

## EPIDEMIOLOGICAL INVESTIGATION ON DUCK SALMONELLOSIS IN SOME SELECTED AREAS OF BANGLADESH

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### ABSTRACT

The study was performed with a view to collect epidemiological data to proper control of duck salmonellosis in Bangladesh. A total of 12 small to medium sized duck farms and 28 individual households were visited for data collection. On the basis of history and clinical signs and as per the information provided by the farmers the prevalence rate of duck salmonellosis was recorded as 38.1% and the *P* value was calculated as 0.003 ( $p < 0.01$ ) which was noted as highly significant. Based on tentative occurrences of duck salmonellosis as per information taken from the structured questionnaire a total of 120 fecal samples were collected from apparently healthy and diseased ducks on the basis of age, sex, season, location and health status. The isolated bacteria were identified by studying cultural properties on different selective media, biochemical tests, and finally by PCR. The test results of cultural and biochemical exhibited the typical characteristics of bacteria. On the basis of their cultural and biochemical characteristics it was found that among 120 fecal samples 32 (26.67%) were found to be positive for *Salmonella* and the *P* value was recorded as 0.0019 ( $p < 0.01$ ) which was also considered as highly significant. In motility test duck *Salmonella* were identified as motile and all tested duck *Salmonella* showed indole and VP test negative with MR test positive. In PCR reaction, the organism was further confirmed as *Salmonella* species using the SAL-G primer. Results of antibiotic susceptibility test shows that the selected isolated *Salmonella* were highly sensitive to ciprofloxacin and azithromycin, intermediate sensitive to tobramycin and gentamicin and resistant to oxacillin.

**Key words:** Salmonellosis, *16S rRNA* gene, prevalence

### INTRODUCTION

Duck salmonellosis is a disease of economic importance which occurs sporadically or enzootically all over Bangladesh. Mortality may vary from 10% to 80% or higher in severe outbreaks (Kumar and Kaushi, 1988; Kaura *et al.*, 1990; Kleven and Yoder, 1998). Duck salmonellosis is mainly caused by many types of *Salmonella* species. As a species next to chicken, duck production plays a significant role in the rural economy of Bangladesh. It contributes a major source of animal protein in Bangladesh. Ducks are mainly reared in waterlogged areas of Bangladesh such as Netrokona, Kishoregonj, Brahmanbaria, Habigonj, Habiganj, Gaibandha, Noakhali, Mymensingh. Huque and Sultana (2002) reported that natural water areas in different districts of Bangladesh vary from 151 to 12731 hectares.

But there are many obstacles for the establishment of large scale duck farming both in rural and urban area of Bangladesh. The reasons of mortality of duck in Bangladesh are Bird flu, duck plague, duck viral hepatitis, duck cholera, duck salmonella, duck septicemia, mycoplasmosis-colibacillosis complex, tapeworm infestation, fluke infestation, heat stroke, and ascariasis (Sarkar 1980, 1982; Mustafa *et al.*, 1985; Baki *et al.*, 1986; Das *et al.*, 2005; Islam *et al.*, 2007; Haque *et al.*, 2011; Nooruzzaman *et al.*, 2012). Including several numbers of infectious diseases Duck Salmonellosis has been considering is a major disease, hampering the duck industry throughout the world. Price and Berry (1962) have shown that *Salmonella anatum*, is the most important infectious agent in case of Duck Salmonellosis. Others related species were *Salmonella enteritidis*, *Salmonella bredeney*, *Salmonella panama*, *Salmonella give*, *Salmonella senftenberg*, *Salmonella newport*, *Salmonella oregon*, *Salmonella saintpaul*, *Salmonella manhattan* and *Salmonella manchester*.

The members of the genus *Salmonella* were identified by studying cultural properties on different selective media such as selenite broth, *Salmonella-Shigella* (SS) agar, XLD agar, MacConkey agar, Brilliant green agar (BGA); biochemical tests, and finally by PCR. Antibioqram study, serum agglutination test and PCR (Polymerase chain reaction) are widely being used to identify and characterize *Salmonella* species in the laboratories (Deighan *et al.*, 2000; Veling *et al.*, 2000; Buerfeind *et al.*, 2001). *Salmonella* organisms were isolated from various hosts such as from chickens (Begum, 1992), cattle (Islam, 2007), goat (Rahman, 2006), sheep (Karim, 2007) and other animals in Bangladesh and from chicks of Japan by Begum in 2005.

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Although a few research works were done on the basis of this topic internationally but nationally there is no research work was done related to the mentioned title. So, the present work was undertaken to study the prevalence of Duck Salmonellosis considering age, sex, season and location; to isolate and characterize the etiological agent of Duck Salmonella from the collected sample from apparently healthy and diseased Ducks through agent surveillance using cultural, biochemical and molecular technique and to study the antibiogram of the selected isolated species of Duck *Salmonella* species.

## MATERIALS AND METHODS

### Sample collection

At first the epidemiological data and subsequently cloacal swab samples were collected from Mohongonj of Netrokona, Tarail of Kishoregonj, Chunarughat of Habiganj, Ramgonj of Lakshmipur and Phulpur of Mymensingh, Bangladesh. A total number of 120 field samples (cloacal swab) from apparently healthy and diseased ducks. The samples were collected from April 2014 to March 2015 and investigation was carried out following collection. The samples were aseptically collected and then these samples were carried to the Bacteriology Laboratory at the Department of Microbiology and Hygiene of BAU, Mymensingh through Nutrient broth for bacteriological analysis.

### Epidemiological investigation on Duck Salmonellosis

Epidemiological data and cloacal swab were collected from selected areas of Netrokona, Kishoregonj, Habiganj, Lakshmipur and Mymensingh districts of Bangladesh. Epidemiological data were collected through structured Questionnaire and epidemiological investigation was carried out based on some epidemiological parameters such as age, sex, seasons, location and health status of the ducks followed by isolation, identification, molecular characterization and antibiogram study of Duck Salmonella bacteria (Table 1 and Table 2).

Table 1. Number of cloacal swab samples collected from apparently healthy and diseased ducks of Mymensingh, Netrokona, Habiganj, Kishoregonj and Lakshmipur

Sl. No.	Study areas	Number of Cloacal Swabs collected
1.	Mymensingh	32
2.	Lakshmipur	24
3.	Kishorgonj	16
4.	Netrokona	22
5.	Habiganj	26
	Total	120

Table 2. Summary of cloacal samples collected from duck based on age, sex, season and health status

Sl. No.	Epidemiological parameters	Level of patterns	Apparently healthy	Sick	Number of animal examined
1.	Age	1-12 month	44	28	72
		13-24 month	44	04	48
2.	Sex	Male	36	2	38
		Female	52	30	82
3.	Season	Summer	32	13	45
		Rainy	26	12	38
		Winter	30	7	37
4.	Health status	Apparently healthy			88
		Sick			32

### **Isolation and identification of *Salmonella***

Cloacal swabs were collected and each of swabs was inoculated into freshly prepared Nutrient broth. Then the tubes were marked properly 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 test tubes and the smears were fixed. The fixed smears were stained with Gram's Method of staining and examined under microscope at 100 magnifications using immersion oil. In presence of gram negative rods in the smears, the materials from the tube corresponding to the smears were streaked onto MacConkey 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. Motility test and Gram's staining test were performed to identify the plates containing *Salmonella* accurately. Sub culturing on Salmonella-Shigella agar was performed from the suspected plates containing *Salmonella* to obtain a pure culture. (Cheesbrough, 1985). Further the isolates were confirmed by amplification of *Salmonella* species specific *16S rRNA* gene.

### **Bacterial Genomic DNA extraction**

A pure bacterial colony of *Salmonella* species was mixed with 200 µL of distilled water which were boiled for 10 minutes then immediately kept on ice for cold shock. Finally centrifugation was done at 10000 rpm for 10 minutes. The supernatant were collected and used as DNA template for PCR.

### **Identification of *Salmonella* by PCR**

PCR reaction was performed to detect *Salmonella* species specific *16S rRNA* gene from isolated *Salmonella* in a thermal cycler. Two different primers pairs were used for this purpose, *16S rRNA* gene (F 5'-ACTGGCGTTATCCCTTCTCTGGTG -3' and R 5'-ATGTTGTCCTGCCCCTGGTAAGAGA-3') according to the methods described by Noah *et al.*, 1993. Each 20 µl reaction mixture consists of 3 µl genomic DNA, 10 µl PCR master mixtures (Promega, USA), and 1 µl of each of the two primers with the final volume adjusted to 20 µl with 5µl of nuclease free water. Amplification was done by initial denaturation at 94°C for 3 minutes, followed by denaturation at 94°C for 30 sec, annealing temperature of primers was 60°C for 60 sec and extension at 72°C for 45 sec. The final extension was conducted at 72°C for 10 minutes. The total reaction was performed at 30 cycles. The amplified PCR products were resolved by electrophoresis in 2% agarose gel at 100v for 30 minutes, stained with ethidium bromide and finally visualized under UV trans-illuminator.

### **Antibiotic Sensitivity Test**

A total of 5 Positive isolates were tested for antimicrobial drug susceptibility against five commonly used antibiotics by disc diffusion method (Bauer *et al.*, 1966). Sensitivity pattern of the isolates were determined against oxacillin, gentamicin, tobramycin, azithromycin and ciprofloxacin. Antimicrobial testing results were recorded as sensitive, intermediate sensitive and resistant according to zone diameter interpretative standards provided by CLSI, 2012.

## **RESULTS AND DISCUSSION**

The present study was performed for epidemiological investigation of Duck Salmonellosis in some selected areas of Bangladesh followed by isolation, identification and antibiogram study of *Salmonella* species. For this purpose at first data were collected through structured questionnaire. After consideration of collected data a total of 120 cloacal swab samples were collected and subjected to various cultural, biochemical's, molecular examinations and antibiogram study. The prevalence rate of Duck Salmonellosis from different location was shown in Table 3. Prevalence rate was recorded as 40.2% at farm level and 36% was recorded as household level. Overall prevalence rate was recorded as 38.1% and the *P* value was calculated as 0.003 ( $p < 0.01$ ) which was noted as highly significant.

Table 3. Demonstration of Prevalence rate of Duck Salmonellosis as per collected data considering the epidemiological parameters and clinical signs

Location	Visited farms	No. of Ducks/ farm	Sick birds/100	Prevalence	Overall prevalence	Households	No. of Ducks/ households	Sick birds/10 birds	Prevalence	Overall prevalence	Clinical signs
Mymensingh	3	101-500	38	38%	40.2%	7	2-20	3	30%	36%	Whitish to straw colored diarrhoea Weight loss Reduced production Ruffled feathers
Lakshmipur	3	100-300	37	37%		8	2-10	4	40%		
Kishoregonj	2	100-500	42	42%		6	2-30	4	40%		
Netrokona	2	100-600	44	44%		4	4-30	4	40%		
Habiganj	2	100-400	40	40%		3	4-30	3	30%		
Overall prevalence					38.1%						
P value					0.003**						

\*\*means 1% level of sig. (p<0.01)

Farms were classified as 1-50 ducks= Household duck keepers; 1-100 ducks= Small farms; 1-500= Medium farms and Over 500= Large farms

For this study the samples were collected on some epidemiological parameters such as age, sex, season, health status and location. The prevalence of *Salmonella* from different location was shown in Table 4 & 5, Figure 1 & 2. Out of 120 samples examined 32 were found to be positive for *Salmonella*. Among the positive samples, 6 were from Mymensingh, 4 were from Lakshmipur, 9 were from Habiganj, 8 were from Netrokona and 5 were from Kishoregonj. The prevalence rate of *Salmonella* in Duck of these study areas were 18.75%, 16.67%, 34.61%, 36.36%, 31.25% respectively. Overall prevalence rate was 26.67%. *P* value was recorded as 0.0019 (p<0.01) which indicate that the result was highly significant.

Table 4. Results of isolation of *Salmonella* species from feces sample of apparently healthy and diseased ducks

Study areas	No of collected sample	No. of positive sample for <i>Salmonella</i>	Prevalence rate (%)	Overall prevalence rate (%)	P value
Mymensingh	32	6	18.75%	26.67% (32/120)	0.0019**
Lakshmipur	24	4	16.67%		
Habiganj	26	9	34.61%		
Netrokona	22	8	36.36%		
Kishoregonj	16	5	31.25%		

\*\* means 1% level of sig. (p<0.01)

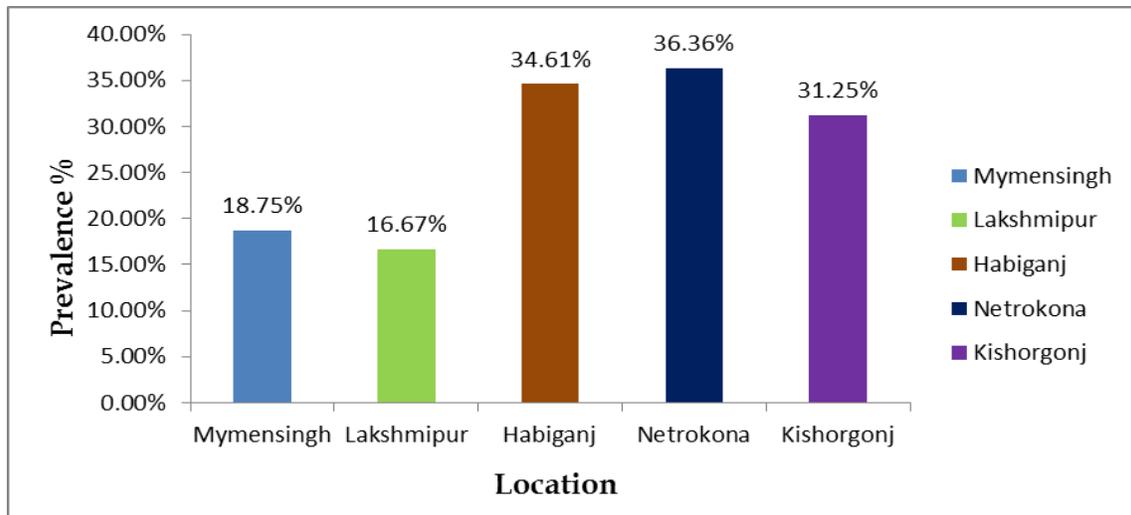


Figure 1. Graphical representation of the prevalence of *Salmonella* species at different locations (Results shown in table 4)

This figure represents that out of 120 samples 18.75%, 16.67%, 34.61%, 36.36% and 31.25% were *Salmonella* positive at Memensingh, Lakshmipur, Habiganj, Netrokona and Kishoregonj respectively.

Table 5. Results of prevalence of *Salmonella* species at different epidemiological parameters

Epidemiological parameters	Level of patterns	No. of animal examined	No. of Positive	Prevalence (%)	P value
Age	1-12 month	72	23	31.94	0.041*
	13-24 month	48	9	18.75	
Sex	Male	38	4	10.52	0.004*
	Female	82	28	34.14	
Season	Summer	45	13	28.88	0.001**
	Rainy	38	12	31.57	
	Winter	37	7	18.92	
Health status	Apparently healthy	88	7	7.95	0.007**
	Sick	32	25	78.12	

\* means 5% level of sig. ( $p < 0.05$ )

\*\* means 1% level of sig. ( $p < 0.01$ )

This table represents that out of 120 samples prevalence of salmonellosis was 31.94% in 1 year aged ducks, 18.75% in more than 1 year aged ducks. *P* value was calculated as 0.041 ( $p < 0.05$ ) that means the result was significant. Salmonellosis was 10.52% prevalent in male birds whereas 34.14% were in female; and the *P* value was calculated as 0.004 ( $p < 0.01$ ) which indicate the result was highly significant. Salmonellosis was also prevalent 31.57% in Rainy season, 28.88% in summer and 18.92% in winter; and the *P* value was calculated as 0.001 ( $p < 0.01$ ) that means the result was highly significant. At finally Salmonellosis was prevalent 78.12% in sick birds and 7.95% were in healthy ducks where *P* value was calculated as 0.007 ( $p < 0.01$ ) which also indicate the result was highly significant.

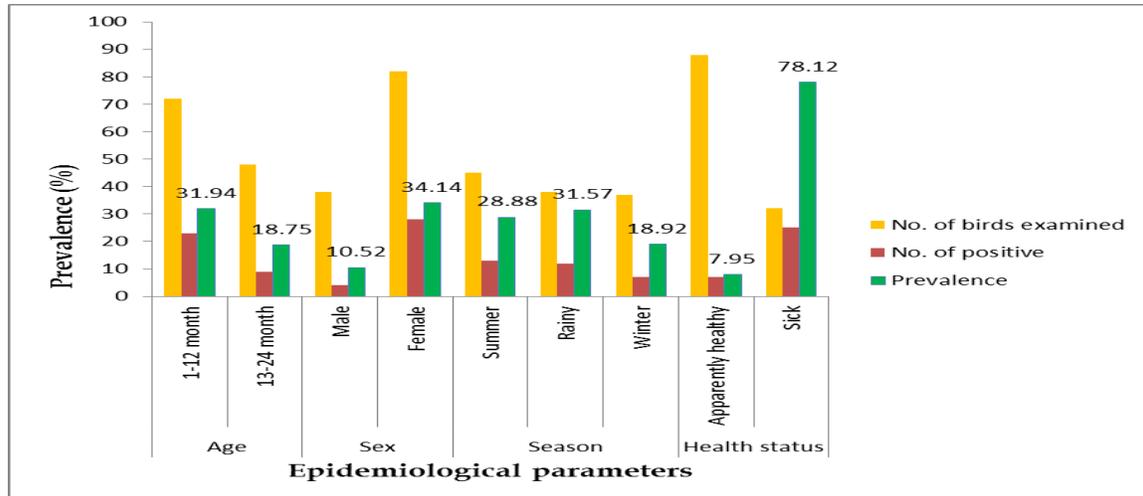


Figure 2. Graphical representation of prevalence rate of Salmonella according to age, sex, season and health status (Results shown in Table 5)

The collected swab samples were subjected to various cultural, biochemical, molecular examinations and antibiogram study to identify and characterization of the isolates. In Nutrient broth the inoculated swab samples revealed the growth of bacteria after 24 hours of inoculation at 37°C. The growth of bacteria was indicated by the presence of turbidity (Figure 3). On NA plates, the isolates produced round, smooth, opaque and translucent colonies (Figure 4). On SS agar plates, the isolates produced pinhead or lentil sized, raised, round or circular smooth, glistening, opaque, black, transparent or translucent colonies (Figure 5). On XLD agar plates, the isolates produced black centered colonies (Figure 6). On EMB agar plates, the isolates produced pink, smooth colonies (Figure 7). On blood agar isolates produced non hemolytic and gray colonies (Figure 8). On MC agar plates, the isolates produced colorless, smooth colonies (Figure 9). The microscopic examination of Gram's staining smears from SS, MC and BGA revealed short plum rod shaped organism, arranged in single and pairs (Figure 10). All of the isolates were found to be motile with hanging drop slide. The isolates were identified as *Salmonella* by biochemical test. All of the isolated *Salmonella* fermented dextrose, maltose, mannitol and dulcitol with the production of acid and gas but did not ferment lactose and sucrose. Acid production was indicated by the color change from reddish to yellow and gas production was noted by the presence of gas bubbles in the inverted Durham's tubes. These isolates were positive for MR test (development of red color indicated positive test) and negative for VP and Indole test. The optimized PCR assay was able to successfully amplify the target *16S rRNA* gene (496 bp fragment) from the DNA templates of all isolated *Salmonella*. Result of PCR for *Salmonella* is shown in Figure 11. The organism was subjected to antimicrobial susceptibility test by disc diffusion method against 5 most commonly used antimicrobial agents. From the antibiogram study, it was revealed that selected isolated of Mymensingh, Lakshmipur, Habiganj, Netrokona and Kishoregonj were highly sensitive to ciprofloxacin and azithromycin, moderately sensitive to gentamicin and tobramycin and were resistant to Oxacillin.

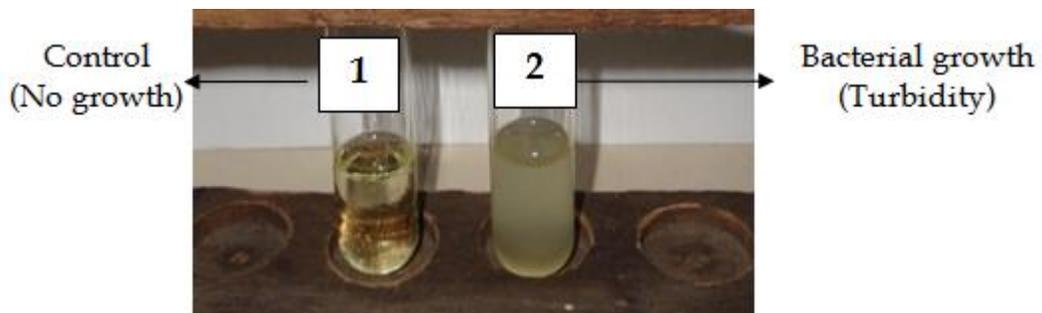


Figure 3. Growth of bacteria in nutrient broth indicated by turbidity  
1= Control, 2= bacterial growth

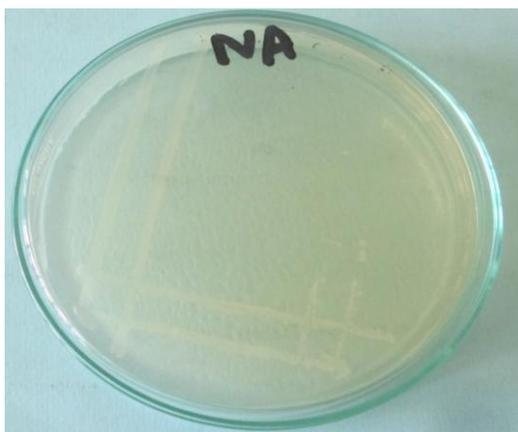


Figure 4. *Salmonella* colonies on Nutrient



Figure 5. *Salmonella* colonies on S-S agar



Figure 6. *Salmonella* colonies on XLD agar



Figure 7. *Salmonella* colonies on EMB agar

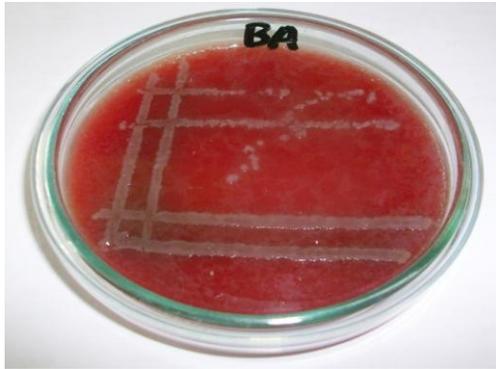


Figure 8. *Salmonella* colonies on Blood agar



Figure 9. No growth of bacteria on MacConkey agar

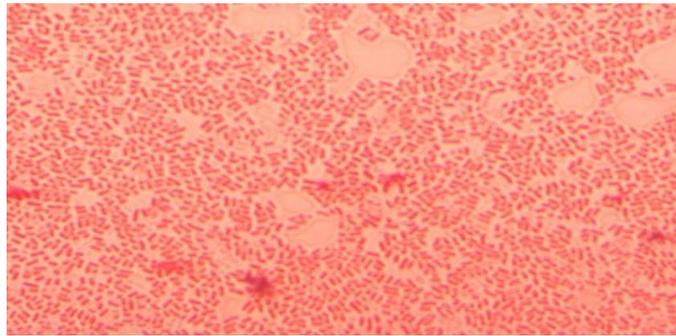


Figure 10. Gram staining of *Salmonella* isolates (100X)

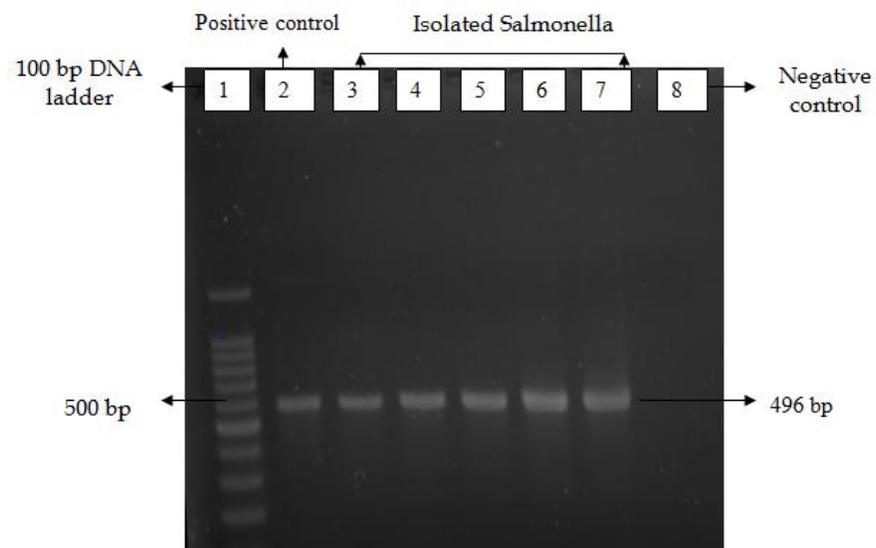


Figure 11. PCR image of *Salmonella* species Lane 1: 100 bp DNA ladder, Lane 2-Positive control, Lane 3-7: Isolated sample of *Salmonella* species and Lane 8: Negative control

The present research work was selected and performed with the title ‘‘Epidemiological Investigation on Duck Salmonellosis in some selected areas of Bangladesh’’ considering the specific objectives as the study of prevalence of duck salmonellosis based on age, sex, season and location differences following isolation, characterization using cultural, biochemical, molecular and antibiogram study. Infection by *Salmonella* is a common cause of food poisoning in humans (Hobbs and Robert, 1993). So, it is of great economic concern and public health significance. Isolation of *Salmonella* species from duck was reported in many other countries (Rettger *et al.*, 1920) but there is no available report on prevalence of *Salmonella* species from duck and its comparison with those from other species in Bangladesh. For the study of prevalence at first data were collected through structured questionnaire from duck farmers and house hold duck keepers at selected areas of Bangladesh. On the basis of history and clinical signs and as per the information provided by the farmers the prevalence rate of Duck Salmonellosis was recorded as 38.1% and the *P* value was calculated as 0.0003 ( $p < 0.01$ ) which was noted as highly significant.

Specific enrichment media and biochemical tests were used for the isolation and identification of *Salmonella* which was previously suggested by a number of researchers (Buxton and Fraser 1977; Mallinson *et al.*, 1991; Ruiz *et al.*, 1992; Sharma and Katock 1996; Dhruva *et al.*, 1999; Habrun *et al.*, 2006). In this study, colony characteristics of *Salmonella* from ducks of selected areas on MacConkey agar, SS agar, XLD agar and EMB agar were similar to the findings of other authors (Shaffer *et al.*, 1964; Merchant and Packer, 1967; Buxton and Fraser, 1977). In Gram staining, the morphology of the isolated *Salmonella* exhibited Gram negative, small rod shaped, single or paired in arrangement under microscope which was supported by other researchers (Freeman, 1985; Gene, 2002). In motility test, all the isolates of *Salmonella* from duck have shown swinging movement which differentiates the motile *Salmonella* bacteria from non-motile and others (Merchant and Packer, 1967; Buxton and Fraser, 1977). Differentiation of *Salmonella* into species level was difficult to identify based on their sugar fermentation pattern (Freeman, 1985). In sugar fermentation test, all of the isolated *Salmonella* fermented dextrose, maltose and mannitol and produced acid and gas but did not ferment lactose which satisfies the statement of Buxton and Fraser (1977). Again, all the isolates were positive to methyl red test and negative to indole and V-P test (Buxton and Fraser, 1977).

The prevalence of Duck Salmonellosis in location parameters was recorded as 18.75% in Mymensingh, 16.67% in Lakshmipur, 34.61% in Habiganj, 36.36% Netrokona and 31.25% in Kishoregonj. Overall prevalence rate of Duck Salmonellosis was recorded as 26.67%. *P* value was recorded as 0.0019 ( $p < 0.01$ ) which indicated that the result was highly significant (Table 4).

On the basis of cultural, biochemical and molecular confirmation the prevalence of Duck Salmonellosis was differed depending on different epidemiological parameters. Out of 120 fecal samples the prevalence of Duck Salmonellosis was 31.94% in 1 year aged ducks and 18.75% in more than 1 year aged ducks. *P* value was calculated as 0.041 ( $p < 0.05$ ) that means the result was significant. Duck Salmonellosis was 10.52% prevalent in male birds whereas 34.14% were in female birds; and the *P* value was calculated as 0.004 ( $p < 0.01$ ) which indicated the result was highly significant. Duck Salmonellosis was also prevalent 31.57% in Rainy season, 28.88% in summer and 18.92% in winter; and the *P* value was calculated as 0.001 ( $p < 0.01$ ) that means the result was highly significant. At finally Duck Salmonellosis was prevalent 78.12% in sick birds and 7.95% were in healthy birds where *P* value was calculated as 0.007 ( $p < 0.01$ ) which also indicate the result was reported as highly significant (Table 5).

On the basis history and clinical signs and as per the information provided by the farmers the prevalence rate of Duck Salmonellosis was recorded as 38.1% but after cultural, biochemical and molecular conformation it was revealed only 26.67% which showed similarity with the findings of other researchers (Snow *et al.*, 2007; Limawongpranee *et al.*, 1999). It also showed more or less similar results with other researchers who worked with cloacal samples of poultry. For instance, Haque (2011) isolated 48.07% *Salmonella* from cloacal samples of apparently healthy ducks; Aktar (2011) found 59% prevalence from cloacal swabs of quails and Sarkar (2009) recovered 20.83% from fecal samples and 75% from cloacal swabs of apparently healthy water birds. Conversely, Chiu *et al.* (2010) found 0.3% prevalence for cloacal swabs of breeder broiler and 11.3% for broiler, Mondal (2007) recorded prevalence of 13.07% from cloacal swabs of apparently healthy and diarrheic ducks. The differences among the prevalence percentages might be due to the species differentiation, hygienic, environmental and geographic variation and limitations in the laboratory of the study.

In molecular characterization, a 496-bp band was seen in each lane with the product of the PCR for *Salmonella* species. The isolates of *Salmonella* species in this study were similar to the findings of the researcher (Noah *et al.*, 1993).

In this study, total 5 isolates were investigated for susceptibility and resistance patterns by disc diffusion method using 5 commonly used antibiotics belonging to different criteria which were followed during sample collection such as age, sex, season, location and health status. The highest resistance was found with Oxacillin at all epidemiological criteria. This finding satisfy the result of Chugh and Suheir (1983), Banani *et al.* (2003), Lee *et al.* (2005), Kobayashi *et al.* (2007) and Haque (2011). Among 5 antibiotics the selected isolates showed 100% sensitive to Ciprofloxacin and Azithromycin. On the other hand 80% were Intermediate to Tobramycin and Gentamycin and 100% were resistant to oxacillin which showed similarity with the findings of (Soomro *et al.*, 2010). Notably the sensitivity and resistant pattern of the isolates against the 5 selected antibiotics were shown various results at different age, sex, location and season groups. Results of the study indicated that some isolates were more likely to be multi-drug resistant. Therefore, more sensible use of antibiotics can be strongly suggested for the veterinarians since drug resistance could be a major public health concern as fluoroquinolones are important antimicrobial compounds in the treatment of *Salmonellosis* in humans (CLSI, 2012).

## CONCLUSION

Results of this study concluded that *Salmonella species* was found to be an important cause of duck diseases in Bangladesh resulting significant economic losses through mortality, morbidity and reduced production. Epidemiological parameters were recorded as significant factors on Duck Salmonellosis in Bangladesh and the prevalence was varied in different age, sex, location, season and health status. *Salmonella* were successfully isolated and identified from the collected cloacal swabs from apparently healthy and diseased ducks by cultural, morphological, biochemical and molecular techniques. On the basis of history and clinical signs and as per the information provided by the farmers the tentative prevalence rate of Duck Salmonellosis was recorded as 38.1% and the *P* value was calculated as 0.0003 ( $p < 0.01$ ) which was noted as highly significant but after cultural, biochemical and molecular confirmation it was revealed only 26.67% and the *P* value was calculated as 0.0019 ( $p < 0.01$ ) which indicated that the result was highly significant. Antibiotic sensitivity was measured by their zone of inhibition and it might be suggested that ciprofloxacin and azithromycin could be used at any stage of duck rearing. Other drugs are not recommended for treatment and prevention of Salmonellosis at any stage of duck rearing. The isolated and characterized etiological agent of Duck Salmonellosis could be *Salmonella anatum*, *Salmonella enteritidis*, *Salmonella enterica*. The final and most important outcome of this study is the surveillance techniques of Salmonellosis in ducks of selected areas of Bangladesh were developed for the proper control and prevention of this economically important duck disease of the country.

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