An Official Journal of Bangladesh Livestock Research Institute (BLRI)

Bangladesh Journal of Livestock Research

Journal Homepage: https://www.banglajol.info/index.php/BJLR

ISSN 1022-3851

Short communication

Investigation of duck plague virus in hoar areas of Bangladesh

A. Hossen[1], M.H. Rahman[2], M.Z. Ali[2], M.A. Yousuf[2], M.Z. Hassan[2] and M. Giasuddin[2*]

[1]Bangladesh Livestock Research Institute Regional Station, Naikhongcahri, Bandarban, Bangladesh.
[2]Animal Health Research Division, Bangladesh Livestock Research Institute, Savar, Dhaka 1341, Bangladesh.

Abstract

Duck plague (DP) is the most important infectious disease of geese, ducks and free-ranging water birds. The present study was conducted to determine the prevalence of duck plague virus followed by isolation and identification. For these purposes, a total of 155 cloacal swabs samples were collected randomly from duck of different haor areas of Bangladesh including 45 (41 surveillance and 4 clinical) samples from Netrokona; 42 (40 surveillance and 2 clinical) samples from Kishoregonj; 30 samples from Brahmanbaria and 38 samples from Sunamganj. The samples were processed and pooled (1:5 ratio) for initial screening of target *polymerase gene* of duck plague virus by polymerase chain reaction (PCR) method. All the samples of a positive pool were then tested individually for identifying the individual positive samples. The result showed that out of 155 samples, 41 (26.45%) were found positive in which 17 were from Netrokona, where 15 (36.58%) were from surveillance samples and 2 (50%) were from clinical sample; 16 were from Kishoregonj, where 14 (35%) were from surveillance samples and 2 (100%) were from clinical sample; 2 (6.6%) were from Brahmanbaria and 5 (13.15%) were from Sunamganj. These positive samples were inoculated into 9-10 days embryonated duck eggs (EDE) through chorioallantoic membrane (CAM) route for the isolation of virus. The EDE died earlier was also chilled, and in a similar way, the CAMs were collected and again performed PCR for id entification of virus. Out of 41 PCR positive samples, 26 samples were isolated and reconfirmed by PCR. Subsequently, DPV was isolated in primary duck embryo fibroblasts cell culture and confirmed by observing cytopathic effect (CPE).

(Key words: Duck Plague Virus, Isolation, PCR, Cell culture)

Bang. J. Livs. Res. Vol. 26 (1&2), 2019: P. 73-78 https://doi.org/10.3329/bjlr.v26i1-2.49939

Introduction

Duck plague (DP) is an acute, sometimes chronic, contagious viral disease that occurs naturally only in ducks, geese and swans, all members of the family *Anatidae* of the order *Anseriformes*. Duck plague is caused by duck herpesvirus 1 (anatid herpesvirus 1) is a member of the *Alphaherpesvirinae* sub-family

*Corresponding author: mgias04@yahoo.com

of the family *Herpesviridae* (OIE, 2018). In domestic ducks and ducklings, DP has been reported in birds ranging from 7 days of age to mature breeders. In domestic ducks the incubation period ranges from 3–7 days. Mortality usually occurs 1-5 days after the onset of clinical signs and is often more severe in susceptible adult breeder ducks. Most infected ducks that show symptoms eventually die; morbidity and mortality may reach 90-100 percent (Campagnolo *et al.*, 2001, Carter *et al.,* 2006). Recovered birds may be latently infected carriers and may shed the virus in the faeces or on the surface of eggs over a period of years (Richter and Horzinek, 1993; Shawky and Schat, 2002). So, it`s a great concern for the duck industry in the world including Bangladesh. In Bangladesh duck population is about 57.752 million (DLS, 2019; Ali, 2018). Major portion of the ducks are reared in semi intensive methods in the Haor areas of Bangladesh. Lands in these areas remain under water for 4-5 months of the year. These enormous water bodies in the areas are very convenient for duck rearing. Poor and ultra-poor people depend on duck as a sole source of their livelihoods (Bary *et al.*, 2018; Bhuiyan *et al.*, 2019; Ali and Hasan, 2018). In addition to these, ducks are the major source of duck meats and eggs for other areas of the country. However, there are several constraints of which infectious diseases are considered as the most leading causes of economic loss and discouraging duck rearing in this country (Das *et al.*, 2005; Ali *et al.*, 2019). The objectives of the study were to determine prevalence, isolation and molecular identification of Duck plague virus in selected areas of Bangladesh.

Materials and Methods

Study areas

The four Haor areas including Mohanganj upazilla of Netrokona; Itna upazilla of Kishoregonj, Nasirnagar upazilla of Brahmanbaria and Taherpur upazilla of Sunamganj districts in Bangladesh were selected on the basis of the highest density duck population.

Collection of samples and processing

A total of 155 samples were collected from duck species of different haor areas of Bangladesh including 45 (41 surveillance and 4 clinical) samples from Mohanganj, Netrokona; 42 (40 surveillance and 2 clinicals samples from Itna, Kishoregonj; 30 samples from Nasirnagar, Brahmanbaria and 38 samples from Taherpur, Sunamganj. After collection, the samples were stored locally in veterinary hospitals at -20°C and then transported to the Virology Laboratory of the Animal Health Research Division, BLRI, Savar, Dhaka and stored at -80°C until tested. The samples were processed according to the protocol described by OIE Terrestrial Manual 2012 (OIE, 2018) and surveillance samples were pooled (1:5 ratio) for initial PCR screening of DPV. Samples from positive pools were then tested individually for identifying the individual positive samples.

Detection of duck plague virus

DNA was extracted from the processed samples by using QIAamp DNA Blood Mini Kit (Qiagen, USA) according to the manufacturer instructions. Then the extracted DNA was subjected to PCR for detection of duck plague virus targeting the *polymerase gene* (446 bp) by using specific primers described by OIE (2018) (Table 1). The

master mix was prepared by GoTaq® G2 Green Master Mix kit (ThermoFisher, USA) according to the manufacturer instructions. The PCR product was analyzed in 1.5% agarose in TAE buffer gel containing 0.5 µ l/ml ethidium bromide and visualized by UV transilluminator.

Isolation and identification of duck plague virus

All individual positive field samples (either cloacal or tissue) by PCR test were treated with antibiotic (2000 iu/ml Penicillin and 200 mcg/ml Streptomycin) and tested for sterility in fresh blood agar media at 37°C for 24h. Sterile inoculums were then inoculated into chorioallantoic membrane (CAM) of 9-10 day-old duck embryonated eggs using standard techniques (OIE, 2018). Each of the inoculated embryos was monitored daily for 6 days. After 6 days of post-infection (PI), all live EDEs were chilled overnight and CAM were collected separately from embryos and again performed PCR test for identification and confirmation of virus. Subsequently, isolation of DPV in primary duck embryo fibroblasts cell culture was done by observing cytopathic effect (CPE).

Table 1. Primer sequences for the detection of duck plague virus from field samples

Primer name	Sequence	Product size	References
Forward	5′-GAA-GGC-GGG-TAT-GTA-ATG-TA-3′	446 bp	OIE, 2018
Reverse	5′-CAA-GGC-TCT-ATT-CGG- TAA-TG-3′		

Results and Discussion

The prevalence results showed that out of 155 samples, 41 (26.45%) were found positive in which 17 were from Mohongonj, Netrokona, where 15 (36.58%) were from surveillance samples and 16 were from Itna, Kishoregonj, where 14 (35%) were from surveillance samples and 2 (100%) were from clinical sample; 2 (6.6%) were from Nasirnagar, Brahmanbaria and 5 (13.15%) were from Taherpur, Sunamganj (Table 2).

The surveillance samples also showed considerable presence of duck plague virus in the studied areas, which is almost similar to the findings of Ahamed *et al.* (2015), where they showed the overall prevalence rate of DPV was 18.1% (17/94) in three districts (Rajshahi, Mymensingh and Netrokona) of Bangladesh. Haque *et al.* (2006) revealed that the most important causes of duck mortality were duck plague. Duck plague together with duck cholera in Chatkhil Upazila in Bangladesh where the prevalence of these disease were 25.3% for both.

The positive samples were then inoculated into 9-10 days embryonated duck eggs (EDEs) through chorioallantoic membrane (CAM) route for isolation of the virus. After 6 days of post-infection (PI), all live EDEs were chilled overnight and CAM were collected separately from the embryos. The EDE that died earlier was also chilled, and in a similar way, the CAMs were collected (OIE, 2018) and again the virus was identified through PCR (Figure 1).

Out of 41 PCR positive samples, 26 isolated samples were confirmed by PCR. The

Table 2. Prevalence of duck plague virus in selected Haor areas of Bangladesh

Source of sample	Sample type	Sample No.	PCR test result		Virus isolation
			Positive	% Prevalence	
Mohanganj, Netrokona	Cloacal	41	15	36.58	11
	Clinical	4	2	50	2
Itna, Kishoreganj	Cloacal	40	14	35	9
	Clinical	2	2	100	2
Nasirnagar, Brahmanbaria	Cloacal	30	2	6.6	1
Taherpur, Sunamganj	Cloacal	38	5	13.15	2
Total		155	41	26.45	26

present isolation results were also similar to the results found by Hansen *et al.* (2000), Wallace *et al.* (2000), Ahmed *et al.* (2015) and Campagnolo *et al.* (2001). Subsequently, isolation of DPV in primary duck embryo fibroblasts cell culture was done by observing cytopathic effect (Figure 2). CPE is characterized by the appearance of rounded clumped cells that enlarge and become necrotic 2– 4 days later. Wang *et al.* (2013) found that a distinct CPE of duck embryonic fibroblast cells inoculated with samples was observed, whereas cells in the negative control group grew normally.

Conclusion

This study indicates the high prevalence of duck plague virus among duck population in the Haor areas of Bangladesh. Therefore, further studies are warranted to investigate the epidemiology of DPV, in Bangladesh.

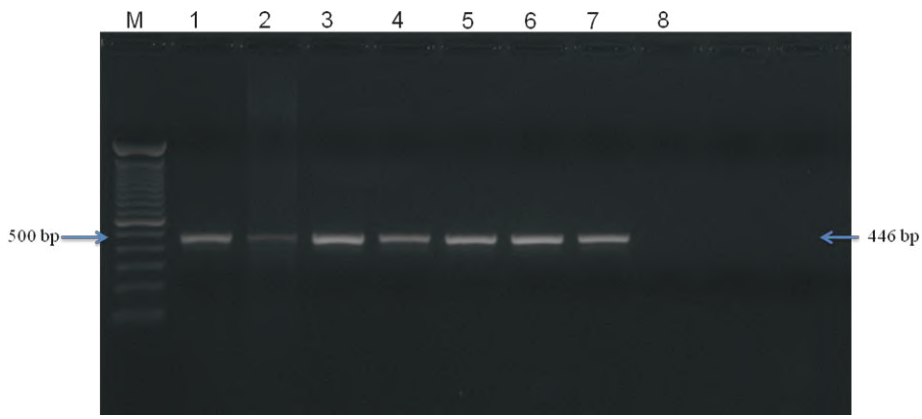

Figure 1. Image showing the PCR products of DPV with specific primer. Lane M:100-bp ladder; Lane 1-6 samples of DPV; Lane 7: positive control (Advanced vaccine strain, vaxiduck®) and Lane 8 negative control.

References

Ahamed, M.M., Hossain, M.T., Rahman, M., Nazir, K.H.M.N.H., Khan, M.F.R., Parvej, M.S., Ansari, W.K., Chiste, M.N.NA.A., Amin, K.B., Hossen, M.L., Ahmed, S. and Rahman,

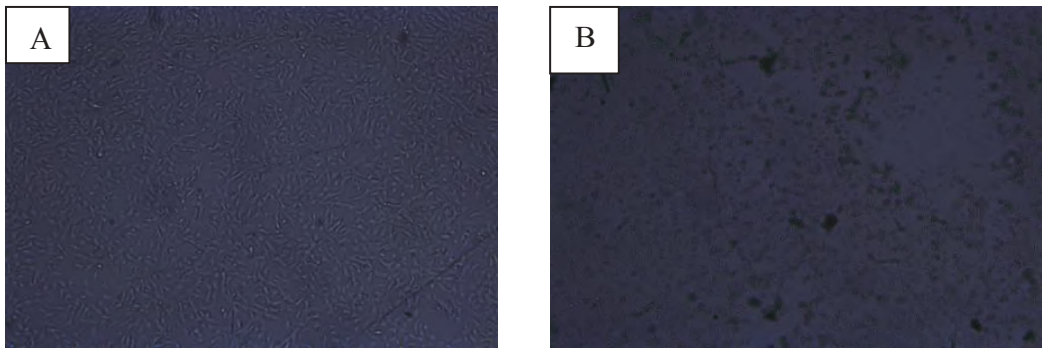

Figure 2. Culture of duck plague virus in primary duck embryo fibroblasts cell line. (A) normal cell, (B) Infected cell showing cytopathic effect.

M.B. 2015. Molecular characterization of Duck Plague virus isolated from Bangladesh. J. Adv. Vet. Anim. Res. 2(3): 296-303.

Ali, M.Z. 2018. The seroprevalence study of Reticuloendotheliosis virus infection in chicken in Bangladesh. Egyptian J. Vet. Sci. 49(2): 179-186.

Ali, M.Z. and Hasan, B. 2018. Follow up of maternally derived antibodies titer against economically important viral diseases of chicken. Poultry Sci. J. 6(2), pp.149-154.

Ali, M.Z., Park, J.E. and Shin, H.J. 2019. Serological survey of avian Metapneumovirus infection in chickens in Bangladesh. J. Appl. Poultry Res. 28(4): 1330-1334.

Bary, M.A., Ali, M.Z., Chowdhury, S., Mannan, A., Azam, M.N.E., Moula, M.M., Bhuiyan, Z.A., Shaon, M.T.W. and Hossain, M.A. 2018. Prevalence and molecular identification of haemoprotozoan diseases of cattle in Bangladesh. Adv. Anim. Vet. Sci. 6(4): 176-182.

Bhuiyan, Z.A., Ali, M.Z., Moula, M.M., Giasuddin, M. and Khan, Z.U.M. 2019. Prevalence and molecular characterization of infectious bronchitis virus isolated from chicken in Bangladesh. Vet. World. 12 (6): 909-915.

Campagnolo, E.R., Banerjee, M., Panigrahy, B. and Jones, R.L. 2001. An outbreak of duck viral enteritis (duck plague) in domestic Muscovy ducks (Cairina moschata domesticus) in Illinois. Avian Dis. 45: 522–528.

Carter, G.R., Flores, E.F. and Wise, D.J. 2006. Herpesviridae'. A concise review of veterinary virology. Ithaca, NY: International Veterinary Information Service.

Das, P.M., Rajib, D.M.M., Noor, M. and Islam, M.R. 2005. Retrospective analysis on tile proportional incidence of poultry diseases in greater Mymensingh district of Bangladesh. 4th International Poultry show and seminar, Bangladesh China Friendship Conference Centre, Agargaon, pp. 35-39.

Department of Livestock Services (DLS). 2019. Livestock Economy at a glance 2018-2019. http://www.dls.gov.bd/site/page/22b1143b-9323-44f8-bfd8-647087828c9b/Livestock-Economy (Accessed at 12 May 2020)

Hansen, W.R., Brown, S.E., Nashold, S.W. and Knudson, D.L. 1999. Identification of duck plague by polymerase chain reaction. Avian Dis. 43: 106-115.

OIE. 2018. Manual of diagnosis tests and vaccines for terrestrial animals; Chapter 2.3.7. Available at link: https://www.oie.int/ fileadmin/Home/eng/Health_standards/tahm/2.03.07_DVE.pdf (Accessed at 4 August 2020)

Richter, J.H.M. and Horzinek, M.C. 1993. Duck Plague. in: Virus Infections of Birds, McFerran J.B. & McNulty M.S., eds. Elsevier Science Publishers B.V., Amsterdam, the Netherlands, pp.77–90.

Shawky, S., Sandhu, T.S. and Shivaprasad, H.L. 2001. Pathogenicity of low virulence duck virus enteritis isolates with apparent immunosuppressive ability. Avian Dis. 44: 590-599.

Wallace, R., Hansen, A., Sean, W., Nashold, A., Douglas, E., Docherty, A., Susan, E., Brown, B., Dennis, L. and Knudson, B. 2000. Diagnosis of Duck Plague in Waterfowl by Polymerase Chain Reaction. Avian Dis. 44: 266-274

Wang, G., Qu, Y., Wang, F., Hu, D., Liu, L., Li, N., Yue, R., Li, C. and Liu, S. 2013. The comprehensive diagnosis and prevention of duck plague in northwest Shandong province of China. Poultry Sci. 92: 2892-2898.