International Journal of Animal Biology
Vol. 3, No. 3, 2017, pp. 12-17
http://www.aiscience.org/journal/ijab
ISSN: 2381-7658 (Print); ISSN: 2381-7666 (Online)

Isolation and Molecular Characterization of *E. coli, Salmonella* spp. and *Pasteurella* spp. from Apparently Healthy Duck

Hasna Banu[1], Md Zulfekar Ali[2, *], Sonia Akther[3], Parbati Paul[4], Md. Alimul Islam[5], Mst. Minara Khatun[5]

[1]ACI Animal Health, ACI Limited, Dhaka, Bangladesh
[2]Animal Health Research Division, Bangladesh Livestock Research Institute (BLRI), Dhaka, Bangladesh
[3]Goat and Sheep Production Research Division, Bangladesh Livestock Research Institute (BLRI), Dhaka, Bangladesh
[4]Department of Livestock Services (DLS), Ministry of Fisheries and Livestock Khamarbari, Dhaka, Bangladesh
[5]Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh, Bangladesh

Abstract

The present study was conducted for the isolation, identification and molecular detection of the bacterial flora present in the respiratory tract of apparently healthy 4 breeds (Khaki Campbell, Pekin, Jinding and Muscovy) of ducks. Out of 60, a total of 48 respiratory samples from Bangladesh Agricultural University poultry farm (BAUPF) and 12 from scavenge area were collected. Three bacteria were isolated and identified from samples by cultural, staining biochemical tests and molecular detection. The prevalence of *E. coli, Salmonella* spp. and *Pasteurella* spp. of Khaki Campbell duck were 50%, 41.67% &58.33% and 50%, 50% & 66.67% recorded from BAUPF and scavenging area, respectively. Out of 60 samples, the overall prevalence of *E. coli*, *Salmonella* spp. and *Pasteurella* spp. were 50%, 31.67% & 50%. In both sources, *E. coli* and *Pasteurella* spp. shown high prevalence in BAUPF and scavenging area. In BAUPF the isolation of *E. coli, Slamonella* spp. and *Pasteurella* spp. were 0, 2 & 2 and 2, 0 & 1from both adult and young Khaki Campbell duck. On the other hand, in scavenge area the isolation of *E. coli, Slamonella* spp. & *Pasteurella* spp. were 1, 2 & 2 and 2, 1 &1 from both adult and young Khaki Campbell duck. So it was found that the isolation of bacterial species shown higher in scavenge area than in BAUPF. The highest number of bacteria were recovered from lungs (27 isolates from 8 samples) while the lowest number were from tracheal washing and swab of infraorbital sinus (14 isolates from 8 sample in both cases). Total bacterial isolates were 6 isolates *E. coli* 3, *Salmonella* spp. 2 and *Pasteurella* spp. 2 were found positive by conventional PCR. The results of this study would be helpful for prevention and control of bacterial respiratory diseases in ducks.

Keywords

E. coli, *Salmonella*, *Pasteurella*, Duck, PCR

Received: August 28, 2017 / Accepted: October 11, 2017 / Published online: November 1, 2017

1. Introduction

Ducks are considered as a second widespread species among poultry following the chickens in Bangladesh. Duck rearing is an integral part of poultry husbandry. Duck population of Bangladesh is estimated to be 35.53 million [1] of which 95% are of indigenous type [2]. Household indigenous domestic ducks are of 39.8 million in the world [3]. Diseases affecting the respiratory system are generally the most important in all

* Corresponding author
E-mail address: zulfekar@blri.gov.bd (Md Z. Ali), zulfekarvet@gmail.com (Md Z. Ali)

species of domestic animals [4]. Bacterial infections of the respiratory tract are of major importance in poultry production as it can cause severe economic losses with mortality, loss of egg production or impaired growth. Both Gram-positive andGram-negative bacteria are found in the upper respiratory tract of psittacoses birds. Various pathogenic microbes, such as *Escherichia coli, Salmonella spp., Bacillus spp., Streptococcus spp., and Staphylococcus spp.,* have been implicated to reduce the growth of poultry including duck [5]. All of these organisms have been reported to be associated with upper respiratory disease under certain conditions such as stress, viral infections etc [6]. *Escherichia coli* associated with respiratory infection in chickens have also been reported [7]. *E. coli* infection is by far the most common bacterial infection of all age of commercial ducks and can certainly have the largest economic effect [8]. Initial infection is most commonly via the respiratory tract and air sacs and is usually secondary to infection by mycoplasma or a virus. Acute infections may only show a congested carcass, congested lungs and small hemorrhages of the heart and air sacs. However, more chronic infections will show the characteristic lesions of pericarditis, perihepatitis and enlargement of the liver, air sacculitis and pneumonia [8]. Respiratory colibacillosis is a respiratory disease caused by secondary infection with pathogenic *Escherichia coli*. Any insult to the respiratory tract creates a climate for potential colonization of the respiratory tract by *E. coli* [9]. *Salmonellosis* is one of the most important diseases that cause serious economic loss due to mortality and reduced egg production [10]. *Salmonella* are Gram-negative, small rod-shaped, non spore forming, non capsulated, aerobic and facultative anaerobic organisms and classified under the family Enterobacteriaceae [11]. True Salmonellosis is comparatively rare in ducks but is often due to the serotype *Salmonella typhimurium* [8]. Mortality level in a flock can be quite significant, sometimes reaching 15%. Avian *pasteurellosis* (Fowl cholera/duck cholera) is a contagious bacterial disease of domesticated and wild avian species caused by infection with *Pasteurella multocida* which hamper the profitable poultry production [11]. Losses usually occur in ducks over 4 weeks of age and mortality may reach 50% [12]. *Pasteurella* sp. are Gram-negative rod with a tendency to bipolar staining, non-spore forming, non-motile and capsulated organisms. The most important species are *P. multocida, P. anatipestifer and P. haemolytica.* This can infect chickens, turkeys, ducks, quails and other bird species. *P. multocida* can be harbored in the respiratory tract or cloacal mucosa of asymptomatic birds and these strains can become sources of outbreaks [13].

2. Materials and Methods

Study design: The collection of 48 samples from BAU poultry farm and 12 samples from scavenging duck (Khaki Campbell, Pekin, Jinding and Muscovy ducks) (Table 1). Then inoculated into nutrient broth (NB), incubate at 37°C and 24 hours for enrichment. Inoculated into the specific media Salmonella-shigella agar (SSA), Eosin methyline blue agar (EMB), Mc-Conkey agar and XLD agar media for identification of *E. coli, Salmonella* spp. *and Pasteurella* spp. Then morphological characterization by standard staining techniques and biochemical tests (Coagulase test, Catalase test, Sugar fermentation test, MR-VP test and Indole test. Testing motility of the bacteria by hanging drop method and finally molecular detection of *E. coli, Salmonella* and *Pasteurella* spp. by conventional PCR test method.

Table 1. Summary of respiratory samples of Khaki Campbell, Pekin, Jinding and Muscovy ducks in BAU poultry farm.

Samples	Age	Sample no	Grand total
Tracheal swab	Adult (8 months)	10	60
	Young (1 month)	10	
Tracheal washing	Young (1 month)	10	
Air sacs	Young (1 month)	10	
Lung	Young (1 month)	10	
Swab of Infraorbital sinus	Young (1 month)	10	

Isolation and identification of Escherichia coli

The samples were first inoculated into NB and incubated at 37°C for 24 hours. Then it was inoculated into NA and incubated at 37°C for 24 hours aerobically. To identify *E. coli* and other coliforms lactose fermenting white colonies from the NA were sub-cultured on EMB agar. Colonies on EMB agar with metallic sheen were suspected as positive for *E. coli* and were confirmed by biochemical test. *E. coli* was found positive to indole and MR tests but negative to VP tests and was motile [14].

Isolation and identification of Salmonella spp.

Processed samples were inoculated into NB and incubated at 37°C for 24 hours. Then it was inoculated into NA and incubated at 37°C for 24 hours aerobically. To identify *salmonella* and other coliforms lactose fermenting white colonies from the NA were sub-cultured on SS agar. Translucent, round and colorless colonies on SSA were suspected to be *Salmonella* and/or *Shigella*, which were late confirmed by biochemical test and the motility test. Organisms were Gram negative rod, motile, indole and VP negative and MR positive [14].

Isolation and identification of Pasteurella spp.

Pasteurella spp. were easily isolated and identified by their morphological and cultural characteristics. The organisms were gram negative, non motile, non-spore forming, bipolar rod occurring in pairs, singly or in chains. The organisms produced moderate size, round and grayish colonies in blood agar medium. A characteristic musty odor was found.

3. Molecular Detection

Method of isolation of genomic DNA

Pure broth culture, centrifuge at 8000 rpm for 5 minutes, discarded supernatant and pellet collected. Add 200µl of distilled water in each eppendrof tube of pellete thenresuspend the pellet by vortexing andplaced the eppendrof tube in the boiling water for 10 minutes (Table 2). After boiling transferred the tube into the ice and centrifuge immediately at 10000 rpm for 10 minutes andcollected the supernatant containing genomic DNA of each eppendrof tube of the separatedfresh which will act as template DNA for PCR reaction (Table 3).

Table 2. List of primers used for genomic detection.

Specificity	Primers	Sequence(5'-3')	Ampliconsize(bp)	Reference
Escherichiacoli	EC16srRNA/F	5'-GACCTCGGTTTAGTTCACAGA-3'	585	[15]
	EC16srRNA/R	5'-CACACGCTGACGCTGACCA-3'		
*Salmonella*spp.	SdiA1	5'-AATATCGCTTCGTACCAC-3'	274	[28]
	SdiA2	5'-GTAGGTAAACGAGGAGCA-3'		
*Pasteurella*spp.	KMT1T7	5'-ATCCGCTATTTACCCAGTGG-3'	460	[29]
	KMT1SP6	5'-GCTGTAAACGAACTCGCCAC-3'		

Table 3. Preparation of reaction mixture for PCR.

Composition	Amount
Taq-buffer (10X PCR buffer)	4 µl
50 mM $MgCl_2$	4 µl
10 mMdNTP	1·5 µl
FPrimer	3 µl
R Primer	3 µl
LA Taq	1 µl
Deionized Distilled water	31·5 µl
DNA/PC-DNA	2 µl
Total	50 µl

Protocol used for PCR

At first, the required number of PCR tubes were labeled and kept on ice. Then 48 µl of reaction mixture was dispensed into each of the PCR tubes and 2 µl of eluted DNA from each sample was added to that respective tube and mixed properly with the help of the micropipette. The tubes were placed in a forty wells Thermocycler (MJ Mini Thermocycler, BIO-RAD, USA). Then the temperature of the Thermocycler was set according to the thermal profile mentioned below. After completion of PCR, PCR products were kept in 0 C for Agarose gel electrophoresis.

Table 4. PCR condition against different primer.

Primer	Initial denaturation		Denaturation		Annealing		Elongation		Final extension	
	Tem	Time	Tem	Time	Tem	Time	Tem	Time	Tem	Time
EC 16sr RNA	95°C	5m	95°C	30s	50°C	2m	65°C	2min	65°C	10m
SdiA1and SdiA2	95°C	5m	95°C	30s	50°C	2m	65°C	2min	65°C	10m
KMT1T7and KMT1SP6	95°C	5m	95°C	30s	50°C	2m	65°C	2min	65°C	10m

Gel documentation

Preparation of 1.5% Agarose media then added 5 µl of ethidium bromide and shacked for mixing. Then the melted gel was poured on the agar gel plate. Comb containing various numbers of teeth was placed on the gel at the beginning of the solidification. Then it was kept for half an hour for solidification. Then 6× loading dye was taken on parafilm for each PCR product. Each PCR product was mixed with loading dye and was placed in well of electrophoresis chamber. The marker dye was placed in wells of each side of the chamber and was run at 90 V for 40 minutes. Finally observed under UV transilluminator (Figure 1).

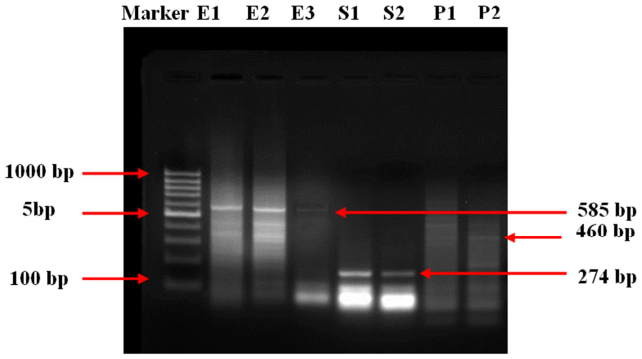

Figure 1. 2% Agarose gel Electrophoresis shows band of DNA for the selective bacteria (*E. coli*, *Salmonella* spp. and *Pasteurella* spp.) in PCR method. M: 100bp DNA ladder; Lanes E1-E3: PCR product of *E. coli* (585bp); Lanes S1-S2: PCR product of *Salmonella* spp. (274bp); Lanes P1-P2: PCR product of *Pasteurella* spp. (460bp).

4. Results

Prevalence of bacteria isolated from all respiratory samples (n=48) of different breeds of ducks in BAUPF. The results were expressed in percentage. In Khaki Campbell duck, out of 12 samples the prevalence of *E. coli, Salmonella* spp. & *Pasteurella* spp. were 50%, 41.67% & 58.33% respectively. In Pekin duck, out of 12 samples the prevalence of *E. coli, Salmonella* spp. and *Pasteurella* spp. were 58.33%, 16.67% & 41.67 respectively. In Jinding duck, out of 12 samples the prevalence of *E. coli, Salmonella* spp. and *Pasteurella* spp. were 50%, 25%, & 33.33% respectively. In Muscovy duck, out of 12 samples the prevalence of *E. coli, Salmonella* spp. and *Pasteurella* spp. were 41.67%, 25%, & 50% respectively. Comparative prevalence study of bacteria isolated from respiratory samples of Khaki Campbell ducks from BAU Poultry Farm (n=12) and scavenge area (n=12). In BAU poultry farm the prevalence of *E. coli, Salmonella* spp., *Pasteurella* spp., *Bacillus* spp. *and Staphylococcus* spp. were 50% (6 of 12), 41.67% (5 of 12), 58.33% (7 of 12), 25% (3 of 12) and 41.67% (5 of 12), respectively. On the other hand, in scavenge area the prevalence of *E. coli, Salmonella* spp., *Pasteurella* spp., *Bacillus* spp. *and Staphylococcus* spp. were 50% (6 of 12), 50% (6 of 12), 66.67% (8 of 12), 33.33% (4 of 12) and 75% (9 of 12), respectively. Prevalence of bacteria isolated from all respiratory samples (n=60) of different breeds of ducks in BAUPF and scavenge area. Out of 60 samples 50% positive for *E. coli,* 31.67% were positive for *Salmonella* spp. 50% was positive for *Pasteurella* spp. respectively. Age dependent distribution of overall prevalence of bacteria isolated from tracheal swab samples of different breeds of adult (n=10) and young (n=10) ducks. In adult duck the prevalence of *E. coli, Salmonella* spp. and *Pasteurella* spp. were 20%, 80% and 70%, respectively. On the other hand, in young ducks the prevalence of *E. coli, Salmonella* spp. &*Pasteurella* spp. were 90%, 30% & 50%, respectively. Number of bacterial isolates recovered from internal organs of respiratory tract. The highest number of bacteria were recovered from lung (27 isolates from 8 samples) followed by air sacs (16 isolates from 8 samples), tracheal washing (14 isolates from 8 samples) and swab of infraorbital sinus (14 isolates from 8 samples).

Three isolates of *E. coli*, two isolates of *Salmonellae,* two isolates of *Pasteurella* spp., showed positive with molecular detection through PCR.

5. Discussion

In this study, three different types of bacteria were isolated from respiratory samples of different breeds of duck. The isolated bacteria were *E. coli*, *Salmonella* spp. and *Pasteurella* spp. The results of isolation are in agreement with the findings of [16]. The prevalence of bacteria isolated from all respiratory samples (n=48) of different breeds of duck in BAUPF were recorded. In Khaki Campbell duck, out of 12 samples the prevalence of *E. coli, Slamonella* spp. & *Pasteurella* spp., *Bacillus* were 6 (50%), 5 (41.67%) & 7 (58.33%). In Pekin duck, the prevalence of *E. coli, Slamonella* spp. & *Pasteurella* spp. were 7 (58.33%), 2 (16.67%) & 5 (41.67%) found out of 12 sample. In Jinding duck, out of 12 samples the prevalence of *E. coli, Slamonella* spp. & *Pasteurella* spp. were 6 (50%), 3 (25%) & 4 (33.33%). In Muscovy duck, out of 12 samples the prevalence of *E. coli, Slamonella* spp. & *Pasteurella* spp. were 5 (41.67%), 3 (25%) & 6 (50%). So it was shown that the highest prevalence of *E. coli* was found in Pekin duck (58.33%) and lowest in Muscovy duck (41.67%), the highest prevalence of *Salmonella* spp. was found in Khaki Campbell duck (41.67%) and lowest prevalence of *Salmonella* spp. was found in pekin duck (16.67%) in *Pasteurella* spp. In BAUPF (n=12), the prevalence of E. *coli, Slamonella* spp. & *Pasteurella* spp. were 50% (6 of 12), 41.67% (5 of 12) & 58.33% (7 of 12), respectively. On the other hand, in scavenge area (n=12) the prevalence of *E. coli, Slamonella* spp. & *Pasteurella* spp. were 50% (6 of 12), 50% (6 of 12) & 66.67% (8 of 12), respectively. So it was shown that the highest prevalence of *E. coli* (50%) was found in both BAUPF and scavenges area. On the other hand, the prevalence of other isolated bacteria were higher in scavenge area than in BAUPF. It may be due to improper biosecurity, lack of nutritional management and immune status [17]. This study recorded the highest bacterial prevalence in lungs (27 isolates from 8 samples) followed by air sacs (16 isolates from 8 samples), tracheal washing (14 isolates from 8 samples) and swab of infraorbital sinus (14 isolates from 8 sample) in BAUPF and scavenge area. [18] also reported highest prevalence of bacteria in lungs (14.4%) followed by trachea (7.2%), swab of infraorbital sinus (6.4%) and air sacs (4.8%). It may be due to anatomical location, physiological composition and food intake behavior [17]. In this study, colony characteristics of *E. coli* observed in NA, EMB and SS agar were similar to the findings of [19], [20]. In Gram's staining, the morphology of the isolated bacteria exhibited Gram negative short rod arranged in single or paired and motile which was supported by several authors [21]. The colony characteristics of *Salmonella* spp. observed in NA, EMB agar and SS agar were similar to the findings of [22]. In Gram's staining, the morphology of the isolated bacteria exhibited Gram negative small rod arranged in single or paired and motile which was supported by several authors [23]. The motile *salmonella* isolated in this study might belong to serovars other than *S. pullorum and S. gallinarum* [24]. The morphology of the isolated *Pasteurella* spp. in

Gram's staining exhibited gram negative, coccobacillary organism and arranged singly or in paired which was supported by [25]. Bipolar appearance of *P. multocida* recorded in this study is in agreement with the findings of other workers [26]. In the present study, multiplex PCR assay was performed for confirmation of bacterial isolates. Based on analysis 2 isolates of *Salmonella* spp., 3 isolates of *E. coli* and 2 isolates of *Pasteurella* spp. showed 100% specificity. The results of PCR of the present study closely agree with the findings of [27] [30] [31] [32] [33]. This method can be applied to other bacterial species directly obtained from affected tissues as well as grown on media.

6. Conclusion

Considering the findings of this research work, it may be concluded that- Respiratory tract of animals harbor five genera of bacteria such as: *E. coli, Salmonella spp., Pasteurella* spp*., Bacillus* spp. *and Staphylococcus* spp. Recovery of bacteria was higher in adults as compared to young ducks. Lung harbor highest number of bacteria followed by air sacs, tracheal washing and swab of infraorbital sinus. Bacteria isolated from respiratory tract showed three antimicrobial sensitivity profiles such as: sensitive, intermediate and resistant. All of the isolates were sensitive to Amoxycillin, Ciprofloxin, Gentamycin, and Kanamycin. The most resistant antibiotic was Tetracycline and Erythromycin. Multidrug resistant bacteria isolated from respiratory tract of ducks might have resulted from indiscriminate use of antibiotics for treatment.

References

[1] Department of Livestock Services (DLS), 2003. Annual report of Department of Livestock Services, Khamarbari Road, Farmgate, Dhaka.

[2] Huque, K. S., & Sultana, N. (2003). Organic duck farming in Bangladesh and Entrepreneurship Development. In *Proceeding of 3rd Int. Poultry Show and Seminar. Bangladesh China Friendship Conference Center, Dhaka, Bangladesh* (pp. 279-287).

[3] Ali, M. Z., Rahman, M. M., & Sultana, S. (2015). Seroprevalence of Mycoplasma gallisepticum antibody by ELISA and serum plate agglutination test of laying chicken. *Veterinary world*, 8(1), 9.

[4] Russo, T. A., Davidson, B. A., Genagon, S. A., Warholic, N. M., MacDonald, U., Pawlicki, P. D.& Knight, P. R. (2005). E. coli virulence factor hemolysin induces neutrophil apoptosis and necrosis/lysis in vitro and necrosis/lysis and lung injury in a rat pneumonia model. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, *289*(2), L207-L216.

[5] Duke, G. E. (1986). Alimentary canal: secretion and digestion, special digestive functions, and absorption. In *Avian Physiology* (pp. 289-302). Springer New York.

[6] Jesus, S. O., & Correia, J. H. D. (1998). Potential pathogens recovered from the upper respiratory tract of psittacine birds. In *Proceedings of International Virtual Conference in veterinary medicine: diseases of Psittacine birds. University of Georgia College of Veterinary Medicine*.

[7] El-Sukhon, S. N., Musa, A., & Al-Attar, M. (2002). Studies on the bacterial etiology of airsacculitis of broilers in northern and middle Jordan with special reference to Escherichia coli, Ornithobacterium rhinotracheale, and Bordetella avium. *Avian diseases*, *46*(3), 605-612.

[8] Johnston, A. (2007). Controlling duck diseases-Current diseases of ducks and their control-How to identify and control the most common diseases of ducks. *Poultry international*, *46*(1), 24.

[9] Calnek, B. W., Barnes, H. J., Beard, C. W., McDougald, L. R., & Saif, Y. M. (1997). Diseases of poultry 10th ed.

[10] Khan, M. F. R., Rahman, M. B., Khan, M. S. R., Nazir, K. H. M. N. H., & Rahman, M. (2005). Antibiogram and plasmid profile analysis of isolated poultry Salmonella of Bangladesh. *Pakistan Journal of Biological Sciences*, *8*, 1614-1619.

[11] Office International Des Epizooties (OIE), 2006. http://www.oie.int/chapter x.4. *Salmonellosis*.

[12] Calnek BW, Barnes HJ, Beard CW, McDouglad LR and Saif YM, 1997. Diseases of poultry.10th edi. Lowa State University Press, Ames, Iowa. pp. 143-150.

[13] Muhairwa, A. P., Christensen, J. P., & Bisgaard, M. (2000). Investigations on the carrier rate of Pasteurella multocida in healthy commercial poultry flocks and flocks affected by fowl cholera. *Avian pathology*, *29*(2), 133-142.

[14] Merchant IA, Packer RA, 1967. Veterinary Bacteriology and Virology. 7th edi. Lowa State University Press, Ames. Lowa, USA. pp. 286- 306.

[15] Magray, M. S. U. D., Kumar, A., Rawat, A. K., & Srivastava, S. (2011). Identification of Escherichia coli through analysis of 16S rRNA and 16S-23S rRNA internal transcribed spacer region sequences. *Bioinformation*, 6(10), 370.

[16] Thenmozhi, V., Malmarugan, S., Suresh, P., & Jeyanthi, C. (2010). Isolation and identification of bacterial respiratory pathogens in Japanese quails. *Indian Journal of Field Veterinarians*, 6(1), 54-56.

[17] Tizard IR, 1997. Veterinary immunology: An Introduction. 5th edi, chapter I. W. B Saunders Company Philadelphia, P. 3-5.

[18] Murthy, T. R. G. K., Dorairajan, N., Balasubramanium, G. A., Dinakaran, A. M., & Saravanabava, K. (2008). Pathogenic bacteria related to respiratory diseases in poultry with reference to Ornithobacterium rhinotracheale isolated in India. *Veterinarski Arhiv*, 78(2), 131.

[19] Kalin, R., Ongor, H., & Cetinkaya, B. (2012). Isolation and molecular characterization of Escherichia coli O157 from broiler and human samples. *Foodborne pathogens and disease*, *9*(4), 313-318.

[20] Hasina B, 2006. Enteropathotypic characterization of *Escherichia coli* isolated from diarrhoeic calves and their antibiogram study. MS. Thesis, Department of Microbiology and Hygiene, BAU, Mymensingh, pp. 51-53.

[21] KHMNH, N., Rahman, M. B., Nasiruddin, K. M., Akhtar, F., Khan, M. F. R., & Islam, M. S. (2005). Antibiotic sensitivity of Escherichia coli isolated from water and its relation with plasmid profile analysis. *Pakistan Journal of Biological Sciences*, 8(11), 1610-1613.

[22] Ali MZ, Sultana S. Determination of humoral immune response in chickens against formalin-inactivated alum-precipitated fowl cholera vaccine. International Journal of Animal Biology. Vol. 1, No. 4, 2015, pp. 114-117.

[23] Sultana S, Rahman MM, Ali MZ. Evaluation of gentian violet and copper sulphate as fungi inhibitor in broiler diet. International Journal of Animal Biology. Vol. 1, No. 4, 2015, pp. 146-149.

[24] Sultana S, Rashid SM, Islam MN, Ali MH, Islam MM, Azam MG. Pathological investigation of avian aspergillosis in commercial broiler chicken at chittagong district. International Journal of Innovation and Applied Studies. 2015 Jan 1;10(1): 75-85.

[25] Woo YK and Kim JH, 2006. Fowl cholera outbreak in domestic poultry and epidemiological properties of *P. multocida* isolate. *Microbiology,* 44: 344-353.

[26] Chawak MM, Verma KC, Kataria JM and Kumar AA, 2000. Characterization of indigenous isolates of avian *Pasteurella multocida. Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases,* 21:111-114.

[27] Yuan W, Tang SH and Cen LJ. 2010. Detection of *Salmonella typhimurium* in Duck-slaughtering Chain by PCR. *Food Science*, 31: 326-331.

[28] Halatsi, K., Oikonomou, I., Lambiri, M., Mandilara, G., Vatopoulos, A., & Kyriacou, A. (2006). PCR detection of Salmonella spp. using primers targeting the quorum sensing gene sdiA. *FEMS microbiology letters*, *259*(2), 201-207.

[29] Townsend, K. M., Frost, A. J., Lee, C. W., Papadimitriou, J. M., & Dawkins, H. J. (1998). Development of PCR assays for species-and type-specific identification of Pasteurella multocidaisolates. *Journal of clinical microbiology*, *36*(4), 1096-1100.

[30] Khokon, M. S. I, Azizunnesa, Islam M. M, Chowdhury, K. B., Rahman, M. L. & Ali, M. Z. (2017). Effect of mastitis on post-partum conception of cross bred dairy cows in Chittagong district of Bangladesh. *Journal of Advanced Veterinary and Animal Research, 4*(2): 155-160

[31] Ali MZ, Sultana S (2012). Isolation and identification of bacteria from tracheas and lungs of buffaloes in Dinajpur. Stamford Journal of Microbiology, 2(1):31-33.

[32] Ali MZ, Sultana S, Rahman MT, Islam MS (2013). Economics of Fertility Management of Small Holding Dairy Farms in Bangladesh. Iranian Journal of Applied Animal Science, 3(3): 509-512.

[33] Ali, M. Z., & Sultana, S. (2013). Isolation and identification of bacteria from tracheas and lungs of buffaloes in Dinajpur. *Stamford Journal of Microbiology*, *2*(1), 31-33.