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Prevalence and Risk factors of Newcastle Disease in Chickens of Live Bird Markets, Commercial Poultry Farms and Backyard in Selected Areas of Bangladesh

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ABSTRACT

The present study was carried out to determine the prevalence and potential risk factors of ND in poultry in selected areas of Bangladesh. A total of 77 pooled oropharyngeal swab samples were randomly collected from live bird markets (LBM), backyard chickens, and commercial poultry farms around Savar, Tangail, and Gazipur. The risk factor data were collected using farmer's interviews and close observation. All swab samples were subjected to RNA extraction followed by qRT-PCR with reference primers and probe to detect NDV. Overall, 28.58% samples were detected NDV positive with highest in backyard chickens 40% (OR:2.16; 95%CI:0.65-7.15) and lowest (23.53%) in commercial farms chickens. NDV prevalence was found highest (41.67%) in Gazipur and minimum (20%) in Tangail. Chickens that consume hand-mixed feed were found more susceptible to develop NVD (OR:12.28; 95%CI:1.26-25.6; n=5) compared with commercial and scavenging types of chicken feed. Educationally qualified people were found more successful to prevent the breakout of ND and chickens reared on floor were significantly prone to NDV than chickens reared in the cage. The odd ratio of NDV infection was 3.27 in dead chickens thrown away vs buried and OR:4.75 when sold sick chickens vs eat and medication. These findings suggest that NDV infections are prevailing in the chickens and may appear as a potential threat to the poultry industry. Extensive surveillance and strict farm biosecurity practices along with proper vaccination are recommended to prevent NDV.

Introduction

Newcastle disease (ND) is a contagious viral disease that can be transmitted to humans and affects many domestic and wild avian species (Abdisa and Tagesu, 2017). The World Organization for Animal Health (OIE) has listed the disease as a notifiable disease because it has such a large economic effect. In 1926, the first outbreaks of ND were observed in poultry in Java, Indonesia, and Newcastle-upon-Tyne, England (Capua *et al*., 1993). Newcastle disease virus (NDV) is caused by the avian paramyxovirus serotype 1 virus, which belongs to the genus Avulavirus, subfamily Paramyxovirinae, family Paramyxoviridae, together with the other eight serotypes (avian paramyxovirus 1-9). The NDV is a single-stranded, negative-sense enveloped RNA virus with a genome length of 15,186 base pairs (Wajid *et al*., 2017; Ali, 2020). In most of Asia, Africa, and some countries in North and South America, virulent NDV strains are endemic in poultry. NDV alone is responsible for at least 40-60% of the overall mortality of Bangladesh's poultry population (Ali and Hasan, 2018). The transmission of ND occurs through respiratory aerosols, exposure to fecal and other excretions from infected birds, through newly introduced birds, selling and giving away sick birds and contacts with contaminated feed, water, equipment, and clothing. Asymptomatic enteric strain, lentogenic strain, mesogenic stain, viscerotropic velogenic strain, and neurotropic velogenic strain are the five pathotypes of NDV pathogenicity (Bary *et al*., 2018; Ali *et al*., 2019a; Bhuiyan *et al*., 2019a; Bhuiyan *et al*., 2019b)

Hygiene and vaccination are the two primary strategies for avoiding ND. In addition to avian species, humans are one of the many species that can be affected by ND (Ali *et al*., 2021a; Ali *et al*., 2019b). When a person is exposed to large amounts of the virus, ND can cause conjunctivitis in humans. Among infection diseases, ND is the one that causes economical loss of poultry and its products (Yusoff and Tan, 2001; Ali *et al*., 2021b).

Confirmation of the ND virus can be done using hemagglutination and hemagglutination inhibition tests, virus neutralization tests, Enzyme associated immune-sorbent assays, plaque neutralization tests, and reverse-transcriptase polymerase chain reaction (RT-PCR) (Wajid *et al*., 2017; Ali *et al*., 2021a). To evaluate the virulence of a NDV isolate, RT-PCR with sequencing of the F cleavage site is now the most widely used

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process. In comparison to other lab diagnostic approaches such as virus isolation, the RT-PCR assay is more sensitive, precise, and time-consuming. The use of rRT-PCR, first with a primer and probe set to diagnose the virus as an NDV and then with a second primer and probe set to identify the virus as virulent, is the primary molecular basis for evaluating NDV virulence in several laboratories (Ali, 2018; Ali *et al*., 2021b). While a vaccine program is widely used in Bangladesh to avoid and monitor ND, the disease is still enzootic throughout the country and poses a constant threat to commercial poultry. Well after routine vaccination of chickens with live NDV vaccines prepared from mesogenic and lentogenic strains, records of serious ND outbreaks are sometimes produced. The pathology, serology, and genetics of the dominant strain of NDV and vaccine viruses may vary significantly, which is thought to be one of the key explanations for the disease's repeated outbreaks (Rahman *et al*., 2018). The biology, serology, and genetics of the current strain of NDV and vaccine viruses may vary significantly, which is thought to be one of the key reasons for the disease's repeated outbreaks in Bangladesh's vaccinated poultry flocks. There are few studies on the molecular and pathological properties of NDVs circulating in Bangladesh (Qin *et al*., 2008; Khokon *et al*., 2017).

This study has been designed for molecular detection of NDV isolated from Tracheal swab of live bird markets, commercial and backyard chickens from three different areas of Bangladesh. The objective of this study was to get the prevalence of NDV among the study chickens of selected areas.

Materials and methods

Sample collection

A total of 77 pooled tracheal swab samples were collected from live bird markets (LBM), backyard chickens and commercial poultry farms around Savar, Tangail, and Gazipur district of Bangladesh (Fig. 1). The study chickens have included broilers, layers and native chickens during 2020. For obtaining risk factors information, a structured and pretested questionnaire had been deployed during the sample collection. Oropharyngeal swabs samples from LBM, commercial and backyard chickens were pooled (five chickens into one pool) and collected into a vial containing 5 ml of sterile phosphate buffer saline. Collected samples were immediately stored in Icebox and transported to the laboratory within 6 hours and stored at -80 °C until further testing.

Laboratory testing

For molecular testing, oropharyngeal swab samples were undergone RNA extraction by using the MagMAX™-96 AI/ND Viral RNA Isolation Kit (Applied Biosystems™, San Francisco, CA) in KingFisher™ Flex 96-well robot (Thermo Scientific™, Waltham, MA) according to the manufacturer's protocol.

RNA extracts were then screened for NDV by using real-time reverse transcription PCR (rRT-PCR) test with published primers and probe specific for matrix gene of NDV (APHA, 2015). The AgPath-ID One-Step RT-PCR kit (ThermoFisher Scientific, Waltham, MA, USA) was used for qRT-PCR testing.

Statistical evaluation

Data were analyzed using STATA13 (STATA Crop, 4905, Lake way drive, College station, Texas 77845, USA) for performing prevalence and risk factors analysis. Descriptive statistics was used to summarize the data and compute ratios and percentages. Chi-square test and logistic regression were used to assess the association of risk factors with the prevalence of NDV. For all risk factors, the level with the lowest prevalence was used as a reference category. Those variables with a p-value of less than 0.25 in the unavailable analysis were further analyzed by multivariable logistic regression after checking for confounders. In all tested cases, 95% confidence intervals and p< (0.05) were set for significance.

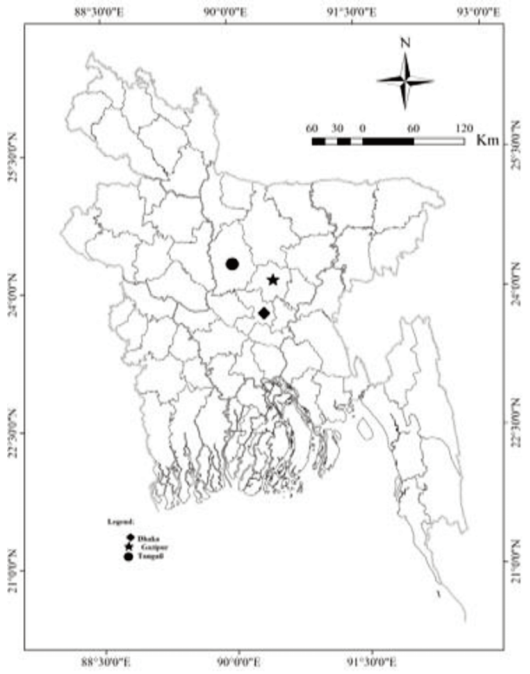

Fig. 1. Geographical location of study the areas in Gazipur, Tangail and Savar, Bangladesh.

Results

Prevalence of NDV

The overall prevalence of NDV in chickens of the study areas was found to be 28.57%. The prevalence of NDV in backyard chickens was 40% (OR: 2.16; 95% CI: 0.65-7.15; n=20), in commercial poultry farms was 23.53% (OR:1.0; n=34) and in LBM was 26.03% (OR:2.14; 95% CI: 0.33-3.89; n=23). In location, NDV positive samples was 41.67% in Gazipur (OR: 2.85; 95% CI: 0.85-9.56; n=24), in Savar was 26.09% (OR: 1.41; 95% CI: 0.38-5.13; n=23) and 20.00 % (OR: 1.0; n=30) in Tangail. Among sample types, the presentence of NDV in broilers was 18.18% (OR: 1.0; n=22), in native chickens was 40.00 % (OR: 3.00; 95% CI: 0.73-12.22; n=20), and in layers was 33.33% (OR: 2.25; 95% CI: 0.44-11.33; n=12). Chickens that consumed hand mixed feed were more susceptible to develop NVD 80% (OR;

Table 1. Primers and probes required for real time RT-PCR

Assay	Type	Sequence (5′-3′)	References
Newcastle diseases virus	Forward primer	GAG CTA ATG AAC ATT CTT TC	APHA, 2015
	Reverse primer	AAT AGG CGG ACC ACA TC TG	
	LproMGB	[6FAM] CCA ATC AAC TTC CC [MGBNFQ]	
	LproMGB2	[VIC] AAT AGT GTA TGA CAA CAC [MGBNFQ]	

12.28; 95% CI: 1.26-25.6; n=5) compared with commercial ready feed 24.56% (OR: 1.0; n=57) and scavenging feeding types 26.67% (OR: 1.11; 95% CI: 0.3-4.07; n=15). Educationally qualified people were more successful to prevent the breakout of ND compared with less qualified people. In this study, the prevalence of NDV was 50% (OR-1.0; n=16) among those poultry farmers, who studied in secondary level, 25 % (OR: 0.33; 95% CI: 0.09-1.22; n= 28) for those poultry farmers who studied in higher secondary and for those poultry farmers who have graduated the prevalence of NDV was 21.21% (OR: 0.26; 95% CI: 0.07-0.97; n=33). The prevalence of NDV was 15.63% when the dead chickens were buried and 37.78% (OR: 3.27; 95% CI: 1.06-10.13; n=45) when the dead chickens were thrown away. Infection rate was 7.69% (OR-1.0; n=13) for those chickens who lived in the cage and 33.33% (OR-6.0; 95% CI: 0.64-55.94 and n=21) for those chickens who lived on the floor (Table 2).

Risk factors associated with NDV infection

The multivariable logistic regression analysis revealed that feed habit of chickens, sick chicken and dead chicken were significantly and independently associated ($p<0.05$) with the development of NDV. The multivariable regression model showed that hand-mixed feed consuming chickens was 12.28 (95% CI:0.68-22.31; p= 0.001) times more likely to be NDV positive than scavenging feed consuming chickens. In case of dead chicken disposals, throwing away dead chickens were 3.27 (95% CI:1.06-10.13; p =0.009) times more likely to be NDV prevalent than burying dead chickens. Also, sold sick chickens were found at 4.75 (95% CI: 1.18-19.06; p = 0.043) times more likely to suffer from NDV infection than medicated sick chickens.

Discussion

Newcastle disease (ND) is a threat to the poultry industry which causes high mortality and morbidity. This disease is said to occur in the poultry population throughout the year in most of the country (Ali and Hasan, 2018; Belgrad *et al*., 2018; Ali, 2020). NDV infects a wide variety of avian species but this study was conducted to investigate the prevalence of NDV only in chickens in three different areas of Bangladesh through qRT-PCR. The results showed that the overall tracheal swab positive percentage of NDV was 28.58%. It was observed that the prevalence of NDV was highest in backyard chickens. Poor sanitary conditions, continuous exposure of chickens to range conditions and wild birds, nutritional deficiencies, the absence of vaccination in traditionally managed chickens, and contact of chickens of one village with those in other villages may facilitate the spread of ND. This is in concurrence with the reports by Hossain *et al*. (2013). LBM also had a significant percentage of NDV prevalence as chickens from different areas come to local bird markets facilitate the spread of NDV in market birds. About 23.53% from commercial poultry farms which was moderately low compared with backyard chickens as commercial poultry workers always maintain healthy sanitary conditions, feed quality, and vaccination process.

In this study, the educational qualification of the person who rear chickens was considered as an important risk factor. The result indicated that less educated persons recorded the highest occurrence of ND and undergraduates and graduates recorded the lowest prevalence of ND. Educated farmers can control ND better because they know about different types of poultry diseases and technology. So, they are aware of biosafety, medication, and vaccination. On other hand, less educated poultry farmers don't know much about poultry diseases and are unable to control the diseases. But the result was contradictory with - Thomazelli *et al*. (2012), who reported First School Leaving Certificate holders recorded the lowest occurrence of ND (10.5%), while the tertiary institution graduates recorded the highest cases (34.2 %) followed by Secondary School Certificate holders (29 %) and those with technical training (26.3%).

Table 2. Logistic regression analysis of potential risk factors for the occurrence of Newcastle Diseases in the study area.

Factor	Categories	Tested	Positive	Univariable			Multivariable		
			n (%)	OR	95% CI	p value	OR	95% CI	p value
Sample source	Commercial Farm chicken	34	8(23.53%)	1	-	-			
	Backyard chicken	20	8(40.00%)	2.16	0.65-7.15	0.205			
	LBM chicken	23	6(26.09%)	2.14	0.33-3.89	0.82			
Education level	SSC	16	8(50.00%)	1	-	-			
	HSC	28	7(25.00%)	0.33	0.09-1.22	0.097			
	Graduate	33	7(21.21%)	0.26	0.07-0.97	0.045			
Study area	Tangail	30	6(20.00%)	1	-	-		Ref.	
	Savar	23	6(26.09%)	1.41	0.38-5.13	0.608	1.1	0.22-3.61	0.889
	Gazipur	24	10(41.67%)	2.85	0.85-9.56	0.08	2.14	060-7.67	0.23
Feeding habit	Ready feed	57	14(24.56%)	1	-	-		Ref.	
	Home made	5	4(80.00%)	12.28	1.26-25.6	0.031	7.11	0.68-22.31	0.001
	Scavenging	15	4(26.67%)	1.11	0.3-4.07	0.867	1.08	0.66-2.63	0.041
Sick chicken	Eat	23	4(17.39%)	1	-	-		Ref.	
	Medication	34	8(23.53%)	1.46	0.38-5.57	0.57	3.77	0.69-20.48	0.124
	Sold	20	10(50.00%)	4.75	1.18-19.06	0.028	4.27	1.04-17.44	0.043
Dead chicken disposal	Bury	32	5(15.63%)	1	-	-		Ref.	
	Throw	45	17(37.78%)	3.27	1.06-10.13	0.039	3.03	0.86-10.66	0.009
Sample type	Broiler	22	4(18.18%)	1	-	-			
	Native	20	8(40.00%)	3	0.73-12.22	0.125			
	Layer	12	4(33.33%)	2.25	0.44-11.33	0.325			
Housing system	Environment	23	6(26.06%)	1.58	0.38-6.26	0.525			
	Caged	13	1(7.69%)	1	-	-		Ref.	
	Floor	21	7(33.33%)	6	0.64-55.94	0.116	3.98	0.33-9.73	0.275

CI: confidence interval; OR: odds ratio

In this study, 3 different types of chickens (broiler, layer, and native) were considered for collecting tracheal swab samples. Environment (knife, cage, feed container, and water container) is also considered as a source for sample collection. NDV infections were observed as 18.18% in broilers, 33.33 % in layers, 40.00% in native chicken, and 26.09% in environment samples. Among them, the prevalence of NDV was the highest in layers and the lowest in broilers. Hassan *et al*. (2016) reported 14.28% prevalence of NDV in cockerel. In sonali chickens, prevalence of NDV was reported as 11.24%, 7.5%, 14.1%, and 8.8% by Talukdar *et al*. (2017), Giasuddin *et al*. (2002), Islam *et al*. (2014), and Saleque *et al*. (2003) respectively in different regions of Bangladesh. But in this study, the prevalence of ND in cockerel was 100% (1/1) and sonali was 0% (0/2) this might be due to the small number of samples. Rahman *et al*. (2012) revealed the prevalence of NDV in layers, broilers, native bird and ducks were 37.5%, 32.5%, 55.0%, and 27.5%, respectively.

In the present study, feed habit of the birds was considered as potential risk factors for the development of NDV. In the total of 22 positive samples, 24.56% chickens consume commercially available ready feed, 80% chickens intake hand-mixed feed and 26.67% chickens manage their feed through scavenging. Results indicated that there was significant association of NDV infection with the feeding habit of chickens.

For LBM, Savar Upazila was prevalent of ND as 26.09%. Gazipur and Tangail were considered as the location for the collection of commercial poultry farms and backyard samples because of a large number of poultry farms and rural areas. The prevalence of ND in Gazipur and Tangail was 41.67% and 20.00% respectively. Among them, Gazipur was the region that had the highest percentage of NDV positive samples, which might be due to mass gathering of different types of birds since major poultry industries are located in these areas in Bangladesh (Bhuiyan *et al*., 2019b; Ali and Islam, 2021). Rahman *et al*. (2012) reported ND as 45.0% in Gazipur district. Tangail was the region that had the lowest percentage of NDV positive samples.

There was a significant association of NDV infection in dead chicken disposal practices. Dead chicken thrown away was found as a risk of NDV infection by 3.03 times higher than dead chicken bury practice. The findings were agreed with Rahman *et al*. (2019) and who stated that dead chickens thrown away causes spread of diseases. The prevalence rate of ND was found low (7.69%) when chickens lived in the cage and high (33.33%) when chickens lived on the floor. Chicken rearing in cage system reduce diseases prevalence significantly compared to open floor system due to birds can be isolated from each other (Paul *et al*., 2017; Rahman *et al*., 2018).

Conclusion

Overall results indicated that the prevalence of NDV was mediocre in this selected study location. This study shows that the prevalence of ND was the highest in Gazipur and the lowest in Tangail. Maximum prevalence of NDV is found in layer chickens than broiler chickens. Educated farmers record the lowest occurrence of ND in their farms. In case of chickens feed habits, the prevalence of NDV is high in hand-mixed feed consuming chicken compared to scavenging and ready feed consuming chickens. The prevalence of NDV is high in backyard chickens and low in commercial poultry farms. The feeding habits of chickens, dead chickens, and sick chickens are significantly related to the occurrence of NDV.

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Conflict of interest

The authors declare no conflict of interests.

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