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Research article

Determinants of Newcastle disease in commercial layer chicken farms in two districts of Bangladesh: A case-control study

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ABSTRACT

Newcastle disease (ND) is a real threat for commercial layer chicken farms in Bangladesh. However, only few studies have focused on exploring the epidemiology of this disease. A case-control study was conducted to identify determinants of Newcastle disease in commercial layer chicken farms in Kishoreganj and Gazipur district of Bangladesh between September 2019 and February 2020. Farms with birds diagnosed as ND positive based on clinical history, clinical signs and postmortem findings were considered as case and farms that did not have such ND positive chickens were the control for this study. Farmers of 56 case farms and 56 control farms were interviewed face to face using a structured questionnaire. The association between Farms' ND status and determinants was assessed by multivariable logistic regression with backward elimination. In the final model, six variables were found to be associated with the risk for ND outbreak: age of the farmers (Odds Ratio [OR] 0.94; 95% Confidence Interval [CI] 0.87–0.99), distance from the nearest poultry farms (OR = 3.23, 95% CI 1.27–8.39), number of houses in the farms (OR = 3.06, 95% CI 1.06–8.83), surrounding environments (OR = 5.27, 95% CI 1.96–14.20), rearing different aged bird together (OR = 4.76, 95% CI 1.25–18.19), and no isolation of sick birds (OR = 2.85, 95% CI 1.07–7.55). Alteration of these determinants should reduce the ND burden in commercial layer chicken farms.

1. Introduction

Newcastle disease (ND), a contagious viral infection of domestic and wild avian species, caused by a virulent strain of avian paramyxovirus type 1 (APMV-1), belongs to the family Paramyxoviridae (Abdisa and Tagesu, 2017). The global monetary impact of the disease is enormous that results in serious losses with high mortality, morbidity, growth retardation, drop in egg production, and poor quality meat (Wiseman et al., 2018). Alongside, ND is a major constraint reported by industry players that kills up to 80% of unprotected poultry in rural areas (Alexander et al., 2004). The most susceptible bird species are chickens, turkey, peafowl, guineas, pheasants, quails, and pigeons (Ashraf and Shah, 2014). However, ducks and geese are the least vulnerable and act as natural reservoirs or carriers (Elbestawy et al., 2019). The virus itself causes harm in the gastrointestinal, respiratory, and neurological systems according to their pathogenicity, host physiology, and their immune status (Wiseman et al., 2018).

The virulent strains of Newcastle disease virus (NDV) are frequently manifested in Asia, Africa, and some parts of America, but the major endemics prefecture imply the African and Asian subcontinent including China, India, Pakistan, Malaysia, and Bangladesh (Abdisa and Tagesu, 2017). Countries with high ND prevalence including 82.3% in Pakistan (Aziz-ul-Rahman et al., 2017), 63.5% in Nigeria (Ameh et al., 2016), 33.8% in Brazil (Marks et al., 2014), 53% in Libya (Gedara et al., 2020) and so on. This disease is capable of causing 100% mortality in non-vaccinated chicken (Zhang et al., 2011). A number of determinants such as cleaning of farm once a week or never practiced (Chaka et al., 2013), multi-age production system (Jaganathan et al., 2015), poor biosecurity and insufficient vaccination program (Messaï and Salhi, 2019), access to visitors (Alsahami et al., 2018), contact with migratory wild birds (Otim et al., 2007), were shown to have influence on the occurrence of this disease.

Poultry industry in Bangladesh is an established commercial platform, performing collaterally in income generation, profit-making, and

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supplement of animal protein at a cheaper rate (Rahman et al., 2017a, b). The overall gross national income of this particular trade unit infers 13% per annum (Mandal and Khan, 2017). Regardless of all successes, outbreak of different infectious diseases including ND hinders the average national productivity, that accounts for around 30% of the birds' death annually (Al Mamun et al., 2019). In Bangladesh, seroprevalence of ND was reported to be 21.2% in domestic chickens (Belgrad et al., 2018) and 37.5% in commercial layer chicken (Rahman et al., 2012). The mortality of ND has been counted as 15.81% in semi-scavenging layer chicken (Biswas et al., 2005) and 13.4% in commercial layer birds (Rashid et al., 2013). Former history of ND in current study areas are documented with 17.54% in Kishoreganj and 36.1% in Gazipur (Al Mamun et al., 2019; Rahman et al., 2017a,b). At large, the country expenses on an average US$ 288.49 million per annum for this disease (Khatun et al., 2018). Despite reports on high burden in domestic and commercial layers in Bangladesh (Al Mamun et al., 2019; Rahman et al., 2017a,b; Hasan et al., 2012; Biswas et al., 2005), the epidemiology of ND has not been completely explored. Thus, investigation of the factors influencing this disease burden is necessary. Although, previous studies have provided framework of exploring the determinants of ND in poultry (Messaï and Salhi, 2019; Wiseman et al., 2018; Jaganathan et al., 2015; Chaka et al., 2013; Otim et al., 2007; East et al., 2006), findings and control strategies suggested by these studies may not suit Bangladesh in consideration of variations in climate, poultry husbandry practice, and demography of the population of this region. Therefore, the aim of the present study was to investigate determinants of ND in commercial layer chicken farms using a case-control study design.

2. Materials and methods

2.1. Study period and location

This case-control study was conducted on commercial layer chicken farms of Kishoreganj and Gazipur districts of Bangladesh in between September 2019 and February 2020. The status of ND is endemic in Bangladesh. It occurs throughout the year in poultry population. We selected this time frame for the convenience of work schedule. The locations were selected purposively as these two districts have relatively high density of commercial chicken farms and commonly recognized as the major poultry hubs of the country. As of 2016, Kishoreganj has 6602 commercial poultry farms of which 1400 were commercial chicken farms (Rahman et al., 2019). On the other hand, the total number of registered commercial poultry farms in Gazipur district is 2164, of which 1331 are commercial layer farms (Veterinary Surgeon, Department of Livestock service, Gazipur, Personal communication, 2020). The first author of this manuscript was assigned to work at Kishoreganj District Veterinary Hospital (KDVH) and Gazipur District Veterinary Hospital (GDVH), two potential internship placements for Doctor of Veterinary Medicine (DVM) program of Sylhet Agricultural University (SAU), Bangladesh. These two veterinary hospitals provide free veterinary services to the livestock farmers of the catchment areas of hospitals. However, they also receive cases from the other parts of the districts referred by the government and private veterinarians and also from non-veterinarians. The catchment areas of KDVH are Kishoreganj Sadar, Hossainpur, Kuliarchor, Pakundia, Nikli, Katiadi, and Bajitpur upazilla, whereas the catchment areas for GDVH are Gazipur Sadar, Kaliganj and Kaliakoir upazilla (Figure 1).

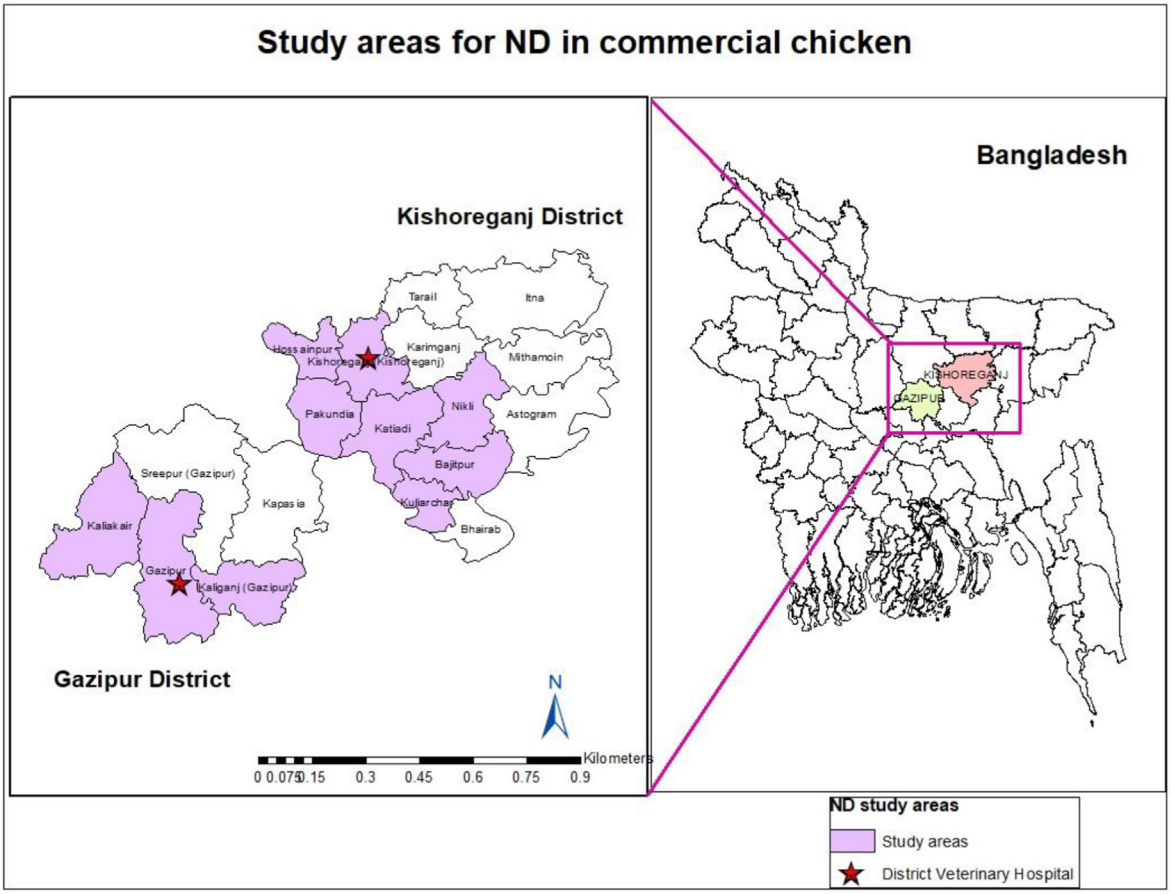

Figure 1. Catchment areas of Kishoreganj District Veterinary Hospital and Gazipur District Veterinary Hospital from where the case and control farms were selected.

During the first three months of study period, from September, 2019 to November, 2019 birds from 32 farms were diagnosed as ND positive in KDVH. But farmers of four ND positive farms refused to participate in the study, thus 28 NDV positive farms from Kishoreganj were included in this study.

At GDVH from December, 2019 to February, 2020, birds of 30 commercial chicken farms out of 70 were diagnosed ND positive. As we got 28 ND positive farms from our first study area, we decided to include same number farms from Gazipur. Thus, first 28 farmers, who accepted our invitation to participate in the study, were included.

2.2. Selection of case and controls

In both of these two veterinary hospitals, diagnosis of poultry diseases are mostly presumptive i.e., are based on the clinical history described by the farmers, clinical signs of sick birds, and postmortem lesions observed in dead or sick birds. Thus, for this study, a farm was considered case i.e. ND positive if, (i) the clinical history described by the farmer resembled those of ND such as a state of prostration and depression, ruffled feathers, greenish-white diarrhea, lack of appetite (Getabalew et al., 2019) (ii) the sick birds showed clinical signs like coughing, sneezing, gasping, rales, paralyzed wings, ataxia, twisted neck etc. (Abdisa and Tagesu, 2017) and (iii) postmortem lesions included pin-point hemorrhages in the proventricular tip, breast and thigh muscle, intestinal ulcers and ileocecal tonsil's hemorrhages (Sedeik et al., 2019). All these clinical examinations were performed by the first author under the supervision of the Veterinary Surgeons (VS) of the veterinary hospitals. However, VS made the final decision on the diagnosis. After being diagnosed as ND positive, the farmer of that farm was invited to take part in the questionnaire interview and farmers who showed interest were only included as case. Following these selection criteria, we included 56 case farms from the two study areas (28 from each district).

We aimed to conduct a 1:1 case control study. Hence, we selected the same number of control farms i.e., 56 farms. We selected most of the control farms from the group of commercial chicken farms came to veterinary hospitals for receiving veterinary services, but birds of those firms were not diagnosed to have ND based on the criteria described above. We included a control farm after selecting a case farm on the same day. In case, a control was not appeared in the hospital premises on the same day of case selection, we included controls from the farms of the catchment areas of the hospital. Therefore, we included 112 commercial chicken farms in this study.

2.3. Questionnaire interview

For collecting data of epidemiological interest, we designed, validated and applied a structured questionnaire during the farmers' visit at veterinary hospitals for receiving veterinary services, when framers are interviewed face-to-face. We interviewed some of the participating farmers at farm premises during the farm visit. The questionnaire included open and close ended questions. It was reviewed by a panel of epidemiologists. Where farmers refused to participate, the farms were excluded and the next farm was selected. We were opting to collect information about demography, general farm management and farm biosecurity practice through this farmers' questionnaire interview. A comprehensive list of variables included in the questionnaire is listed in Table 1. The data were entered into Microsoft Excel 2010 spread sheet, doubled checked by the authors, coded and prepared for further analyses.

2.4. Statistical analyses

All the statistical analysis of this study was accomplished with Statistical Analytical System (SAS) version 9.4. Statistical analysis started with the execution of descriptive analyses of all categorical and continuous variables for exploring the distribution. To account for possible nonlinear relations, continuous variables with skewed distribution, for instance, the

Table 1. Characteristics of significant variables (N = 112) considered during the field survey of ND among commercial layer farms.

Variable	Category	N	%
Educational status of farmer	Illiterate/Basic R&W	23	20.54
	Educated	89	79.46
Poultry farming as a primary occupation	Yes	70	62.50
	No	42	37.50
Experience of poultry farming (Year)	≤6	58	51.79
	>6	54	48.21
Number of workers	1	67	59.82
	>1	45	40.18
Number of houses in the farm	1	85	75.89
	>1	27	24.11
Cleanliness of farmyard	Yes	46	41.07
	No	66	58.93
Ventilation status of farm	Poor	87	77.68
	Satisfactory	25	22.32
Condition of feeder and waterer	Good	80	71.43
	Defective	32	28.57
Empty resting period after selling each batch (Days)	0–30	55	49.11
	>30	57	50.89
Presence of fence	Yes	16	14.29
	No	96	85.71
Maintaining all-in all-out system	Yes	32	28.57
	No	80	71.43
Floor condition	Proper	90	80.36
	Impaired	22	19.64
Frequency of cleaning floor	Once a day	77	68.75
	One or two-day interval	35	31.25
Previous history of ND	Yes	30	26.89
	No	82	73.21
Flock size	≤1500	59	52.68
	>1500	53	47.32
Distance from the nearest road or pathway (Meter)	≤100 m	58	57.79
	>100 m	54	48.21
Distance from the nearest farm (Meter)	≤175 m	56	50.00
	>175 m	56	50.00
Poultry house density per km^2	≤10	65	58.04
	>10	47	41.96
Surrounding environment	Damp	52	46.43
	Dry	60	53.57
Proper drainage system	Yes	46	41.07
	No	66	58.93
Rearing different-aged birds together	Yes	23	20.54
	No	89	79.46
Isolation of sick birds	Yes	50	44.64
	No	62	55.36
Access of visitor into the farm	Yes	31	27.68
	No	81	72.32
Farmers visiting other farms	Yes	37	33.04
	No	75	66.96
Contact with backyard chicken	Yes	82	73.21
	No	30	26.79
Entry of vehicles inside the farm premises	Yes	47	41.96
	No	65	58.04
Disposal of carcass	Thrown openly	61	54.46
	Dispose properly	51	45.54
Regular use of disinfectant	Yes	62	55.36
	No	50	44.64
Washing equipment's with disinfectant	Yes	92	82.14
	No	20	17.86

(*continued on next page*)

Table 1 (*continued*)

Variable	Category	N %
Age of the farmer (Year)	Mean	38.69
	Minimum	20.00
	Maximum	65.00
	Standard deviation	8.08
Current age of flock (Week)	Mean	40.188
	Minimum	1.00
	Maximum	104.00
	Standard deviation	21.94

number of the employees, flock size, minimum distance from other poultry farms, minimum distance from the road, etc. were categorized taking their median as cut-offs. We further re-categorized variables before further analysis when required (Dohoo et al., 2003). All categorical variables were individually tested for an association with the case-control status of a farm by Pearson's chi-square test. We applied t-test for continuous variable(s) in the univariable analysis. The independent variables with statistical significance at $p \leq 0.20$ were included in multivariable analysis. Pairwise correlation among the explanatory variables was also checked for multi-collinearity and only one variable from each correlated group was used in the multivariable analysis. After these initial screening, a multivariable logistic regression analysis was used to assess the association between dependent variable (Farms' ND status) and the independent variables using PROC LOGISTIC in SAS 9.4. We used backward step-wise procedure to fit the final model. The statistical significance of the explanatory variables was assessed by the likelihood ratio test and an explanatory variable with p-value ≤ 0.05 was considered to be statistically significant. Regression coefficients were converted into odds ratios (ORs; eβ) and their 95% confidence intervals (CIs) (Hosmer et al., 2000). Confounding effects of two independent variables were evaluated by observing the change of parameter estimates before and after the removal of a variable from the model. If the parameter estimate of a variable changed ≥20% after discarding a variable from the model, then the effects of these two variables were likely to be confounded each other. We also assessed the significances of interactions between each pair of factors in the final model. We evaluated the model fitness with the use of the Hosmer-Lemeshow goodness-of-fit test and the ratio of the deviance to the degree of freedom. Area Under the Curve (AUC) value were obtained from the Receiver Operating Characteristic (ROC) analysis to assess the predictive ability of the final model.

3. Result

The study considered 56 ND positive and 56 ND negative commercial chicken layer farms while seeking veterinary service at KDVH and GDVH from September 2019 to February 2020. Rate of positive response among the owners of case farms was 90.32% (56/62) and the control farms was 93.33% (56/60). The average farm size of the study population was 1739.87 with median 1400 ranging from 500 and 7000. The mean size of the farms included from Gazipur district (1783.49) was slightly higher than that from Kishoreganj district (1632.45).

A total of 31 variables were found to have comparable respondents in their categories and included in the analysis (Table 1). Out of these 31 variables, 14 received p-value <0.2 univariable analyses (Table 2). Two from seven demographic variables, for instance, age and education status of the farmers were included in the model as they obtained p-value <0.01 and 0.01, respectively in the univariable analyses. Eventually, 14 explanatory variables became the candidates for further multivariable logistic regression.

Factors associated with ND in commercial layer farms identified by multivariable logistic regression are displayed in Table 3. The final model

Table 2. Result of univariable analysis of the determinants of Newcastle disease infection in commercial layer chicken farms.

Variables	Case (n = 56)	Control (n = 56)	OR with 95% CI	P-value
Education status of the farmer				0.01
Illiterate/Basic R&W	17	6	3.63 (1.29–10.19)	
Educated	39	50	1	
Number of houses in the farm				0.13
1	46	39	2.01 (0.81–4.93)	
˃1	10	17	1	
Flock size				0.19
≤1500	33	26	1.65 (0.78–3.52)	
˃1500	23	30	1	
Distance from the nearest road or pathway (Meter)				0.01
≤100 m	36	22	2.78 (1.28–6.03)	
>100 m	20	34	1	
Distance from the nearest farm (Meter)				0.03
≤175 m	34	22	2.38 (1.11–5.14)	
˃175 m	22	34	1	
Condition of feeder and waterer				0.04
Defective	21	11	2.45 (1.05–5.76)	
Good	35	45	1	
Floor condition				˂0.01
Impaired	17	5	4.45 (1.51–13.10)	
Proper	39	51	1	
Regular use of disinfectant				0.06
No	30	20	2.07 (0.97–4.43)	
Yes	26	36	1	
Surrounding environment				˂0.01
Damp	36	16	4.50 (2.01–10.07)	
Dry	20	40	1	
Rearing different-aged birds together				˂0.01
Yes	18	5	4.83 (1.63–14.34)	
No	38	51	1	
Isolation of sick birds				0.03
No	37	25	2.41 (1.12–5.23)	
Yes	19	31	1	
Cleanliness of farmyard				0.05
No	38	28	2.11 (0.98–4.55)	
Yes	18	28	1	
Disposal of carcass				0.04
Throw away	36	25	2.23 (1.04–4.77)	
Dispose properly	20	31	1	
Age of the farmer (Year)*				<0.01**

*continuous variable; ** OR was calculated for increasing each unit.

Table 3. Result of multivariable logistic regression analysis of determinants of Newcastle disease infection in commercial layer chicken farms.

Variables	OR	95% CI for OR	P-value
Age of the farmer (Year)*	0.94**	0.87–0.99	0.04
Distance from the nearest farm			0.01
≤100m	3.23	1.27–8.39	
˃100m	1		
Number of houses in the farm			0.04
1	3.06	1.06–8.83	
>1	1		
Surrounding environment			0.001
Damp	5.27	1.96–14.20	
Dry	1		
Rearing different-aged birds together			0.02
Yes	4.76	1.25–18.19	
No	1		
Isolation of sick birds			0.04
No	2.85	1.07–7.55	
Yes	1		

*continuous variable; ** OR was calculated for increasing each unit.

contained six variables, i.e., age of the farmers (Odds Ratio [OR]: 0.94; 95% CI 0.87–0.99), distance from the nearest poultry farm (OR 3.23; 95% CI 1.27–8.39), number of houses on the farm (OR 3.06; 95% CI 1.06–8.83), surrounding environments (OR 5.27; 95% CI 1.96–14.20), rearing different-aged birds together (OR 4.76; 95% CI 1.25–18.19), and isolation of sick birds (OR 2.85; 95% CI 1.07–7.55). The Hosmer–Lemeshow test statistics for this model has a chi-square value of 2.57 and $p = 0.96$ and the predictive ability of this model denoted by AUC was 0.83.

4. Discussion

The epidemiologic research method we used in this study to identify the potential determinants of ND in commercial layer chicken farms in two districts with high poultry density in Bangladesh from September 2019 to February 2020 proved suitable. We relied on the farms whose owners came to hospitals for receive veterinary services for selecting cases and controls. It was documented that country's existing passive surveillance system based on the reports from sub-district veterinary hospitals might encounter under reporting and/or over reporting (Chowdhury et al., 2018). However, such probability did not affect the selection of case/control farms included in this study. This study endorses that ND in commercial layer farms was associated with lower biosecurity standard of the farms like rearing different-aged birds together; not isolating sick birds from healthy birds; and damp surroundings around the shed. In addition, previously known factor such as distance from the nearest poultry farm (Wiseman et al., 2018) was also associated with ND status of layer farms. This study did not find the associations of ND status with some of the already known important factors like frequency of floor cleaning (Chaka et al., 2013), distance from highway (Wiseman et al., 2018), flock size (Chaka et al., 2013), etc. These discrepancies may not only be due to differences in study designs and differences in study population but also probably for differences in demography of the study locations.

It was observed that the shorter the distance from the nearest poultry farm, the higher the risk of infection. This finding is in line with the findings of some other studies (East et al., 2006; Wiseman et al., 2018). It is proven that NDV is able to transmit through air, especially in humid and cool environment (Bhutia et al., 2017). Thus, risk of horizontal transmission of the virus increases between the farms with shorter distance between them. Closer farms might also encounter increasing risk of mechanical transmission of virus through contacts between farm personnel, utensils and vehicles (East et al., 2006). Further, wild or stray animals like cats, dogs and rodents spread the virus among farms with compromised biosecurity while roaming between farms (East et al., 2006).

We observed that rearing birds of different ages together increased the odds of having ND in the commercial layer farms. It was shown in a previous study that the occurrence of ND was strongly associated with the age of birds, and older birds had a relatively higher chance of having ND than relatively younger birds (Biswas et al., 2005). Thus, while mixing different aged birds together in a single farm, the older infected or carrier birds might also increase the risk of ND in younger birds.

In this study, we also found higher risk of ND in the farms where sick birds were not isolated from the healthy birds than the farms where isolation of sick birds were practiced (Table 3). Sick infectious birds shed viruses and might act as the source of infection for the rest of the birds on the farm (Zhou et al., 2020). Hence, keeping such birds with the healthier birds in the same place increased the risk of disease in the farms.

This study revealed that a farm with damp surroundings was more likely to have ND than a farm in dry surroundings. Moist and dampness might favor the propagation and existence of the NDV in the environment. The dampness and wet environment especially in wet seasons might increase the susceptibility of the birds (Bhutia et al., 2017). Moreover, wet and damp environments might attract wild scavenging birds and ducks. These birds might play roles in the transmission of the virus (Onapa et al., 2006).

One of the interesting findings of this study was odds of having ND was higher in farms with a single house than farms having multiple houses. This finding was opposite to our initial hypothesis. Providing a biologically plausible explanation of this finding was difficult as no such findings from previous studies were retrieved in our literature search. Farms having multiple houses might be relatively larger farms and have better farm management and biosecurity facilities than the farms with a single house. This finding could also be an effect of the characteristic of the study population. Descriptive analysis presented in Table 1 shows that most of the farms (≈76%) included in this study had a single house.

It was demonstrated that risk of ND was lower in farms managed by older farmers than farms managed by relatively younger farmers. We assume that farmers' age was positively correlated with their farming experience and the experienced farmer had good management techniques to mitigate the disease burdens. This statement was in line with the result of Susilowati et al. (2013). They found that older poultry farmers had comparatively a higher experience of farm management and biosecurity practice than the fresh entrepreneurs in this platform. However, our assumption could not be validated as the variable "experience of farming" was not included in the questionnaire.

Although insignificant in the final model, some of the variables were seemed to have significant relations with the ND infection in the farm in univariable analyses such as Distance from the nearest road or pathway; Cleanliness of farmyard; Floor condition; Condition of feeder and waterer; Disposal of carcasses. Some of the recent researches have also pointed out the role of these factors in the occurrence and spread of ND in commercial poultry farms (Alsahami et al., 2018; Belgrad et al., 2018; Wiseman et al., 2018; Chaka et al., 2013).

There were some limitations in this study. Diagnosis of ND based on clinical history, clinical signs and postmortem of dead birds carried out in veterinary hospitals might lack diagnostic accuracy. Similarities of postmortem findings of ND with other diseases could have introduced misclassification bias in this study. Mohammed et al. (2013) showed 76.48% agreement between clinical diagnosis and molecular diagnosis of ND, whereas Hasan et al. (2012) observed that clinical diagnosis of ND had 73.03% agreement with both serological and molecular diagnosis. Information about farms was achieved through face-to-face questionnaire interview where some information was relied on farmers' memory. These might introduce recall bias in the study. To the best of our knowledge, this was the first study which attempted to explore extensively to identify the determinants of ND in commercial layer farms in

Bangladesh. We used a generic epidemiologic research approach to fulfill the study objectives. Although farms were selected from two districts only, we believe that study outcome would not be varied that much if farms were selected from other parts of Bangladesh.

The present study identified a series of determinants such as farmer's age, the minimal distance from the nearest poultry farm (≤100m), presence of a single house in the farm premise, presence of damp environment surrounding the farms, rearing different aged birds together, and isolation of sick birds were associated with ND in the commercial layer farms of Bangladesh. These results are the important inclusions in our knowledge of ND. Alteration or manipulation of these factors might reduce the risk of ND in commercial layer farms in Bangladesh and in other developing countries, particularly in South Asia.

Ethical statement

Ethical approval is not required for this study. No animals were killed for the scientific purpose of this study.

Declarations

Author contribution statement

Shamsul Alam Roky: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Moumita Das, Suman Paul: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Sharmin Akter: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Aminul Islam: Performed the experiments.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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