± 2.08), followed by GPC (8) TM ® (11.33 ± 1.53) , and more or less similar patterns with TH 4 ® (10.50 ± 0.71), Virocid ® (10.50 ± 0.71) and Lysol ® (10.00 ± 0.00). It may be concluded from these results that Desinkap® is the most effective disinfectant against Salmonellae as it contains multiple ingredients.

Key words: Disinfectants, salmonella, isolates, effectiveness, chickens

INTRODUCTION

The importance of salmonellosis in poultry industry has increased to be growing concern day by day throughout the world during the last decades. The etiologic agents are responsible for various pathogenic processes in man and animals including poultry (Freeman, 1985). With the great expansion of poultry rearing and farming, Fowl Typhoid caused by *Salmonella gallinarum* is the most devastating disease in Bangladesh (Begum *et al.,* 1993 and Hoque *et al.,* 1992).The disease can be controlled by maintaining of high level of biosecurity, routine serological test and screening of birds positive to *Salmonella*. The epidemiology of Fowl typhoid and Pullorum disease in poultry, particularly with regard to transmission from one generation to the next is known to be associated with infected eggs (Hofstad *et al.,* 1992). Although more than 2300 serotypes of *Salmonella* have been identified, only 10% of these have been isolated from poultry (Gast, 1997). *Salmonella gallinarum* can produce lesions in chicks, which are indistinguishable from those associated with Pullorum disease. With great expansion of the poultry rearing and farming, Pullorum disease and Fowl typhoid have become wide spread problem in Bangladesh like other area of the world (Sarker, 1976 and Rahman *et al.,* 1979). Heavy economic losses occur both in broiler and layer flock due to morbidity, mortality, reduced production and poor chick quality. Mortality may vary from negligible to higher (10% to 80%) in severe outbreaks (Kumar and Kaushi, 1988; Kaura *et al.,* 1990; Kleven and Yoder, 1998). The poultry industry is intensive and consistently applies an all-in, all-out system with the aim of minimizing infection pressure and targeting specific organisms like *Salmonella*. Therefore, disinfecting during production break is a routine part of the management of poultry houses. Several chemical disinfectants are commercially available in the local markets but their efficacy have not been evaluated in Bangladesh. This paper describes the comparative effectiveness of commercially available disinfectants against Salmonella isolates.

MATERIALS AND METHODS

The study was conducted from July 2009 to June 2010 in 154 field samples comprising liver, spleen, heart & lungs of dead layer chickens aseptically collected from Basherhat, Gobindapur and Mohabolipur of Dinajpur sadar upazila under the district of Dinajpur.

Culture Media

Different solid bacteriological culture media used in this study were Nutrient agar (NA) (Himedia,India), Salmonella-Shigella (S-S) agar (Himedia, India), Brilliant green agar (BGA) (Himedia, India), Mac Conkey agar (Himedia, India), Eosine methylene blue (EMB) agar (Himedia, India) and Simmons citrate agar (Himedia, India) media. The liquid culture media were Nutrient broth (NB) (Himedia, India) and Bacto selenite broth (Himedia, India) and biochemical media were Sugar media (dextrose, maltose, lactose, mannitol and sucrose),Tripple sugar iron (TSI) agar, Motility Indole Urea (MIU). Other solutions for conducting biochemical tests were Methyl Red, Voges Proskauer solutions.

Antisera

Salmonella Polyvalent 'O' (A-I) antisera manufactured by Statens Serum Institute, Copenhagen, Denmark, was used for the sero-grouping of *Salmonella* isolates.

The entire study is divided into two steps. The first step includes the isolation of the bacteria from internal organ of dead birds and identification of *Salmonella* by cultural, morphological and biochemical characteristics. The second step is evaluating the effectiveness of commercially available disinfectants against *Salmonella* isolates.

Collection of samples

Liver, spleen, heart, and lung samples were collected from dead birds of study areas and transferred immediately into a Petridish. The Petridish containing samples were then immediately brought to the Microbiology Laboratory, Department of Microbiology, Hajee Mohammad Danesh Science and Technology University, Dinajpur.

Cultivation and isolation of *Salmonella*

The samples were inoculated into Nutrient agar media and incubated at 37° C for 24 hours aerobically in bacteriological incubator. The incubated tubes were then examined for growth of bacteria. Smears were prepared from each of the petridishes and the smears were fixed. The fixed smears were stained with Gram's Method of staining and examined under microscope at 100 X magnifications using immersion oil. In presence of Gram negative rods in the smears, the materials from the petridishes corresponding to the smears were streaked into Mac Conkey agar, Salmonella-Shigella agar and Brilliant green agar separately. The plates were then incubated at 37° C for 24 hours and the plates containing characteristic colonies of *Salmonella* were selected. Gram's staining tests are performed to identify the plates containing *Salmonella* accurately. Sub-culturing in Salmonella-Shigella (SS) agar was performed from the suspected plates containing *Salmonella* to obtain a pure culture. These pure isolates obtained in this way were used for further study.

Morphological examination

The representative Salmonellae isolates from SS agar were stained by Gram's stain (Marchant and Packer, 1967).

Biochemical examination

Isolated organisms with supporting growth characteristics of *Salmonella* on various media were maintained on Salmonella-Shigella (SS) and Brilliant green Agar (BGA) and were subjected to the different biochemical tests named sugar fermentation test, MR-VP reaction, TSI, MIU and Indole reaction (Marchant and Packer, 1967).

Sero-grouping of *Salmonella*

Sero-grouping of *Salmonella* isolates was performed by slide agglutination test using specific polyvalent 'O' (A-I) antisera. The test was performed according to the protocol of specific Statens Serum Institute, Copenhagen, Denmark (Buxton and Fraser, 1977).

Evaluation of the effectiveness of disinfectants

Preparing agar for petridishes

Before the Petridishes were inoculated with *Salmonella* isolates, selective media (SS agar) was prepared to pour in the bottom of each dish. Approximately 15 ml of agar solution was poured into the bottom of each of the petridishes.

Growing *Salmonella* isolates into petridishes

To grow the *Salmonella,* 1 ml of nutrient broth was pipetted onto the 100% concentration of *Salmonella* and was mixed. After the *Salmonella* was mixed with the nutrient broth, 1 ml of the mixture was removed and placed back into the original stock tube containing the nutrient broth. The mixture was then incubated for 24 hours at 37°C. After the *Salmonella* was incubated for 24 hours, each dish was inoculated with 100 µl of liquid *Salmonella* from the stock tube. After the *Salmonella* were placed on the plate, they were spread over the plate with a glass rod. The plates were then ready to have the disinfectants applied.

Preparation of different concentration of disinfectants

A total of five commercially available disinfectants were evaluated for their efficacy against *Salmonella* isolates. The disinfectants were made in three forms, one (01) according to the manufacturers recommendation, three (03) were higher and one (01) was lower than manufacturers recommendation.

Table 1. Different concentration of commercially available disinfectants for the evaluation of their effectiveness

Disinfectants(Composition and Company)	Tested different concentration of disinfectants(%)				
Desinkap®(Formaldehyde,Glutaraldehyde, Glyoxal, Benzylconium) (Lion Overseas Trading Company)	0.3	0.4*	0.5	0.6	0.7
GPC (8)TM ® (Glutaraldehyde) (Renata Ltd.)	0.4	0.5*	0.6	0.7	0.8
TH 4®(Quaternary ammonium compounds) (Century Agro Ltd.)	0.4	0.5*	0.6	0.7	0.8
Virocid ®(Quaternary ammonium compounds)(ACI Ltd.)	0.5	0.6*	0.7	0.8	0.9
Lysol ®(Tar acid) (Coventry Chemicals Ltd.)	2.4	2.5*	2.6	2.7	2.8

***** Manufacturer's recommendation

Measuring Zones of Inhibition

After the 24-hour incubation time, the dishes were observed. The zones of inhibition were measured by measuring the diameter of the clear inhibition zone surrounding each disk with a ruler.

Statistical analysis

A statistical analysis was performed after collecting the zone of inhibition measurements. An ANOVA statistical analysis was performed for all the zone of inhibition measurements for 24 hours of incubation.

RESULTS AND DISCUSSION

Isolation of *Salmonella spp*

Out of 154 samples 36 (23.38 %) were found to be positive to *Salmonella.* Among the positive samples, 17 (24.29 %) were from Basherhat, 12 (22.22 %) from Gobindapur and 7 (23.33 %) from Mohabolipur area of Dinajpur. The percentages of positive samples (liver, spleen, heart, lungs) from those areas were 24.29 %, 22.22 % and 23.33 % respectively and the average prevalence of *Salmonella* was 23.38 % (Table 2) which was lower than the observation of Habib-ur-Rehman *et al*. (2004), Ahmed *et al.* (2008) might be due to difference in number of birds tested or due to various areas. In case of internal organs, *Salmonella* were detected from 18 samples of liver, 12 samples of spleen, 5 samples of heart and 1 sample of lung and the prevalence was 34.62 %, 23.08 %, 20.00 % and 4.00 % respectively (Table 3). Habib-ur-Rehman *et al*. (2004) described 34.50 % cultural prevalence of *Salmonella* infection in dead birds where 10.50 % in liver and 10.50 % in spleen while Lee *et al*. (2001) described 47.60 % in liver. Ahmed *et al.* (2008) described the average cultural prevalence in the dead bird was 60.50 % in which 64.00 % cultural prevalence in the liver and 57.00 % in the cloaca. Among the 52 samples

tested from liver, *Salmonella* could be isolated from 18 (34.62 %) samples which were higher than the findings of Habib-ur-Rehman *et al*. (2004) and lower than Lee *et al*. (2001) and Ahmed *et al.* (2008). Among the 52 samples tested from spleen, *Salmonella* could be isolated from 12 (23.08 %) samples which were higher than the findings of Habib-ur-Rehman *et al*. (2004). From the above findings it was revealed that liver was the rich source for *Salmonella* contamination other than the spleen, heart and lungs, which was in close agreement with Sujatha *et al*. (2003).

Table 2. Isolation of *Salmonella* from internal organs of 52 numbers of dead birds from the study areas of Dinajpur district

Study area	Sources of samples	No. of sample tested	No. of positive isolates	Prevalence of isolates (%)	Average Prevalence of isolates(%)
Basherhat	Liver	25	8	24.29	23.38
	Spleen	25	6		
	Heart	10	2		
	Lung	10	1		
Sub total		**70**	**17**		
Gobindapur	Liver	17	6	22.22	
	Spleen	17	4		
	Heart	10	2		
	Lung	10	0		
Sub total		**54**	**12**		
Mohabolipur	Liver	10	4	23.33	
	Spleen	10	2		
	Heart	5	1		
	Lung	5	0		
Sub total		**30**	**7**		
	Liver	52	18	34.62	
	Spleen	52	12	23.08	
	Heart	25	5	20.00	
	Lung	25	1	4.00	
Total (Liver, spleen, heart and lungs)		**154**	**36**	**23.38**	

Identification of *Salmonella spp.*

All the isolates produced brick red coloration in Selenite broth and pinhead size to that of a lentil, raised, rounded or circular, smooth, glistening, opaque, colorless, transparent or translucent colonies on SS agar and pink red color colonies against a pinkish background when cultured on BGA and colorless, smooth, pale and transparent raised colonies on MacConkey agar media. These findings support the report of Freeman, (1985) and Jones *et al*., (1997). *Salmonella* isolates on nutrient agar, and other specific media revealed identical cultural characteristics which resemble the findings of Buxton and Fraser (1977). All the isolated *Salmonellae* revealed Gram-negative, pink coloured, short plump rod shaped, single and paired arrangement in Gram's staining technique which were identical to earlier studies of Buxton and Fraser (1977) and Freeman (1985). In biochemical tests all the isolates were positive to MR, TSI tests and negative to VP and Indole tests. All the isolates were negative to MIU media. This study correlates with the study of Pomeroy and Nagaraja (1991); and OIE (2000). Nonmotile organisms were considered to be either *S. pullorum* or *S. gallinarum*. All *Salmonella* isolates were unable to ferment lactose, sucrose, although dextrose, maltose and mannitol were fermented. This result is in agreement with the findings of Freeman (1985) and Jones et al. (1997).

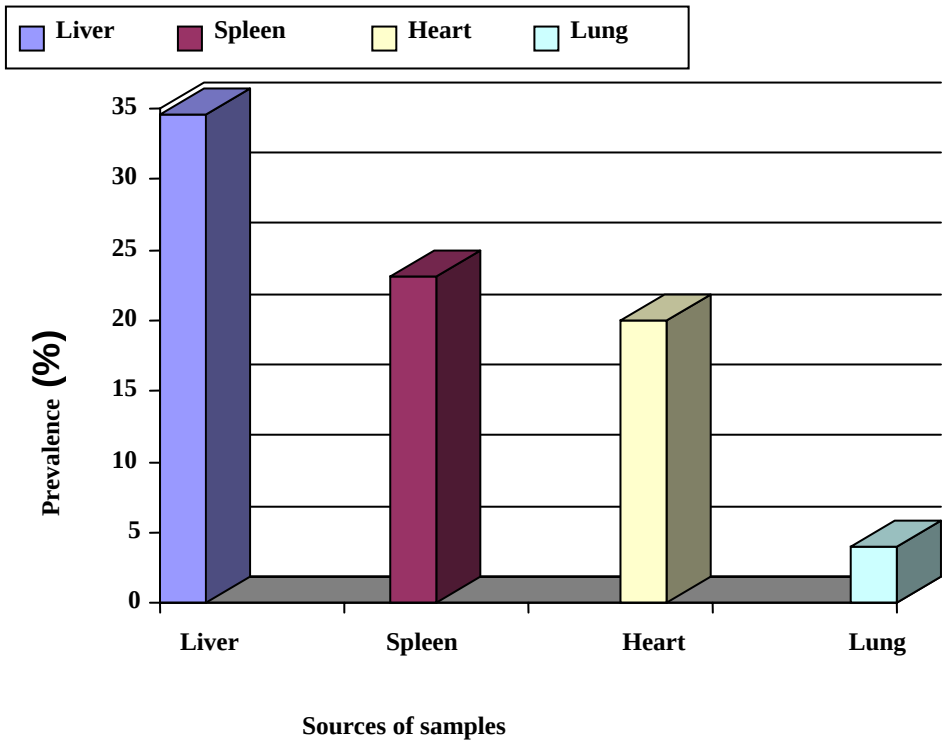

Fig. 1. Prevalence of *Salmonella* isolated from internal organs of dead birds

Evaluation of disinfectants against *Salmonella* isolates

The effectiveness of the disinfectants in preventing the growth of *Salmonella* isolates was tested in the selective media such as SS agar. Among the disinfectants Desinkap® was found most effective of all the disinfectants in preventing the growth of *Salmonella* isolates as measured by zone of inhibition after being incubated for 24 hours (Table 4). GPC (8) TM ® was found to be more effective than Virocid ® and TH 4 ® , but not more effective than Desinkap® but the result was not statistically significant (p> 0.05). These results support the report of Marin *et al.* (2009). TH 4 ® and Virocid ® were less effective than Desinkap® (Table 4). Lysol ® was found less effective than other four disinfectants (Table 4).

Table 4. Evaluation of the effectiveness of commercially available disinfectant against *Salmonella* isolates in different concentration

Name of the disinfectant	Concentration (%)	Time (hour)	Measuring Zones of Inhibition (mm)
Desinkap®	0.3	24	-
	0.4*	24	10
	0.5	24	12
	0.6	24	13
	0.7	24	15
Mean±SD			12.50±2.08
GPC (8) TM ®	0.4	24	-
	0.5*	24	-
	0.6	24	10
	0.7	24	11
	0.8	24	13
Mean±SD			11.33±1.53
TH 4 ®	0.4	24	-
	0.5*	24	-
	0.6	24	-
	0.7	24	10
	0.8	24	11
Mean±SD			10.50±0.71
Virocid ®	0.5	24	-
	0.6*	24	-
	0.7	24	-
	0.8	24	10
	0.9	24	11
Mean±SD			10.50±0.71
Lysol ®	2.4	24	-
	2.5*	24	-
	2.6	24	-
	2.7	24	-
	2.8	24	10
Mean±SD			10.00±0.00
P value			0.459
Levels of significance			NS

NS means (p>0.05)

***** Manufacturers recommendation.

(-) No zone of inhibition.

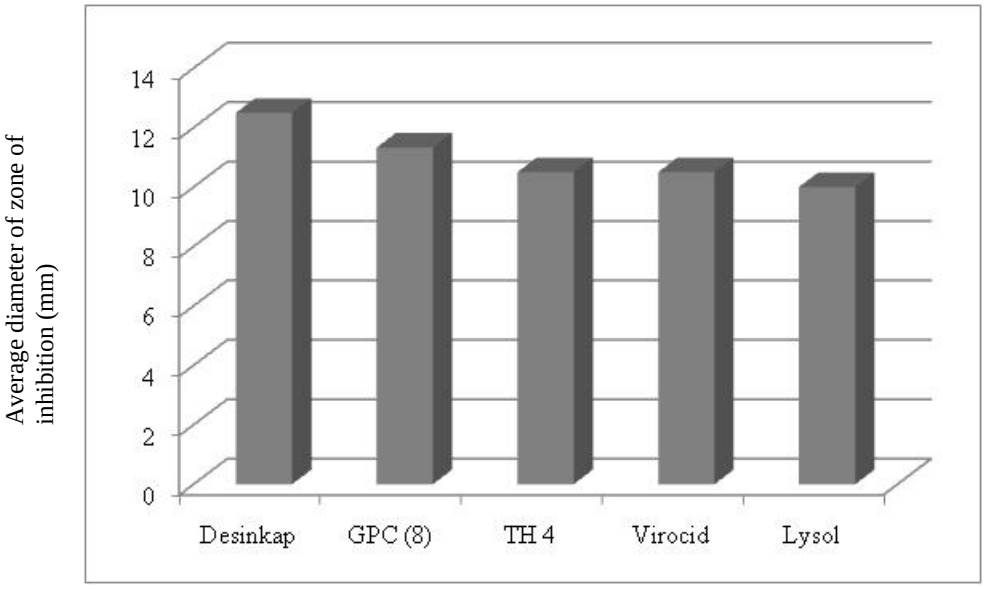

Fig. 2. Comparison of average zone of inhibition values after 24 hours of incubation

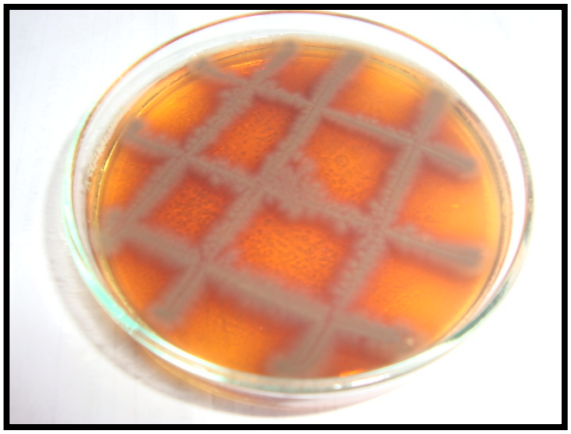

Plate 1. Growth of *Salmonella* in Salmonella- Shigella (SS) agar medium showing raised, rounded or circular, smooth, glistening, opaque, colorless, transparent or translucent colonies

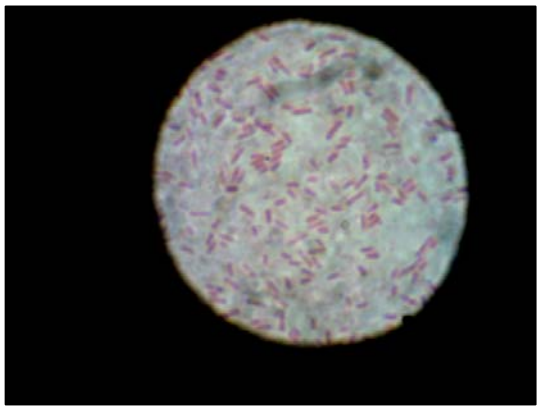

Plate 2. *Salmonella spp.* showing Gram negative small rods arranged singly or in pairs (Gram's staining)

Plate 3. Lower concentration of disinfectants than the manufacturer's recommendation showing no zone of inhibition

Plate 4. Concentration of disinfectants according to the manufacturer's recommendation showing zone of inhibition in case of Desinkap®

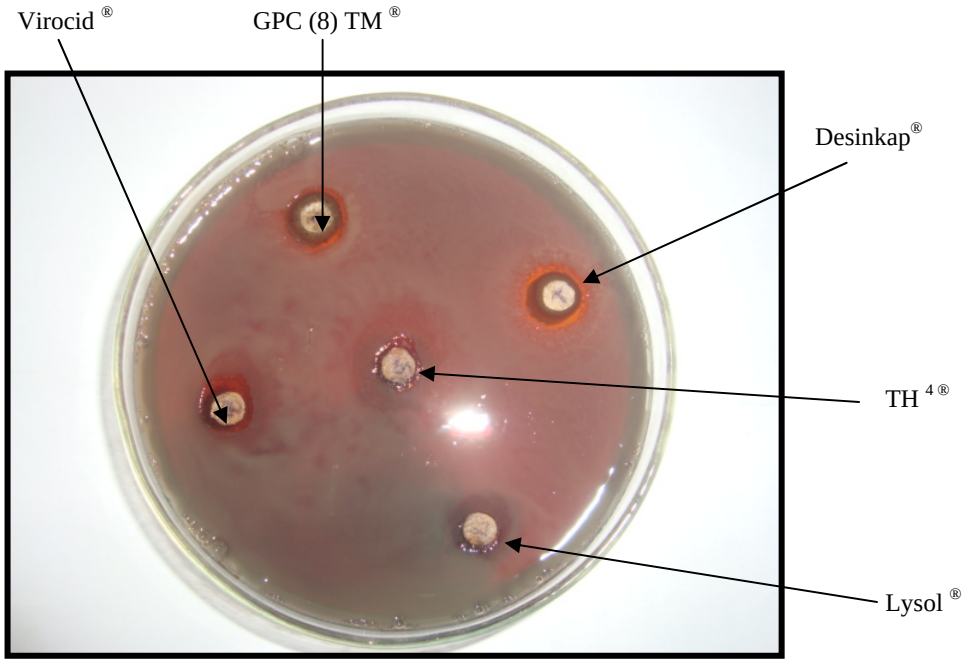

Plate 5. Higher concentration of disinfectants than the manufacturer's recommendation showing zone of inhibition

REFERENCES

1. Ahmed AKM, Islam MT, Haider MG and Hossain MM (2008). Seroprevalence and pathology of naturally infected Salmonellosis in poultry with isolation and identification of causal agents. *Journal of Bangladesh Agricultural University* 6(2): 327–334.
2. Begum F, Khan MSR, Choudhury KA, Rahman MM and Amin MM (1993). Studies on immune response of chickens to fowl typhoid vaccine. *Bangladesh Journal of Microbiology*. 10:51-56.
3. Buxton A and Fraser G (1977). Animal Microbiology. Vol. 1. Blackwell Scientific Publications, Oxford, London, Edinburg, Melbourne. pp. 103-115.
4. Freeman BA (1985). Burrows Textbook of Microbiology, twenty second Edition. W. B. Saunders Company, Philadelphia, London, Toronto, Mexico City, Rio de Janerio, Sydney, Tokeyo, pp. 464-475.
5. Gast IL K (1997). *Salmonella* infection, Diseases of Poultry, 10th edn., P. 8 1.
6. Habib-ur-Rehman *et al*, (2004). Incidence and Gross pathology of *Salmonella gallinarum* Infection in chicken. *Journal of Animal and Veterinary Advances* 3 (3): 175-178.
7. Hofstad MS, John BH, Celnek BW, Reid WM and Yoder Jr. HW (1992). Diseases of Poultry. Eight Edition. Panima Education Book Agreny, New Delhi. Ind. p-73.
8. Hoque MM, Biswas HR and Rahman L (1992). Isolation, identification and production of *Salmonella pullorum* colored antigen in Bangladesh for the Rapid Whole Blood Test. *Asian Australatian Journal of Animal Sciences.* 10: 141-146.
9. Jones TC, Hunt RD and King NW (1997). Veterinary Pathology. 6th edn. Williams and Wilkins Co., Baltimore, USA.
10. Kaura YK, Jagjit SR, Kidsbrestba X, Minakshi RC and Chaturvedi GC (1990). *Salmonella gallinarum* var. *duishurg:* An emerging biotype heavy mortality in poultry birds in northern India. *Indian Journal of Animal Science.* 60(2): 127-130.
11. Kleven SH and Yoder HW (1998). Mycoplasmosis. In: H, G. Purchase, L, H, Arp, C. H. Domermuth and J. E. Pearson (Eds). A Laboratory Manual for the Isolation and Identification of Avian Pathogens, 4hedn. American Association of Avian Pathologists, Kenett Square. pp. 74-80.

12. Kumar A and Kaushi RK (1988). Investigation of fowl typhoid in Haryana State. *Indian Journal of Poultry Science*. 23: 104-106.
13. Lee (2001). Comparative exclusion against *Salmonella gallinarum* in *S. enteritidis* infected chickens. Dissertation Abstracts International, 47(5): 1857.
14. Marin C, Hernandiz A and Lainez M (2009). Biofilm development capacity of Salmonella strains isolated in poultry risk factors and their resistance against disinfectants. *Poultry Science.* 88(2):424-31.
15. Merchant IA and Packer RA (1967). *Veterinary Bacteriology and Virology*. 7 th edn., The Iowa State University Press, Ames, Iowa, USA.
16. OIE (Office International Des Epizooties) (2000). Manual of standards for diagnostic tests and vaccines.
17. Pomeroy BS and Nagaraja KV. 1991. Fowl Typhoid. In: Diseases of Poultry, 9th ed. B. W. Calnek. H. J. Barnes, C. W. Beard, W. M. Reid, and H. W. Yoder, Jr. Eds. Iowa State University Press, Ames, Iowa, pp. 87-99.
18. Rahman MM, Chowdhury TLMF, Rahn-mn MM and Hossain WIMA (1979). Surveillance of *Salmonella* and *Escherichia* organisms in poultry feed *Bangladesh Veterinary Journal.* 15(12): 59-62.
19. Sarker AJ (1976). The prevalence of avian disease in Bangladesh Agricultural University poultry Farm. *Bangladesh Veterinary journal.* 10(1-4): 672-678.
20. Sujatha K, Dhanalakshmi K and Rao AS (2003). Isolation and characterization of *Salmonella gallinarum* from chicken. *Indian Veterinary Journal.* 80(5):473-474.