

Original Article

Epidemiological investigation of recurrent outbreaks of duck plague in selected *Haor* (wetland) areas of Bangladesh

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• Received: April 14, 2018 • Revised: May 5, 2018 • Accepted: May 14, 2017 • Published Online: May 21, 2018



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ABSTRACT

Objective: A cross sectional study was conducted in five *Haor* (wetland) districts of Bangladesh to investigate the epidemiological parameters and associated factors of recurrent outbreak of duck plague (DP) on the basis of molecular detection.

Materials and methods: A total of 450 randomly selected duck farms containing 175,467 ducks were investigated for their morbidity, mortality and case fatality rates from December 2012 to November 2014. The farms were surveyed and the owners were interviewed using a prepared questionnaire about husbandry practice, disease history, bio-security measures, and flock and farmer details. A total of 150 samples (liver=50, intestine=50 and oro-pharyngeal tissue=50) were collected from duck plague suspected sick/dead ducks of north eastern part of Bangladesh during outbreak season. Samples were processed and PCR was done directly from the samples using primers specific for duck plague virus.

Results: Out of 150 test samples of the fifty duck plague suspected ducks of fifty outbreaks, 90 samples (60%) of 36 ducks of thirty six outbreaks (72%) were found positive by PCR. Overall prevalence of duck plague was 8% at farm level and 3.30% at flock level. Morbidity, mortality and case fatality rates of duck plague at farm level were 52.08, 29.62 and 56.86%, respectively. Of the 22 variables selected for this study, 16 were found significant and the remaining 6 were found non-significant statistically.

Conclusion: Results of the epidemiological investigation of the present study regarding duck mortality suggesting that education and training of the farmers on bio-security, modern husbandry practice, regular vaccination and innovation of cost effective intensive duck farming methods are necessary to control recurrent duck plague outbreak in *Haor* (wetland) areas.

KEYWORDS

Associated factors; Bio-security; Duck plague; Epidemiology; PCR

How to cite: Khan KA, Saha S, Hossain MT, Haque ME, Haq MM, Islam MA. Epidemiological investigation of recurrent outbreaks of duck plague in selected *Haor* (wetland) areas of Bangladesh. Journal of Advanced Veterinary and Animal Research. 2018; 5(2):131-139.

INTRODUCTION

Bangladesh is one of the fastest growing developing countries of the world where about 85% of its 150 million people living in rural areas. Poultry rearing is an integral part of rural farming communities in Bangladesh ([Amber and Mia, 2002](#)). Among poultry farming, duck production represent an important complement of sustainable livelihood strategies for rural people ([Hoque, 2006](#)). Duck is the 2nd largest population in poultry, approximately 54.016 million in Bangladesh and 3rd largest duck population in East and South Asia ([FAO, 1991](#)). Duck rearing is being considered as an asset to the rural poor and landless farmers.

The most important constrain in duck rearing is infectious diseases, of which duck plague (DP) is the most important one. The disease is caused by Anatid herpesvirus type 1, a member of the Herpesviridae family and subfamily Alpha-herpesvirinae ([Fadly et al., 2008](#); [Li et al., 2009](#); [King et al., 2011](#)). Duck plague virus (DPV) is a potential threat to all age groups of ducks which is characterized by high morbidity and mortality varying from 5-100% ([Hossain et al., 2004](#)). The disease was first observed in Netherlands in 1923 ([Baudet, 1923](#)) and has been reported in many countries of the world ([Calnek et al., 1997](#)). The DPV was first confirmed in ducks of Bangladesh by [Sarker \(1980\)](#). About 60-75% duck mortality occurs due to duck plague in Bangladesh ([Sarker, 1982](#)). DP frequently occurs in duck population areas of Bangladesh and cause huge economic loss due to high mortality and loss of production. Though vaccine is only specific tool for prevention of DP, but local vaccines are produced without proper surveillance report.

North-Eastern zone of Bangladesh is recognized internationally for the *Haor* basin and its wetland ecosystem which is located in Sunamganj, Habiganj, Moulvibazar, Kishoreganj and Netrokona districts and the Sylhet Sadar Upazila. Among them Sunamganj, Habiganj and Moulvibazar districts, and Sylhet Sadar Upazila forms the core *Haor* area. Total area of the *Haor*-type wetland ecosystem in Bangladesh is 80,000 square kilometers and core *Haor* area is estimated to spread over an area of 25,000 square kilometers ([IUCN, 2007](#)). Most of these areas have pockets of wet lands that stays submerged from April-May to July-August, which are locally termed as “*Haor*”. Every year flash flood results in the *Haor* basin due to the heavy rainfall in the upstream of neighboring upland country ([Islam et al., 2012](#)).

Flood prone wet land areas are suitable for duck production because of its geographical advantages like natural feed availability, abundant water for swimming, and tolerable temperature ([Hoque et al. 2011a](#)). Since first isolation in 1980 until now, limited works on epidemiology, surveillance and molecular characterization of DPV have been carried out on duck mortality of *Haor* areas of Bangladesh. Several investigators have isolated and characterized the DPV in Bangladesh ([Sarker 1980, 1982](#); [Khan et al., 1990](#); [Islam, 1992](#); [Akter et al., 2004](#)). Though DPV is single antigenic type but the reasons for vaccine failure and high rate of duck mortality in Bangladesh are not very clear yet ([Hossain et al., 2004, 2005](#); [Islam et al., 2005](#); [Das et al., 2009](#)). Therefore, it is necessary to study the epidemiological parameters of DPV in Bangladesh which are responsible for duck plague occurrence, transmission and re-emergence. The present study was aimed to conduct an epidemiological investigation of DPV in selected wetland areas of Bangladesh with detection by PCR.

MATERIALS AND METHODS

A cross sectional study was conducted from December 2012 to November 2014 among 450 duck farms of five *Haor* districts (**Figure 1**), namely Kishoreganj (24°22" to 24°32" N latitudes and 90°01" to 91°01" E longitudes), Netrokona (24°47" to 24°58" N latitudes and 90°38" to 90°50"E longitudes), B-Baria (24°20"N to 24°15"N latitudes and 91°02"E to 91°20"E longitudes), Hobiganj (24°28"N to 24°39"N latitudes and 91°11"E to 91°22"E longitudes), and Sunamganj (24°47"N to 25°12"N latitudes and 90°56"E to 90°10"E longitudes) of Bangladesh. These locations were selected giving priority on population density, geographical location, duck husbandry practices and bird movement through the area. One upazila (sub-district) was selected randomly from each of the five districts. From each upazila, 90 duck farms (small-30, medium-30 and large-30) were selected by random selection method.

A questionnaire was designed to collect data through direct interview of the randomly selected 450 farmers of the selected areas. The questionnaire piloting was performed to validate the questionnaire beforehand. The questionnaire included closed, semi closed with space available to record alternatives to the options given, and open ended questions. The questions were designed to collect informations about farmer's details (knowledge about farming, education, income status and training), farm categories, flock details (age, sex, breed and bird movements), housing, season, management variables,

disease prevalence, disease outbreak including duck morbidity and mortality, clinical information along with post mortem lesions. All these information were analyzed for epidemiological investigation of duck plague. A total of 150 samples (liver=50, intestine=50 and oropharyngeal tissue=50) were collected aseptically from duck plague (DP) suspected sick/dead ducks during outbreak season and tested at microbiology lab of Department of Microbiology and Hygiene, Bangladesh Agricultural University. Tentative diagnosis of duck plague was made on the basis of typical symptoms and pathognomonic post mortem lesions observed during the study period. Confirmatory diagnosis was done with PCR.

Chromosomal DNA was extracted from processed samples using the protocol of DNA extraction kit (Promega®, USA). Forward 5'-GAA GGC GGG TAT GTA ATG TA-3', and Reverse 5'-CAA GGC TCT ATT CGG TAA TG-3' primers designed from DNA-directed DNA polymerase gene (OIE, 2012) was used for amplification of the targeted DNA segments of DPV. A 50 µL reaction mixture was prepared by mixing nuclease free water (16 µL), PCR master mixture (25 µL) (Promega-Madison, WI, USA) forward primer (2 µL), reverse primer (2 µL), and DNA template (5 µL). Thermal condition used for the amplification of DNA polymerase gene (UL 30) was: initial denaturation at 94°C for 2 min; followed by 35 cycles of reaction comprising with 94°C for 1 min, 55°C for 1 min, 72°C for 2 min, with a final extension at 72°C for 7 min.

An amount of 5 µL PCR products was mixed with 1 µL 6X loading dye, and the mixture was loaded to the appropriate well of the 2% agar gel. After electrophoresis, the DNA was stained with ethidium bromide, and was visualized using UV trans-illuminator (Biometra, Germany).

Primary and secondary data with confirmatory diagnostic results of duck plague (DP) were analyzed with IBM SPSS Statistics Data Editor Version 20 (SPSS, 2013) by IBM Corp. for epidemiological analysis of different variables and factors associated with DP occurrence. Descriptive statistics were performed to express individual results of each coded category as frequency, percentage, and mean. A model was established with twenty two variables and assessed for overall fit by Pearson *Chi*-square test. The results were presented as *P*-value at 95% confidence interval.

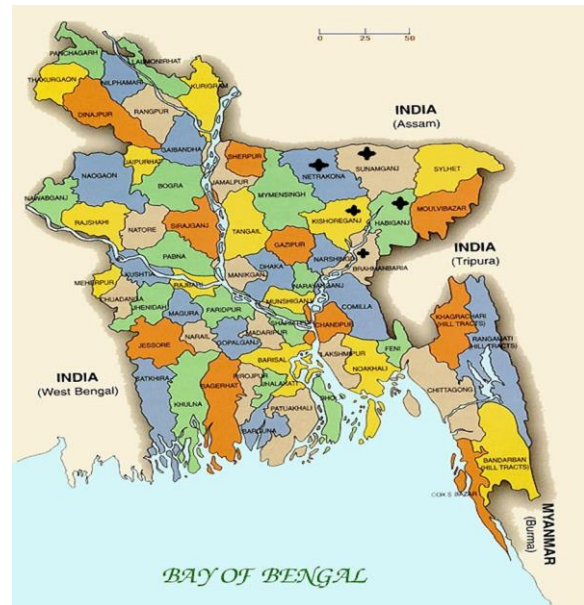


Figure 1. Study area in Bangladesh. Black stars indicated the study area.

RESULTS AND DISCUSSION

A total of 450 duck farms which was 7.40% of total farms of study areas and total duck population 175,467 which was 14.89% of the total number of ducks in the study areas of five *Haor* district were included in the study for survey (**Table 1**). Farms were categorized according to the flock size (**Table 2**). Average flock size for small, medium and large farms was 37, 333 and 800, respectively.

Out of 150 test samples, 90(60%) samples of thirty six ducks of 36(72%) outbreaks were found positive by PCR. The expected PCR amplicon was appeared at 446 -bp for DNA polymerases (**Figure 2**).

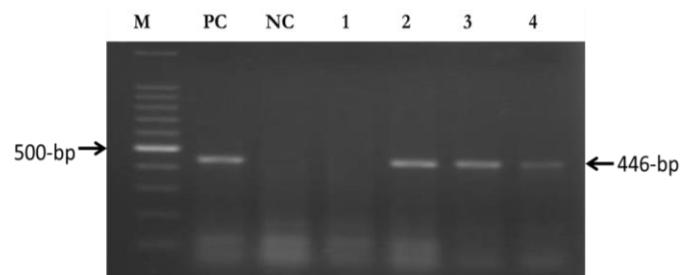


Figure 2: Results of PCR amplification of DPV from field samples. M=100-bp DNA marker; PC=Positive control, NC=Negative control, L1=Negative field sample, L2-L4=Positive field samples of DPV.

Table 1. Duck population in five study areas in *Haor* districts of Bangladesh

Study areas	Duck farms (n)	Visited duck farms (n)	Total ducks (n)	Aver ducks per farm (n)	Total ducks (n)	Total duck farms (%)	Total ducks (%)	Total duck	Total duck (%)
Karimgonj/Kishoregonj	815	90	34300	381	277438	11.04%	12.36%	1631333	2.10%
Netrokona Sadar/Netrokona	1720	90	29155	324	565318	5.23%	5.15%	3849781	0.75%
Nasirnagar/B-Baria	785	90	40898	454	225500	11.46%	17.96%	775690	5.27%
Ajmirigonj/Hobigonj	1056	90	34389	382	400000	8.52%	8.59%	1215287	2.82%
Dhormopasha/Sunamganj	1706	90	36725	408	700000	5.24%	5.24%	1969309	1.86%
Total	6082	450	175467	390	2168256	7.40%	14.89%	9441400	Ave. 1.86%

Table 2. Duck farm categories

Category of farms	Number of farms	Number of ducks	Average flock size	Median	Mode
Small (10-100 ducks)	150	5556	37	27	25
Medium (101-500 ducks)	150	49867	333	350	400
Large (>500 ducks)	150	120044	800	700	600

Table 3. Prevalence, occurrence, morbidity, mortality, case fatality rates of duck diseases and effects of farm categories, study areas on duck plague outbreaks

Parameters	Prevalence		Occurrence (in 36 farms)	Morbidity (in 36 farms)	Mortality (in 36 farms)	Case fatality (in 36 farms)
	Farm	Duck				
Duck diseases						
Duck plague	8% (36/450)	3.30% (5791/175467)	72% (36/50)	52.08% (5791/11119)	29.62% (3293/11119)	56.86% (3293/5791)
Other duck diseases	3.11% (14/450)	0.48% (847/175467)	28% (14/50)	29.54% (847/2867)	10.39% (298/2867)	35.18% (298/847)
Farm category						
Small (10-100 ducks)	10% (15/150)	9.27% (515/5556)	41.67% (15/36)	55.74% (515/924)	33.12% (306/924)	59.42% (306/515)
Medium (101-500 ducks)	8% (12/150)	4.35% (2167/49867)	33.33% (12/36)	53.24% (2167/4070)	29.66% (1207/4070)	55.70% (1207/2167)
Large (>500 ducks)	6% (9/150)	2.59% (3109/120044)	25% (09/36)	50.76% (3109/6125)	29.06% (1780/3109)	57.25% (1780/3109)
Study areas						
Kishoregonj	7.78% (7/90)	3.22% (1103/34300)	19.44% (7/36)	52.28% (1103/2110)	29.29% (618/2110)	56.03% (618/1103)
Netrakona	8.89% (8/900)	4.9% (1428/29155)	22.22% (8/36)	55.78% (1428/2560)	32.27% (826/2560)	57.84% (826/1428)
B-baria	6.67% (6/90)	2.3% (940/40898)	16.67% (6/36)	44.76% (940/2100)	25.48% (535/2100)	56.92% (535/940)
Hobigonj	7.78% (7/90)	2.94% (1012/34389)	19.44% (7/36)	46.96% (1012/2155)	26.59% (573/2155)	56.62% (573/1012)
Sunamganj	8.89% (8/90)	3.56% (1308/36725)	22.22% (8/36)	59.62% (1308/2194)	33.77% (741/2194)	56.65% (741/1308)

Overall prevalence of duck plague among the study population during study period was recorded 8% at farm level and 3.30% at flock level (**Table 3**). Higher case fatality rate (55.86%) was observed in duck plague outbreaks. Although the case fatality rates were almost similar in different farm categories, highest prevalence was recorded in small duck farms (10% at farm level)

(**Table 3**). Highest prevalence was found in Netrokona and Sunamganj districts (8.89% at farm level) (**Table 3**).

Duck farm category or flock size was found to be non-significant by chi-square test at 95% confidence interval. However, the results showed higher prevalence in small duck farms (10%) compared to large duck farms (6%).

Table 4. Effect of phenotypic variables and season on duck plague outbreaks

Variables	Responses	Prevalence (farm)	Prevalence (duck number)	Occurrence (in 36 farms)	Morbidity (in 36 farms)	Mortality (in 36 farms)	Case fatality (in 36 farms)
*Breeds of duck	Khaki	11%	4.31%	61.11%	52.87%	30.28%	57.27%
	Campbell	(22/200)	(5081/117991)	(22/36)	(5081/9610)	(2910/9610)	(2910/5081)
	Xinding	10%	0.65%	5.5%	50.48%	27.62%	54.72%
		(2/10)	(53/8139)	(2/36)	(53/105)	(29/105)	(29/53)
	Cross	7.5%	1.41%	16.67%	47.36%	25.59%	54.02%
	(6/80)	(485/34368)	(6/36)	(485/1024)	(262/1042)	(262/485)	
	Indigenous	4%	1.15%	16.67%	45.26%	24.21%	53.49%
		(6/150)	(172/14969)	(6/36)	(172/380)	(92/380)	(92/172)
*Age of duck	Adult	-	3.68%	63.89%	51.88%	29.51%	56.88%
			(3743/101730)	(23/36)	(3743/7215)	(2129/7215)	(2129/3743)
	Duckling	-	2.78%	36.11%	52.46%	29.82%	56.84%
			(2048/73737)	(13/36)	(2048/3904)	(1164/3904)	(1164/2048)
*Sex of duck	Male bird	-	2.42%	-	51.88%	29.51%	56.88%
			(522/21558)		(3743/7215)	(2129/7215)	(2129/3743)
	Female bird	-	3.42%	-	52.46%	29.82%	56.84%
			(5269/153909)		(2048/3904)	(1164/3904)	(1164/2048)
*Seasons of study	summer	12.9%	5.82%	44.44%	49.65%	28.34%	57.09%
		(16/124)	(2875/50445)	(16/36)	(2875/5790)	(1641/5790)	(1641/2875)
	Rainy	10.1%	4.96%	22.22%	(52.96)%	30.58%	57.12%
		(8/79)	(1271/26792)	(8/36)	(1271/2400)	(726/2400)	(726/1271)
	Autumn	1%	1.52%	2.78%	61.25	30.25%	53.06%
		(1/72)	(245/16068)	(1/36)	(245/400)	(130/400)	(130/245)
	Late-Autumn	3.7%	2.12%	8.33%	58.79%	33.31%	56.85%
	(3/81)	(846/39040)	(3/36)	(846/1445)	(481/1445)	(481/846)	
Winter	8.5%	0.65%	8.45%	47.75%	33.29%	53.07%	
	(6/71)	(309/37290)	(6/71)	(309/647)	(164/647)	(164/309)	
Spring	8.7%	3.48%	5.56%	56.06%	34.56%	61.63%	
	(2/23)	(245/5832)	(2/36)	(245/437)	(151/437)	(151/245)	

* = Indicates statistically non-significant by Chi-square test at 95% confidence interval

Since all of these farms followed traditional open rearing practice and completely dependent upon fate with least biosecurity practice, all flocks are at similar risks of duck plague outbreak. Vaccination is the only preventive measures found infrequently among the farms. However, slight difference among the farm categories suggests that small farms have slightly higher prevalence rate. This could be associated with experience and familiarity of the duck rearing practices of the farmer since most the large duck farm owners are more experienced. Although slight variation had been observed in different districts, there was no significant association found between duck plague prevalence rate and study area (Table 3). Since the geographical and ecological features of the Haor districts are similar, it can be assumed that environmental condition is same for DPV survival and transmission in all areas. The findings of the present study agree with the findings of previous studies (Sandhu and Shawky, 2003; Woźniakowski and Samorek-salamonowicz, 2014).

Indigenous ducks and Khaki Campbell were the most common duck breeds reared in the study areas. Slight differences recorded in prevalence and case fatality rate of duck plague in different breeds (Table 4). But,

statistically no significant association observed between duck plague outbreak and duck breeds. Other than these two, xinding and cross breeds were also found to be reared in different duck farms. Since the farmers raised ducks in same condition with traditional management, therefore the risk of duck plague transmission is almost similar in all breeds of ducks in Haor areas. Similar findings was also mentioned by Leibovitz (1989). Prevalence of duck plague was higher in adult ducks (3.68%) than that of ducklings (2.78%) (Table 4). This variation is not statistically significant which supports the findings of Calnek et al. (1997) and Sandhu and Shawky (2003) who reported that ducks of all ages are susceptible to duck plague. Morbidity, mortality, and case fatality rates of the duck plague were found to be almost similar in ducks of all ages. During the present study, female ducks were found to have slightly higher prevalence rate (3.42%) than the male (2.42%) which was also statistically non-significant (Table 4). In the five haor districts, ducks are raised mainly as layers. Therefore, numbers of females are higher in this study than the male ducks. This could be the reason why slight difference in prevalence was observed.

Table 5: Effects of housing and management variables on duck plague outbreaks

Variables	Responses	Prevalence (farm)	Prevalence (duck number)	Occurrence (in 36 farms)	Morbidity (in 36 farms)	Mortality (in 36 farms)	Case fatality (in 36 farms)
**Idea about duck plague spread	Yes	0% (0/44)	0% (0/19127)	0% (0/36)	0% (0/0)	0% (0/0)	0% (0/0)
	No	8.9% (36/406)	3.70% (5791/156340)	100% (36/36)	52.08% (5791/11119)	29.62% (3293/11119)	56.86% (3293/5791)
**Vaccination status	Vaccinated	4.3% (8/184)	2% (2055/102291)	22.2% (8/36)	49.82% (2055/4125)	26.88% (1109/4125)	53.97% (1109/2055)
	Non-vaccinated	10.5% (28/266)	5.11% (3736/73176)	78.8% (28/36)	53.42% (3736/6994)	31.23% (2184/6994)	58.46% (2184/3736)
**Regular vaccination	Yes	3.73% (5/134)	1.71% (1340/78411)	13.89% (5/36)	47.86% (1340/2800)	26.39% (739/2800)	55.15% (739/1340)
	No	9.81% (31/316)	4.59% (4451/97056)	86.11% (31/36)	53.50% (4451/8319)	30.70% (2554/8319)	57.38% (2554/4451)
**Proper housing	Yes	4.69% (12/256)	2.26% (2803/123912)	33.33% (12/36)	49.79% (2803/5630)	27.90% (1571/5630)	56.05% (1571/2803)
	No	12.37% (24/194)	5.8% (2988/51555)	66.66% (24/36)	54.44% (2988/5489)	31.37% (1722/5489)	57.63% (1722/2988)
**Regular disinfection	Yes	0% (0/36)	0% (0/36)	0% (0/36)	0% (0/0)	0% (0/0)	0% (0/0)
	no	8.85% (32/363)	3.87% (2803/72558)	100% (36/36)	52.08% (5791/11119)	29.62% (3293/11119)	56.86% (3293/5791)
**Idea about bio-security	Yes	0.3% (3/99)	1.28% (778/60857)	8.33% (3/36)	46.59% (778/1670)	25.20% (421/1670)	54.11% (421/778)
	No	9.4% (33/351)	4.37% (5013/114610)	91.66% (33/36)	53.05% (5013/9449)	30.39% (2872/9449)	57.29% (2872/5013)
**Practice of isolation	Yes	0% (0/36)	0% (0/36)	0% (0/36)	0% (0/0)	0% (0/0)	0% (0/0)
	No	9.18% (33/360)	4.03% (2803/70000)	100% (36/36)	52.08% (5791/11119)	29.62% (3293/11119)	56.86% (3293/5791)
**Burial of dead ducks	Yes	0% (0/51)	0% (0/31791)	0% (0/36)	0% (0/0)	0% (0/0)	0% (0/0)
	No	9.02% (36/399)	4.03% (5791/143676)	100% (36/36)	52.08% (5791/11119)	29.62% (3293/11119)	56.86% (3293/5791)
**Supplement with vitamin-minerals	Yes	0% (0/95)	0% (0/57647)	0% (0/36)	0% (0/0)	0% (0/0)	0% (0/0)
	No	10.1% (36/355)	2.99% (5791/117820)	100% (36/36)	52.08% (5791/11119)	29.62% (3293/11119)	56.86% (3292/5791)
**Rearing of recovered ducks	Yes	11.2% (33/295)	4.41% (5593/126784)	91.7% (33/36)	52.47% (5593/10659)	29.89% (3186/10659)	56.96% (3186/5593)
	No	1.9% (3/155)	0.41% (198/48683)	8.3% (3/36)	43.04% (198/460)	23.26% (107/460)	54.04% (107/198)
**Mix with migratory birds	Yes	9.1% (36/394)	3.32% (5791/174274)	100% (36/36)	52.08% (5791/11119)	29.62% (3293/11119)	56.86% (3293/5791)
	No	0% (0/56)	0% (0/1193)	0% (0/36)	0% (0/0)	0% (0/0)	0% (0/0)
**Duck mix with other's	Yes	9.5% (36/377)	3.3% (5791/174094)	100% (36/36)	52.08% (5791/11119)	29.62% (3293/11119)	56.86% (3293/5791)
	No	0% (0/73)	0% (0/1373)	0% (0/36)	0% (0/11119)	0% (0/11119)	0% (0/0)

** = Indicates statistically significant by Chi-square test at 95% confidence interval

A large number of farmers raise ducks according to the season. In late winter, spring, summer, and early rainy season, abundant supply of natural feed resources are available in the *Haor* areas. So, the number of duck population raised in the duck farms was higher during this period. Seasonal variation of duck plague outbreaks was seen but statistically the findings were non-significant. Higher prevalence of duck plague was found in summer (12.9% at farm level and 5.82% at flock level)

and rainy season (10.1% at farm level and 4.96% at flock level) compare to other seasons (**Table 4**). Higher mortality was encountered due to high humidity ([Hoque, 2006](#)). It is because ducks become stressed and susceptible to infectious diseases like duck plague at humid environment, which might have led to higher mortality. But during the late rainy season and autumn, there are least natural feed resources in the *Haor* areas. As a result, traditional duck farmers reduce their flock

Table 6: Effect of farmer's knowledge and training on duck plague outbreaks

Variables	Responses	Prevalence (farm)	Prevalence (duck number)	Occurrence (in 36 farms)	Morbidity (in 36 farms)	Mortality (in 36 farms)	Case fatality (in 36 farms)
**Education status of farmers	Educated	2.27% (5/220)	0.92% (764/82798)	13.89% (5/36)	47.16% (514/1090)	26.51% (289/1090)	56.23% (289/514)
	Un educated	13.48% (31/230)	5.42% (5027/92669)	86.11% (31/36)	52.62% (5277/1029)	29.95% (3004/10029)	56.93% (3004/5277)
**Experience of farmers	Yes	2.6% (8/303)	0.62% (782/126673)	22.22% (8/36)	51.89% (782/1507)	29.53% (445/1507)	56.91% (445/782)
	No	19% (28/147)	10.27% (5009/48794)	77.78% (28/36)	52.11% (5009/9612)	29.63% (2848/9612)	56.86% (2848/5009)
**Training of farmer	Trained	2.88% (3/104)	1.22% (778/63707)	8.3% (3/36)	46.59% (778/1670)	25.21% (421/1670)	54.11% (421/778)
	Non-trained	9.54% (33/346)	4.49% (5013/111760)	91.7% (33/36)	53.05% (5013/9449)	30.39% (2872/9449)	57.29% (2872/5013)
**Income of farmers	High	5.64% (15/266)	2.68% (4356/162648)	41.67% (15/36)	51.37% (4356/8480)	29.41% (2494/8480)	57.25% (2494/4356)
	Low	11.41% (21/184)	11.19% (1435/12819)	58.33% (21/36)	54.38% (1435/2639)	30.28% (799/2639)	55.68% (799/1435)

* = Indicates statistically non-significant by *Chi*-square test at 95% confidence interval

** = Indicates statistically significant by *Chi*-square test at 95% confidence interval

size in this period of the year. This could be the reason why the seasonal variation recorded in duck plague outbreaks.

All recovered duck plague affected birds might act as a carrier for this virus ([Calnek et al., 1997](#); [Shawky and Schat, 2002](#)). Therefore, presence of recovered birds in a duck farm is a definite risk for future disease outbreaks. In this study, presence of recovered birds and intermixing of recovered birds with newly purchased birds were found to be a common practice in the duck farms of *Haor* areas. As a result, multiple outbreaks were very common in a single farm throughout the year. This risky practice also suggests that farmers had lack of knowledge about duck plague transmission and biosecurity practice. Both factors were found to be significantly associated with the outbreaks of duck plague and considered one of the reasons of risky traditional duck rearing practice. Traditional management practice of domestic ducks in *Haor* areas are characterized by faulty housing, no boundary management, and almost non-existent preventive measures. These findings also supports the statement of [Das et al. \(2005\)](#) and [Hoque et al. \(2010\)](#) who reported that proper housing, mix with migratory birds and other flocks, and irregular and/or lack of vaccination had been found significant factors for duck plague outbreaks and transmission (**Table 5**).

Traditional duck house in five *Haor* districts are characterized by low tin made houses with soil floors, overcrowding, lack of ventilation and temperature control, and disinfection use ([Jha et al., 2015](#)). This

condition provides a favorable environment for the establishment and spread of DPV. Proper housing reduces the chance of contamination and spread of the DPV which is absent in studied duck farms. Since there is no intensive duck farming practice in this areas, ducks roam freely in the wetlands and ponds. They mix with migratory and other birds as well as ducks of other farms. This intermixing had created an interface for bird-to-bird transmission of DPV. Proper burial of the dead ducks was almost non-existent in the study areas. Disposing the dead birds into the nearest water bodies is a common practice. All of these findings agree the findings of other researchers ([Shawky and Schat, 2002](#); [Hoque et al. 2011a & b](#); [Woźniakowski and Samorek-salamonowicz, 2014](#)).

Vaccination practice is not frequent in the study area (**Table 5**). Most of the farmers do not have sufficient knowledge about the vaccination schedule. Furthermore, a lot of them do not aware about maintaining the cool chain at the time of transportation of duck plague vaccine. During the wet seasons, the communication system of the *Haor* areas is mostly by boat which is time consuming ([Sarif et al., 2016](#)). Electricity is also not always present. Therefore, while traveling distance, farmers could not be able to maintain cool chain. As they do not have sufficient knowledge about vaccine carrying and maintaining cool chain, quality of vaccines deteriorates while carrying. Significant association was observed between method of vaccine carrying of the farmers and the duck plague outbreaks along with not taking vaccination and irregular vaccination.

A non-trained and un-educated duck farmer is more likely to be ignorant of the biosecurity practice and modern duck farming methods. Hence, it will make their farms prone to disease outbreaks (Hoque et al., 2010; Jha et al., 2015). This study also agrees with this fact because non-trained farmer and un-educated farmer were both found to be the significant risk factors for duck plague outbreaks (Table 6). In current study, significant association between the low income farmer and duck plague outbreaks had been found (Table 6). This probably because a farmer with limited income cannot maintain the biosecurity measures and modern management practice properly. Lack of credit encouraged them to depend more on nature and traditional management practice which is a low cost high risk duck rearing method. This in turn increased the risk of duck plague transmission in their farm. Jha et al. (2015) mentioned that there is least biosecurity practice in the duck farms. Since the farmers do not have sufficient knowledge about duck plague spread, they do not take any attempt to separate the diseased birds or properly dispose the dead birds. This lack of idea about biosecurity is a potential factor for duck plague occurrence and transmission. Sandhu and Shawky (2003) reported that DPV transmit rapidly in those areas where diseased birds are not separated from healthy flock and dead birds are not properly buried. Disinfection of the duck farms with suitable disinfectant is mandatory to control spreading of any infection. Since the DPV spreads through body secretions, without regular disinfection it is not possible to control an outbreak. Unfortunately, irregular disinfection is an important risk for duck plague occurrence in the study areas. Quality veterinary service is essential to control any infection in a farm (Jha et al., 2015).

CONCLUSION

Findings of the epidemiological investigation of duck plague of the present study suggest that the overall prevalence rate of duck plague in the selected areas of five districts at the farm level was 8% and at the flock level it was 3.30%. This result was confirmed by PCR which is suggesting that the outbreak of duck plague is associated with poor management practice, lack of biosecurity measures, well communication, electric facilities and knowledge about modern duck rearing practice on the Haor areas. Considering these factors, recommendations can be made to take necessary steps to improve the management practice, to provide effective sufficient training on biosecurity and effective

vaccination, waste management and dead bird disposal methods.

ACKNOWLEDGEMENT

The research work has been conducted with the financial supports from Project National Agricultural Technology Program (NATP), Department of Livestock Services (DLS), Bangladesh, Dhaka.

CONFLICT OF INTEREST

The authors declare that there is no conflicting interest with regards to the publication of this manuscript.

AUTHORS' CONTRIBUTION

MAI designed the study, interpreted the data and drafted the manuscript. KAK was involved in collection of data and also contributed in manuscript preparation. SS, MTH, MEH and MMH took part in preparing and critical checking of this manuscript.

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