Research in
AGRICULTURE, LIVESTOCK and FISHERIES
ISSN : P-2409-0603, E-2409-9325
An Open Access Peer-Reviewed International Journal

Article Code:463/2024/RALF
Article Type: Research Article

Res. Agric. Livest. Fish.
Vol. 11, No. 3, December 2024: 343-352.

Isolation and Identification of Causal Agents Associated with Duck Mortality in Haor Areas of Bangladesh

Sourav Chakraborty†, Sakib Anzum Pranto, Md. Muket Mahmud, Sharmin Sultana Misty, Md. Mamun-Or-Rashid, Md. Hadiuzzaman, Mohammad Asir Uddin, Mohammad Ferdousur Rahman Khan, Md. Tanvir Rahman, Md. Alimul Islam, and Muhammad Tofazzal Hossain†*

Department of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

†These authors contributed equally to this work.

***Corresponding author:** Muhammad Tofazzal Hossain; E-mail: tofazzalmh@bau.edu.bd

ARTICLE INFO

Received
07 December, 2024

Revised
28 December, 2024

Accepted
30 December, 2024

Key words:
Ducks
Mortality
Causal agents
Haor
Bangladesh

ABSTRACT

The duck population is concentrated mostly in the north and northeast wetland areas in Bangladesh. They are reared in close contact with domestic fowls and can be a potential source of many bacterial and viral diseases as sudden death of ducks is frequently evident. This study aimed to identify the causal agents behind the mortality of ducks in the haor areas of Bangladesh. In total, 66 dead ducks were collected from several locations of different haors in Mymensingh, Kishoreganj, and Netrokona districts. Internal organs of ducklings (n=21) and adult ducks (n=45) were collected as samples. Part of each sample was enriched in nutrient broth for the growth of bacteria followed by culture in specific media, staining, and biochemical tests were conducted for the primary confirmation of the bacterial isolates. Additionally, inoculum was prepared to propagate viruses in duck embryos. Specific primers were used for the detection of bacterial and viral isolates by PCR. Overall, *Escherichia coli* (75.75%), *Salmonella* spp. (12.12%), *Staphylococcus* spp. (7.57%), *Pasteurella* spp. (12.12%) and duck plague virus (13.64%) were detected by PCR. All the bacterial and viral isolates were found to be the cause to different duck diseases and major agents for duck mortality in the haor areas. However, they can also be a potential source of pathogen transmission to other domestic fowls.

To cite this article: Chakraborty S., S. A. Pranto, M. M. Mahmud, S. S. Misty, M. M. O. Rashid, M. Hadiuzzaman, M. A. Uddin, M. F. R. Khan, M. T. Rahman, M. A. Islam and M. T. Hossain, 2024. Isolation and identification of causal agents associated with duck mortality in Haor areas of Bangladesh. Res. Agric. Livest. Fish. 11(3): 343-352.

DOI: https://doi.org/10.3329/ralf.v11i3.78919

INTRODUCTION

Duck farming is becoming popular nowadays among the haor people of Bangladesh. The reason behind this is the low cost of rearing compared to the high-profit margin. The duck population in Bangladesh exceeded 66 million in number mostly concentrated in the inland wetlands (BBS, 2023). The availability of water resources and the abundance of food have influenced the peoples to rear different species of ducks for egg and meat production. Ducks are considered resistant birds compared to chickens because the morbidity and mortality rate in ducks due to disease is minimal (Etriwati *et al.*, 2023; Kuchipudi *et al.*, 2014, Hossain *et al*., 2005). Yet, there are some instances where farmers reported unusually high mortality in ducks but, like many other developing countries, Bangladesh's animal health information system is poorly designed, and that is why those reports are not available in the scientific fields.

The factors associated with loss of potential productivity and increased mortality due to economically important diseases of ducks remain mostly unidentified (Hoque *et al.*, 2011). Predominantly, some bacterial and viral diseases may cause havoc to the duck population. Among the diseases, duck cholera, duck septicemia, colibacillosis, duck viral hepatitis, and duck plague are mainly associated with high morbidity and mortality in ducks worldwide including Bangladesh (Hasan *et al.*, 2022; Khan *et al.*, 2021; Kamruzzaman *et al.*, 2016; Islam *et al.*, 2004). Among the bacterial diseases, duck cholera, caused by *Pasteurella multocida* is an important disease of domestic ducks with mortality varying from 5-100%, duck septicemia is generally caused by *Riemerella anatipestifer* previously known as *Pasteurella anatipestifer,* or *Moraxella anatipestifer* with the mortality rate of 2-75% in ducklings, *Escherichia coli* causes septicemia in ducks at 2-8 weeks of age along with salpingitis and peritonitis in breeder ducks. On the other hand, duck plague (DP) is caused by the duck enteritis virus (DEV) of the family Herpesviridae in all age groups of ducks with mortality of 5-100%, and duck viral hepatitis caused by the duck hepatitis virus of the family Picornaviridae, which is a highly fatal contagious disease of young ducklings with mortality 0-95% in ducklings (Chou and Calnek, 1997). For this reason, the present study was targeted to isolate and identify different causal agents of duck diseases responsible for duck mortality in Bangladesh.

Most of the diseases frequently occur every year in Bangladesh in epidemic form and spread rapidly among the duck-raising areas causing loss of production with high mortality of ducks, which ultimately affect the socio-economy of Bangladesh. Due to the shortage of required vaccines and proper diagnosis, many duck farmers face serious problems with these diseases with their ducks every year. Sometimes, regular vaccination fails to protect the ducks. This might happen due to an inadequate relationship between the vaccine strain and the prevailing strain or some other reasons in field conditions (Rana *et al.*, 2010; Hossain *et al.*, 2005 and 2004). The present research work aimed to isolate and identify the causal agents of dusk diseases responsible for duck mortality in recent outbreak areas.

Materials and Methods

Sample collection

A total of 66 suspected dead birds comprised of 21 ducklings and 45 adult ducks were collected from Mymensingh, Netrokona, and Kishoreganj districts **(Figure 1 and 2)** for the isolation and detection of the causal agents of duck diseases. Liver, heart, lungs, intestine with content, kidneys, lymph nodes, and spleen samples were collected from the dead ducklings and ducks. All the samples were aseptically collected and transported to the Microbiology laboratory, Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh, Bangladesh.

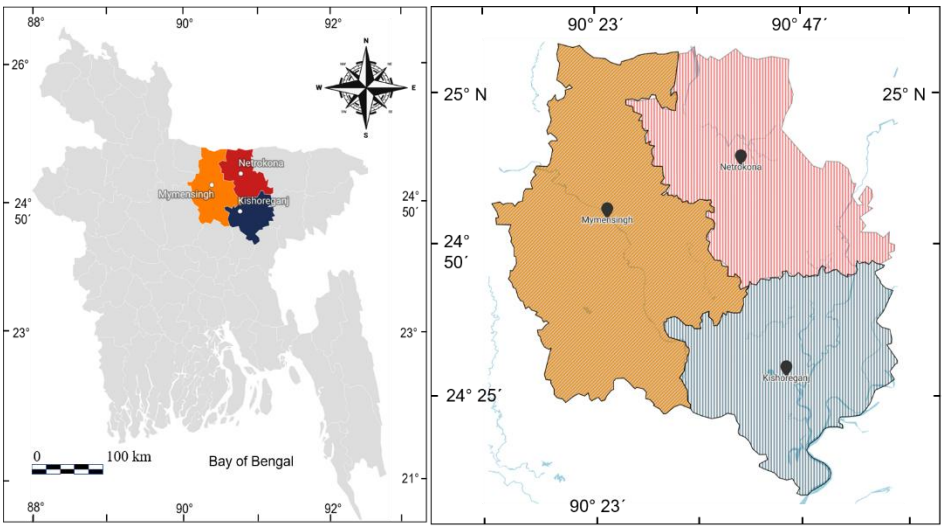

Figure 1. Sample collection areas for isolation of causal agents of duck diseases

Figure 2. Dead ducklings and adult ducks for **s**ample collection.

Isolation and identification of different bacteria

The samples were inoculated in nutrient broth and incubated overnight at 37^{0}C for the growth of the organisms. After the primary growth, a small amount of inoculum was streaked on the blood agar, eosin methylene blue (EMB), mannitol salt (MS), and Salmonella-Shigella (SS) agar media to observe the colony morphology after 24 hours of overnight incubation at 37°C. Subsequent sub-cultures were performed on same agar media to obtain pure isolates. Gram staining and different biochemical tests (Carbohydrate fermentation test, catalase test, MR-VP test, indole test) were performed for initial screening of each isolate according to the methods of Chessbrough (2006).

Isolation of duck enteritis virus using duck embryos

Viral inoculum was prepared from the liver sample following the methods of Akter *et al.* (2004) and Khan *et al.* (2021). Briefly, sterile viral inoculum (0.2 ml) was propagated in 12-day-old embryonated duck eggs through the allantoic cavity or chorio-allantoic membrane (CAM) route. The eggs were incubated at 37°C and candled twice daily up to 5-8 days post inoculation to check the embryo mortality. The embryos that died

during the incubation period were chilled at refrigerated temperature for 2 to 4 hours. The lesions on the embryos and CAM were examined and recorded. Finally, the allantoic fluid and CAM were collected and preserved at -20°C until use.

Molecular detection of bacterial and viral isolates

All the culture-positive bacterial and viral isolates were subjected to molecular detection. The chromosomal DNA of the bacterial isolates was extracted by simple boiling method according to the methods of Hossain *et al.* (2012) and the DNA of the duck plague virus was extracted using the Wizard® Genomic DNA Purification Kit (Promega, Madison, Wisconsin, United States) as per the manufacturer's protocol. Primers of *malB, invA, nuc, kmt*, and DNA polymerase genes were used for the detection of *E. coli*, *Salmonella* spp., *Staphylococcus* spp., *Pasteurella* spp., and duck plague virus (DPV), respectively (**Table 1**) with corresponding thermal profiles for each of the suspected bacterial and viral isolate. A 20 µl reaction mixture was prepared by mixing master mixture (10 µl) (Promega-Madison, WI, USA), nuclease-free water (5 µl), forward (1 µl) and reverse (1 µl) primers, and template DNA (3 µl). The PCR products were analyzed by gel electrophoresis in a 1.5% agarose gel, stained in ethidium bromide solution (approximate concentration 0.2–0.5 µg/ml) for 10 min and finally visualized using a UV transilluminator.

Table 1. Primers with corresponding thermal profiles used in the study

Name of the Bacteria	Targeted gene	Primer name	Sequence (5′-3′)	Thermal profile	Amplicon size (bp)	References
E. coli	*malB*	ECO-F	GACCTCGGTTTAGTTCACAGA	95°C for 5 min, followed by 30 cycles of 94°C for 45 sec, 52°C for 45 sec, 72°C for 1 min; final extension at 72°C for 5 min	585	Hasan *et al.* (2014)
		ECO-R	CACACGCTGACGCTGACCA			
Salmonella spp.	*invA*	INVA-F	CGGTGGTTTTAAGCGTACTCTT	94°C for 2 min, followed by 35 cycles of 94°C for 20 s, 57°C for 1 min, and 72°C for 1 min; final extension of 72°C for 10 min	796	Fratamico *et al.* (1998)
		INVA-R	CGAATATGCTCCACAAGGTTA			
Staphylococcus spp.	*nuc*	SARS-F	GCGATTGATGGTGATACGGTT	94°C for 4 min, followed by 35 cycles of 94°C for 30 sec, 57.5°C for 30 sec, 72°C for 40 sec; final extension at 72°C for 10 min	279	Brakstad *et al.* (1992)
		SARS-R	AGCCAAGCCTTGACGAACTAAAGC			
Pasteurella spp.	*kmt*	KMT1T7	ATCCGCTATTTACCCAGTGG	94°C for 3 min, followed by 35 cycles of 94°C for 45 sec, 50°C for 45 sec, 72°C for 1 min; final extension at 72°C for 10 min	460	Townsend *et al.* (1998)
		KMT1SP6	GCTGTAAACGAACTCGCCAC			
Duck enteritis virus	DNA polymerase	UL30-F	GAAGGCGGGTATGTAATGTA	94°C for 2 min, followed by 35 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 2 min; final extension at 72°C for 7 min	446	Khan *et al.* (2018)
		UL30-R	CAAGGCTCTATTCGGTAATG			

Results and Discussion

The reporting of prevailing duck diseases of haor origin in Bangladesh lacks proper notice. This research gap is responsible for the lack of infrastructure to control high-mortality diseases that occur in ducks. The study aimed to isolate and identify the causal agents that may be responsible for the sudden deaths of the ducks. A total of 66 dead ducks, comprising 21 ducklings and 45 adult ducks were collected from the sampling areas to determine the causal agents responsible for the death of these ducks.

Table 2. Results of biochemical tests of the isolated bacteria

Name of the tests	*E. coli*	*Salmonella* spp.	*Staphylococcus* spp.	*Pasteurella* spp.
Carbohydrate fermentation test				
Glucose	+	+	+	+
Lactose	+	-	+	-
Maltose	+	+	+	-
Mannitol	+	+	+	+
Sucrose	+	-	+	+
Indole	+	-	-	+
MR	+	+	+	-
VP	-	-	+	-
Catalase	+	+	+	+
Coagulase	-	-	+	-

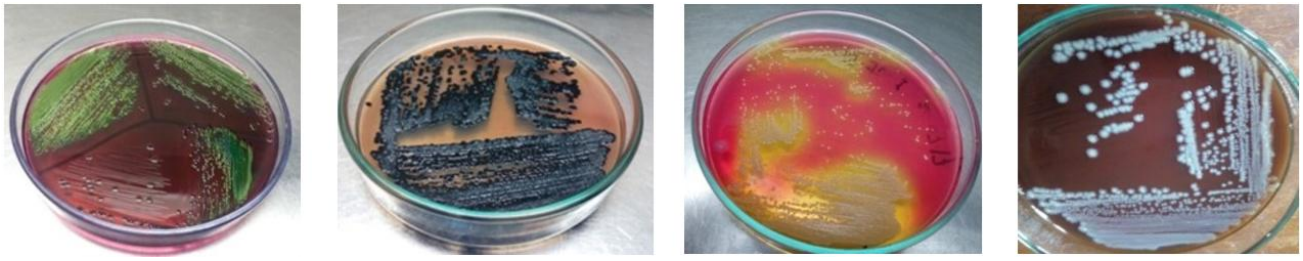

Figure 3. Cultural characteristics observed for *E. coli* (EMB agar media), *Salmonella* spp. (SS agar media), *Staphylococcus* spp. (MS agar media), and *Pasteurella* spp. (Blood agar media)

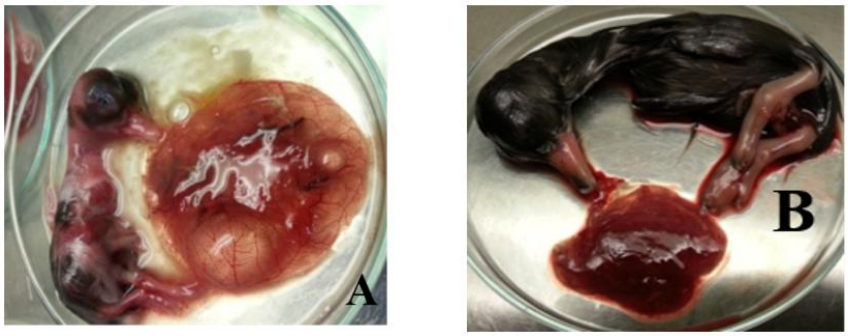

Figure 4. Embryo infected with duck plague virus. (A) Dead hemorrhagic embryo with hemorrhagic voluminous CAM, (B) Duck embryo and CAM (Control)

A variety of bacterial growth (*E. coli*, *Salmonella* spp., *Staphylococcus* spp., and *Pasteurella* spp.) was observed in the specific media used in this study with distinct colony characteristics. *E. coli* produced a green metallic sheen on EMB agar media, *Salmonella* spp. showed black-centered colonies on SS agar media due to H_2S production, *Staphylococcus* spp. developed golden yellow color colonies on MSA media, and lastly, *Pasteurella* spp. produced shiny, opaque, grayish-tinged, non-hemolytic colonies on blood agar media (**Figure 3**). In Gram's staining, *E. coli*, *Salmonella* spp., and *Pasteurella* spp. were observed as Gram-negative rod-shaped bacteria with single or pair in arrangement, whereas *Staphylococcus* spp. was observed as Gram-positive cocci in cluster arrangement. Biochemical tests of the culture-positive isolates correlate with the established findings and are mentioned in **Table 2**. All the cultural and biochemical properties observed in

this study are similar to those observed previously (Chessbrough, 2006). A total of 50 *E. coli* isolates (75.75%), 8 *Salmonella* spp. isolates (12.12%), 5 *Staphylococcus* spp. isolates (7.57%), and 8 *Pasteurella* spp. isolates (12.12%), were confirmed through molecular detection using specific primer sets and the positive band appeared at 585 bp, 796 bp, 279 bp and 460 bp, respectively (**Figure 5-8**).

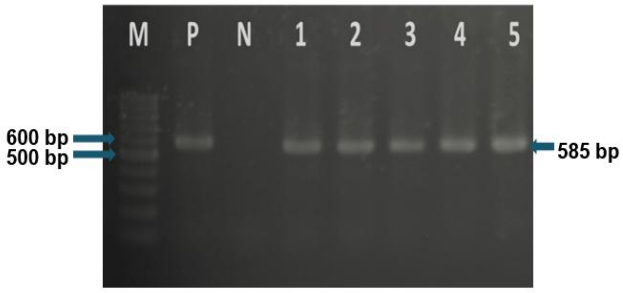

Figure 5. *E. coli* isolates showing positive bands at 585 bp amplifying *malB* gene. Lane M: 100 bp ladder; Lane P: Positive control; Lane N: Negative control; Lane 1-5: *E. coli* isolates of ducks

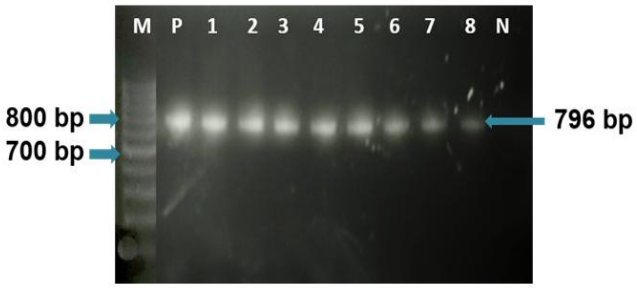

Figure 6. *Salmonella* isolates showing positive bands at 796 bp amplifying *invA* gene. Lane M: 100 bp marker; Lane P: Positive control; Lane N: Negative control; Lane 1-8: *Salmonella* isolates of ducks

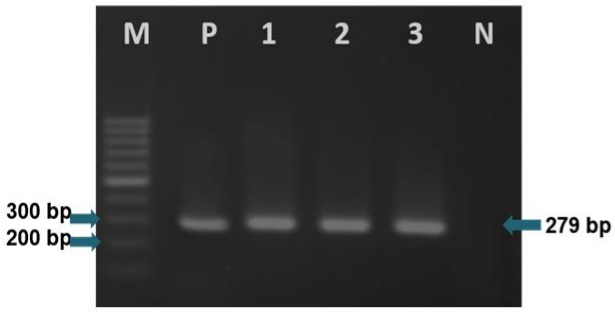

Figure 7. *Staphylococcus* isolates showing positive bands at 279 bp amplifying *nuc* gene. Lane M: 100 bp marker; Lane P: Positive control; Lane N: Negative control; Lane 1-3: *Staphylococcus* isolates of ducks.

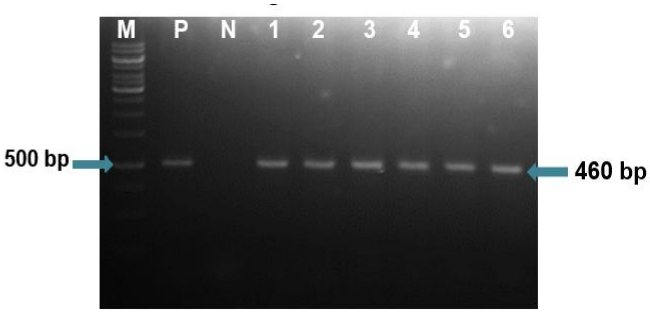

Figure 8. *Pasteurella* isolates showing positive bands at 460 bp amplifying *kmt* gene. Lane M: 100 bp marker; Lane P: Positive control; Lane N: Negative control; Lane 1-6: *Pasteurella* isolates of ducks

According to age, more isolates were found in the adult birds, however, the variations were minimal (**Table 3**). The 80%, 13.13%, 8.88%, and 17.77% of the adult ducks were found positive for *E. coli*, *Salmonella* spp., *Staphylococcus* spp., and *Pasteurella* spp., respectively. On the contrary, the isolation rates of *E. coli*, *Salmonella* spp., and *Staphylococcus* spp. were less in the young as 66.67%, 9.52% and 4.76%, respectively. No *Pasteurella* spp. was isolated from the ducklings in this study (**Table 3**). The isolation rate of *E. coli* is similar to previous studies (Singh *et al.,* 2012; Sarker *et al.,* 2023; Adzitey *et al.,* 2012). However, Majumder *et al.* (2017) accounted for a lower isolation rate of *E. coli* from ducks. Previous studies on isolating *Salmonella* spp. from ducks observed prevalence rates (>30%) three times more than the current research (Mondal *et al.,* 2008; Rahman *et al.,* 2016). Kamruzzaman *et al.* (2016) reported a much higher isolation rate of *Pasteurella* spp. in ducks than in this study. Adult ducks are more susceptible to the *Pasteurella* spp. (Saritha *et al.,* 2024), which is similar to what has been found in this study.

Table 3. Isolation rate of different disease-causing agents in ducks

Origin of samples	Number of samples	Number of isolated pathogens causing disease in ducks				
		E. coli	***Salmonella***	***Staphylococcus***	***Pasteurella***	**Duck enteritis virus**
Duckling	21	14 (66.67%)	2 (9.52%)	1(4.76%)	0 (0%)	3 (14.28%)
Adult ducks	45	36 (80%)	6 (13.33%)	4 (8.88%)	8 (17.77%)	6 (13.33%)
Total	66	50 (75.75%)	8 (12.12%)	5 (7.57%)	8 (12.12%)	9 (13.64%)

Very little information is available about isolating *Staphylococcus* spp. from ducks in Bangladesh. Nevertheless, Wang *et al.* (2024) reported that 70.14% coagulase-negative Staphylococci are the leading causes of bacterial meningitis in ducks. Eid *et al.* (2019) isolated *Staphylococcus* (8.8%-12.2%) from diseased ducks which is similar to the current findings. In earlier research, a higher isolation rate of bacteria was observed in ducklings (Zhao *et al.,* 2019; Hussein *et al.,* 2024). However, in this study, the result contradicts to what has been established. More studies should be conducted in the haor region of Bangladesh to obtain further knowledge on the reasons behind such reports.

Generalized hemorrhages on the whole body with hemorrhagic voluminous CAM was observed in DEV inoculated embryos compared with control (**Figure 4**). These findings were similar with the findings described by Islam *et al.* (2024), Akter *et al.* (2004) and Ahamed *et al.* (2015). Nine DEV isolates were confirmed by PCR, and positive bands appeared at 446 bp (**Figure 9**) with an isolation rate of 13.64% of which 13.13% from adult ducks and 14.28% from ducklings. After molecular detection, 13.64% of isolates were confirmed as DEV in this study. Khan *et al.* (2018) reported a similar isolation rate, whereas, Islam *et al.* (2024) documented a much higher prevalence rate. 3.68% of adult ducks and 2.78% of ducklings were found to contain DPV according to Khan *et al.* (2018). According to the present study, the rates was higher in adult ducks compared to ducklings. The variation among studies may be due to the individual immunological state, age, sex, and other environmental factors.

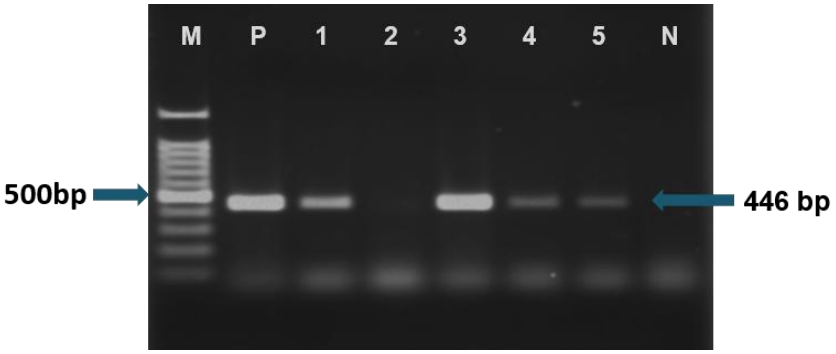

Figure 9. Duck plague virus isolates showing positive bands at 446 bp amplifying the DNA polymerase gene. Lane M: 100 bp marker; Lane P: Positive control; Lane N: Negative control; Lane 1-5: Duck plague virus isolates of ducks

Conclusions

Both bacterial and viral pathogens pose a significant threat to duck health as well as the risk of transmitting these diseases to domestic fowls has economic significance. Multi-dimensional research targeting control of duck diseases in haor areas of Bangladesh should be conducted to save the farmers from economic losses due to mortality.

Competing interest

The authors have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors appreciate the support of the Bangladesh Agricultural University Research System (BAURES), BAU, Mymensingh for project management, monitoring, and evaluation of this research.

References

1. Adzitey F, CY Liew, AP Aronal and N Huda, 2012. Isolation of *Escherichia coli* from ducks and duck related samples. Asian Journal of Animal and Veterinary Advances, 7(4): 351–355.
2. Ahamed MM, MT Hossain, M Rahman, KHMNH Nazir, MFR Khan, MS Parvej, WK Ansari, MNNAA Chiste, KB Amin, ML Hossen, S Ahmed and MB Rahman, 2015. Molecular characterization of duck plague virus isolated from Bangladesh. Journal of Advanced Veterinary and Animal Research, 2(3): 296–303.
3. Akter S, MA Islam, MT Hossain, MM Amin, MIA Begum and M Sadekuzzaman, 2004. Characterization and pathogenicity of duck plague virus isolated from natural outbreaks in ducks of Bangladesh. Bangladesh Journal of Veterinary Medicine, 2(2): 107–111.
4. Brakstad OG, K Aasbakk and JA Maeland, 1992. Detection of Staphylococcus aureus by polymerase chain reaction amplification of the nuc gene. Journal of clinical microbiology, 30(7): 1654–1660.
5. BBS (Bangladesh Bureau of Statistics), 2022-23. Planning Section, Department of Livestock Services (DLS), Dhaka, Bangladesh.
6. Chou KC and Calnek BW, 1997. Diseases of poultry, Iowa state university press. 10th Edition, Ames, Iowa, 131–140.
7. Chessbrough M, 2006. District Laboratory Practices in Tropical Countries, Cambridge University Press, London, 1–266.
8. Dey RK, MSR Khan, KHMNH Nazir, MA Islam and SMSH Belal, 2016. Epidemiological investigation on duck salmonellosis in some selected areas of Bangladesh. Bangladesh Journal of Veterinary Medicine, 14(2): 149–160.
9. Eid HM, AM Algammal, WK Elfeil, FM Youssef, SM Harb and EM Abd-Allah, 2019. Prevalence, molecular typing, and antimicrobial resistance of bacterial pathogens isolated from ducks. Veterinary World, 12(5): 677–683.
10. Elmeslemany RI and AE Yonis, 2023. Pathogenicity of bacterial isolates associated with high mortality in duckling in Behira province. Egyptian Journal of Animal Health, 3(3): 41–53.

11. Etriwati E, D Agungpriyono, S Setiyaningsih, D Darniati, D Ak, E Erwin and E Handharyani, 2023. Comparative pathology and immunohistochemistry of Newcastle disease in domestic chicken (*Gallus-gallus domesticus*) and Alabio duck (*Anas platyrhynchos Borneo*). Open Veterinary Journal, 13(4): 433–442.
12. Fratamico PM and TP Strobaugh, 1998. Simultaneous detection of *Salmonella* spp and *Escherichia coli* 0157:H7 by multiplex PCR. Journal of Industrial Microbiology and Biotechnology, 21(3): 92–98.
13. Hasan B, K Islam, M Ahsan, Z Hossain, M Rashid, B Talukder, KU Ahmed, B Olsen and MA Kashem, 2014. Fecal carriage of multi-drug resistant and extended spectrum β-lactamases producing *E. coli* in household pigeons. Bangladesh. Veterinary Microbiology, 168(1): 221–224.
14. Hasan A, P Bose, MT Aktar, ZF Haque, MR Islam, MT Hossain and MP Siddique, 2022. *groEL* gene-based molecular detection and antibiogram profile of *Riemerella anatipestifer* from duck in Bangladesh. Journal of Advanced Veterinary and Animal Research, 9(4): 684–693.
15. Hoque M, LF Skerratt, MS Rahman, ABMRA Beg and NC Debnath, 2010. Factors limiting traditional household duck production in Bangladesh. Tropical Animal Health and Production, 42(7): 1579–1587.
16. Hossain MT, EY Kim, YR Kim, DG Kim and IS Kong, 2012: Application of *groEL* gene for the species-specific detection of *Vibrio parahaemolyticus* by PCR. Letters in Applied Microbiology, 54(1): 67–72.
17. Hossain MT, MA Islam, MM Amin and MA Islam, 2005. Comparative efficacy of the conventional and experimentally developed duck plague vaccine. International Journal of Poultry Science, 4(6): 369–371.
18. Hossain MT, MA Islam, S Akter, M Sadekuzzaman, MA Islam and MM Amin, 2004. Effect of dose and time of vaccination on immune response of duck plague vaccine in ducks. Bangladesh Journal of Veterinary Medicine, 2(2): 117–119.
19. Hu J, L Chen, G Li, Y Pan, Y Lu, J Chen, W Xiong and Z Zeng, 2023. Prevalence and genetic characteristics of fosB-positive Staphylococcus aureus in duck farms in Guangdong, China in 2020. Journal of Antimicrobial Chemotherapy, 78(3): 802–809.
20. Hussein A, AAM Eid, M Hassaan, E Mohamed, I Elsohaby and M Shawky, 2024. Isolation and identification of multidrug resistance bacterial agents implicated in duck enteritis with first record of *Salmonella enterica* subspecies arizonae in Egypt. Open Veterinary Journal, 14(1): 553–563.
21. Islam J, MM Islam, MS Islam, P Bose, MJ Islam, MR Islam, MM Khatun and MA Islam, 2024. Isolation and characterization of duck viral enteritis (Duck plague) virus from ducks in field outbreaks in Bangladesh. Journal of Research in Veterinary Sciences, 2(1): 29–37.
22. Islam MT, MA Islam, MA Samad and SML Kabir, 2004. Characterization and antibiogram of *Escherichia coli* associated with mortality in broilers and ducklings in Bangladesh. Bangladesh Journal of Veterinary Medicine, 2(1): 9–14.
23. Kamruzzaman M, M Islam, MM Hossain, MK Hassan, MHB Kabir, MS Sabrin and MSR Khan, 2016. Isolation, Characterization and Antibiogram Study of *Pasteurella multocida* Isolated from Ducks of Kishoreganj District, Bangladesh. International Journal of Animal Resources, (1): 69–70.
24. Khan KA, S Saha, MT Hossain, ME Haque, MM Haq and MA Islam, 2018. Epidemiological investigation of recurrent outbreaks of duck plague in selected Haor (wetland) areas of Bangladesh. Journal of Advanced Veterinary and Animal Research, 5(2): 131–139.
25. Khan KA, MA Islam, AAM Sabuj, MA Bashar, MS Islam, MT Hossain and S Saha, 2021. Molecular characterization of duck plague virus from selected Haor areas of Bangladesh. Open Veterinary Journal, 11(1): 42–51.

26. Khan MT, MTR Pavel, A Keya, MAH Shahid, T Ferdausi, Siddique MP, MT Hossain, KHMNH Nazir, MT Rahman and MB Rahman, 2021. Comparative molecular analysis of contemporary isolates of duck plague virus from haor areas of Bangladesh. Journal of Advanced Biotechnology and Experimental Therapeutics, 4(1): 44–52.
27. Kuchipudi S V, M Tellabati, S Sebastian, BZ Londt, C Jansen, L Vervelde, SM Brookes, IH Brown, SP Dunham and KC Cheng, 2014. Highly pathogenic avian influenza virus infection in chickens but not ducks are associated with elevated host immune and pro-inflammatory responses. Veterinary Research, 45(1): 118–128.
28. Majumder S, MM Akter, MM Islam, K Hussain, S Das, I Hasan, KHMNH Nazir and M Rahman, 2017. Prevalence, isolation and detection of virulent gene in *Escherichia coli* from duck. British Journal of Medicine and Medical Research, 20(2): 1–8.
29. Mondal T, MSR Khan, M Alam, M Purakayastha, M Das and MP Siddique, 2008. Isolation, identification and characterization of Salmonella from duck. Bangladesh Journal of Veterinary Medicine, 6(1): 7–12.
30. Rana M, MT Hossain, MA Islam, MM Rahman, MK Alam and UK Dutta, 2010. Comparative immunogenicity study in ducks of different breeds available at coastal regions of Bangladesh against duck plague and duck cholera vaccines. International Journal of BioResearch, 2(8): 23–27.
31. Rahman MM, MM Rahman, MM Meher, MSI Khan and AKMM Anower, 2016. Isolation and antibiogram of *Salmonella* spp. from duck and pigeon in Dinajpur. Bangladesh. Journal of Advanced Veterinary and Animal Research, 3(4): 386–391.
32. Sannegowda SN, SB Raju, K Govindaiah, AR Gomes, C Yadav, YS Laxmanappa and SKR Reddy, 2024. Isolation and molecular characterization of *Pasteurella multocida* from ducks. Indian Journal of Veterinary Sciences and Biotechnology, 20(5): 45–50.
33. Sarker MS, ME Kabir, FA Mahua, A Ahmed, RK Ripon, V Silva, P Poeta and A Ahad, 2023. Antibiotic-resistant *Escherichia coli* isolated from duck cloacal and tap water samples at live bird markets in Bangladesh. Poultry Science Journal, 11(1): 19–27.
34. Singh A, MSR Khan, S Saha, J Hassan and U Roy, 2013. Isolation and detection of antibiotic sensitivity pattern of *Escherichia coli* from ducks in Bangladesh and Nepal. Microbes and Health, 1(1): 6–8.
35. Townsend KM, AJ Frost, CW Lee, JM Papadimitriou and HJ Dawkins, 1998. Development of PCR assays for species- and type-specific identification of *Pasteurella multocida* isolates. Journal of Clinical Microbiology, 36(4): 1096–1100.
36. Wang J, Y Meng, R Zhang, H Yan, G Xu, Y Zhu, Z Xie and S Jiang, 2024. Coagulase-negative staphylococci are the main causes of bacterial meningitis in duck. Poultry Science, 103(5): 103592.
37. Zhao Y, K Li, H Luo, L Duan, C Wei, M Wang, J Jin. S Liu, K Mehmood and M Shahzad, 2019. Comparison of the intestinal microbial community in ducks reared differently through high-throughput sequencing. BioMed Research International, 9015054.