Investigation of Risk Factors and Biosecurity Measures Associated with Prevalence of Newcastle Disease Virus in Broiler Farms

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A R T I C L E I N F O	A B S T R A C T
	Newcastle disease (ND) is one of the major viral diseases of poultry, remains a constant threat in poultry farms that causes huge economic losses every year. However, little is known regarding the potential risk factors of the disease in broiler. The study was undertaken to ascertain the potential risk factors and biosecurity measures in ND prevalent broiler farm. A total of 116 broiler farms were considered as a sample size from a different area of Barishal district of Bangladesh in which 19.83% ND prevalence was found. In the findings, the farmers (33.62%) who had training on poultry farming, were significantly less ND positive case (7.69%). Besides this, among the different risk factors and biosecurity measures only shed location, ventilation, vaccination status, feeder and waterer cleaning frequency, visitors accession, pets in the farm, use of disinfectant and seasonal variation had significant effect on ND outbreak. However, among all the factors only farmers training (OR=0.025; 95% CI: 0.001-0.509), shed location (OR=0.035; 95% CI: 0.003-0.390), vaccination against ND (OR=0.017; 95% CI: 0.001-0.435) and use of disinfectant daily (OR=0.011; 95% CI: 0.000-0.294) made a unique statistically significant contribution to the regression model and the predictors had approximately similar strength on reporting ND outbreak. In completion, ND can be reduced in broiler farms by improving the level of common biosecurity, farm management practices, and minimizing the potential risk factors through training of farmers, awareness build-up, and enforcement of these practices on the farms.

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Introduction

Poultry farming is one of the fast-growing sectors in Bangladesh, plays important role in socio-economic development (Al-Mamun et al., 2019). In the face of the rapid growth of this industry, it is rendered to certain diseases especially viral diseases (Karim 2003). Among the infectious agents, Newcastle disease (ND) is revealed as the significant viral disease of poultry in the world especially in developing countries (Rahaman et al., 2011). The etiological agent of ND is the Newcastle disease virus (NDV), avian paramyxovirus serotype 1 (APMV-1) of the genus Avulavirus belonging to the family *Paramyxoviridae* (Dimitrov et al., 2016).

In Bangladesh, ND is locally familiar as 'Ranikhet' disease (RD) and is highly contagious that susceptible to many species of domestic as well as wild birds (Al-Garib et al. 2003). As is observed, high mortality, morbidity, stress, decreased egg production, and hatchability are the most common in NDV infection, leads to high economic losses (Alexander, 2000). After that, NDV induces mortality up to 100% with or without clinical signs, even amongst vaccinated poultry as a result of faulty administration of vaccine (Chukwudi et al., 2012). The more important is, no treatment for NDV exists, only the use of prophylactic vaccines and biosecurity measures can reduce the likelihood of outbreaks (Orajaka et al., 1999).

Furthermore, several studies narrated the usual transmission of ND from farm to farm through live infected birds, selling or giving away sick birds, inhalation of the virus from contaminated air, and ingestion of contaminated feed and water (Ibitoye et al., 2013). Besides this, the farm equipment, feeder, waterer, clothing, litter or any others contaminated by the exposure to excretions from infected birds have been reported as a potential risk factor of ND transmission (Tu et al., 1998). Additionally, poor

biosecurity measures enhance the transmission leads to an outbreak. Biosecurity aimed at keeping the farms or areas out of viruses, basically all contagious diseases (Swayne and Jackwood, 2008). Nevertheless, in commercial poultry production systems, most of the case minimum biosecurity is practiced or in some cases non-existing and this may drive to the spread of multiple infections within or between farms (Permin, 1997). In commercial poultry farms, faulty disposal of dead poultry and used litter, multi-aged and multi-species poultry on the same farm, poor farm traffic control and sanitation were major biosecurity risks (Ameji et al., 2012; Wakawa et al., 2012). Though during the last few years several emerging diseases and unknown causes threaten the poultry industry and cause huge loss to the farmers (Al-Mamun et al., 2019), indeed that the farmers are not entirely aware of the most important viral diseases of poultry like ND. However, the current information on the occurrence of ND in the broiler, as well as the potential risk factors for the disease in the Barishal district of Bangladesh is limited. Besides this, current reports on biosecurity measures in broiler farms along with farmer's demographic data are insufficient. Meanwhile, updated information is essential for analysis on the epidemiology of ND in broiler to design prevention and control strategies throughout the country. Therefore, this study was aimed to ascertain the prevalence of ND in broiler associated with potential risk factors and biosecurity measures in herd-level as well as the farmer's demographic information in the Barishal district of Bangladesh.

Materials and Methods:

This study was conducted in different areas (Figure 1) of Barishal district of Bangladesh during the period of May 2017 to July 2018. The spatial location of the study sites was identified in the map (Figure 1) using ArcGIS-ArcMap version 10.8 (ESRI, USA) software. Data were collected through an interview schedule involving 116 respondents who were involved in broiler farming. Respondents were randomly selected from different parts of the Barishal district for collecting data to address the objectives. In this study, 116 respondents were interviewed to find out their socio-economic condition associated with the risk factor enhancing the outbreak of ND.
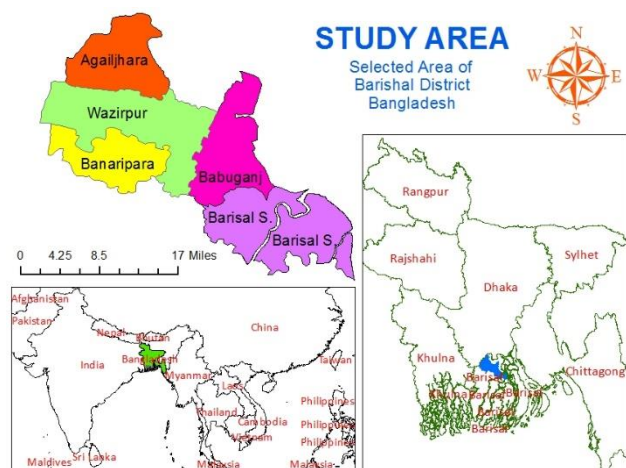

Figure 1. Geographical location of the study areas

Data were collected following the direct interviews by using a previously formed questionnaire that was designed by following the methods described by Thrushfield (2005). Interviews were normally conducted in the respondent's house during their leisure time by following a 'face to face' method. Major focal points of the interview were the general information about the farmers, the number of farms and birds, management of broiler farm, feed source, use of antibiotics, vaccination practice, farm biosecurity, Newcastle disease with its mortality, infectivity and risk factors with the also fundamental idea on the rearing of broiler. Problems and probable solutions related to broiler production were also focused. According to the climatic conditions of Bangladesh, the year was divided into three seasons namely summer, rainy, and winter. The Summer season was considered from March to June, rainy season from July to October, and winter was considered from November to February.

Clinical Diagnosis of ND

The Clinical diagnosis was made on the basis of clinical history from the responsible persons of the farms, recorded clinical signs, and gross lesions of affected chickens. The post-mortem examination of birds was performed and ND was diagnosed according to post-mortem findings which is similar to Hasan et al. (2012).

Statistical Analysis

The data obtained from the study were entered and arranged accordingly into a spreadsheet (Microsoft Excel-2010) and then transferred into Statistical Package for Social Sciences (SPSS) version 25.0 for analysis. Proportionality of outbreak of ND in broiler farm with the potential risk factors and farmer's demographic information were computed by Pearson's Chi-square. On the other hand, when more than 20% of cells of 2×2 contingency table had expected count less than 5, the p-value of continuity correction was considered but when the table was not 2×2 contingency then p value of Fisher exact tests was accounted. Among all variables, those with P<0.05 were subsequently considered for the Binary logistic regression model to screen the association between outcome variable (ND status) and all factors as well as farmer's status. All p-value P<0.05 was considered statistically significant. And lastly, the final Hosmer and Lemeshow-of-fit test were performed to estimate the model for the goodness of fit (Ibrahim et al., 2019).

Result and Discussion

The findings of this study revealed that the prevalence of ND was 19.83% in broiler in Barishal district; with evidence of some potential risk factors and common practices at the farm level. However, the finding was a little bit of higher than reported by Hasan et al. (2012) and Das et al. (2018) where they reported 14.89% and 8.9% respectively. On the other hand, Rahman et al. (2011) was found that 23% ND prevalence in broiler which is some extent of higher than our findings. This variation may be due to different geographical locations with sample sizes.

Table 1, shows the demographic data of farmers indicated that among all the variables only the training status of the farmer had significant (P<0.05) association

with ND positive case in broiler. In our findings, 33.62% of the farmers had training on poultry farming, among them only a small proportion (7.69%) was in ND positive case whether 25.97% farmers of who had no training on poultry farming was in ND positive case. These findings have a close association with Alam et al. (2016) who reported that only 27.5 % of the farmers had received training on poultry farming. Among the broiler farmer, 79.31% of them were male in comparison (P>0.05) to females (20.69%). Though, our findings are close to Islam et al. (2019) where he reported that 18.2 % of rural women had high participation in poultry farming but the findings of Belgrad et al. (2018) was completely reverse than our findings. This may be due to the variation in the socioeconomic conditions in different regions. The people involved in broiler farming in the studied area, the majority (36.28%) of them aged between ≥30 to <40 years that are closely related to the findings of Shahjahan & Bhuiyan, (2016). On other hand, the highest proportion (35.34%) of farmers were SSC passed whereas M. Rahman et al. (2002) found 71.43% and 24.29% of the farmers have a high and medium level of knowledge respectively, but Islam et al., (2019) stated that 43.6% of women farmers had secondary education. The findings of educational status may have variation due to different socioeconomic statuses in different region and may also have their different sample size. However, in our study, the variable "educational status" had no significant (P>0.05) association with ND outbreak (Table 1). In our findings, most of the farmers (37.93%) were engaged in farming about >5 to ≤ 10 years, but Belgrad et al. (2018) narrated that 46.5% of farmers had 3 to 12 years' experience on farming. Additionally, he also reported that 47.3% of farmer's main occupation was self-business. In which, we found highest number of farmers (46.55%) main profession was other than agriculture and services, in this context the findings have a little bit of similarities.

In the study area, the farming condition was considered to determine the ND outbreak in broiler. Among the entire four variables, only two variables (broiler shed location and ventilation facilities) had significant (P<0.01 and P<0.05 respectively) association with ND outbreak (Table 2). The result indicated that, the farm located inside the home yard had approximately five times higher ND positive case (46.88%) than located outside (Table 2). Moreover, the farm had sufficient ventilation, reduced the ND case to 13.51% whereas the farm with insufficient ventilation had 30.95% of ND positive case. Studies have shown that 21.7% of farm had no ventilation (Belgrad et al., 2018). Though in rural area are more prone to conventional farming, our data indicated that conventional farming systems were more ND positive cases (24.05%) than the modern farming system.

The potential risk factor analysis was performed at the farm level. At the same time, the age of birds and seasonal influence were also analyzed to ascertain their rapport with ND exposure in broiler farms. In our findings, among the studied risk factor, only two factors including vaccination status and seasonal influence had a significant association with ND outbreak by, P<0.05 and P<0.01 respectively (Table 3). Broiler farms with no vaccination status had 62.96% of ND positive case, which is close to the findings of other authors (Das et al., 2018) report of 68.7% positive for ND in broiler. On the other side, our data indicated the rainy season had the highest proportion of ND positive cases (35.48%) and the summer the lowest (12.24%). The findings of Rahman and Adhikary (2016) also suggested our result, whereas Islam et al. (2003) reported that ND was higher in summer in comparison to the rainy and winter season. The issue of the seasonal prevalence is controversial and may vary according to the environmental, nutritional, and socioeconomic conditions, under which poultry is kept.

Table 1. Frequencies of ND in broiler according to poultry farmers' demographic characteristics.

Variables	Category Level	RSF		ND Status				P Value
				n=116		%		
		(n)	%	N	P	N	P	
Age (Years)	Less than 20 years	6	5.17	4	2	66.67	33.33	0.567[c]
	From ≥20 to <30	21	18.10	18	3	85.71	14.29	
	From ≥30 to <40	42	36.21	34	8	80.95	19.05	
	From ≥40 to <50	35	30.17	29	6	82.86	17.14	
	≥ 50 years	12	10.34	8	4	66.67	33.33	
Sex	Male	92	79.31	74	18	80.43	19.57	1.000[b]
	Female	24	20.69	19	5	79.17	20.83	
Education	Illiterate	5	4.31	3	2	60.00	40.00	0.529[c]
	Primary	22	18.97	16	6	72.73	27.27	
	Secondary	41	35.34	33	8	80.49	19.51	
	Higher Secondary	34	29.31	29	5	85.29	14.71	
	Bachelor and above	14	12.07	12	2	85.71	14.29	
Farmers Training	No	77	66.38	57	20	74.03	25.97	0.020[a]
	Yes	39	33.62	36	3	92.31	7.69	
Experience on poultry farming (Years)	<1	7	6.03	4	3	57.14	42.86	0.342[a]
	From >1 to ≤ 5	39	33.62	30	9	76.92	23.08	
	From >5 to ≤ 10	44	37.93	37	7	84.09	15.91	
	>10	26	22.41	22	4	84.62	15.38	
Farmers main occupation	Agriculture	46	39.66	41	5	89.13	10.87	0.146[a]
	Service	16	13.79	12	4	75.00	25.00	
	Other than agriculture and services	54	46.55	40	14	74.07	25.93	

RSF: Number of respondent farmers, N: Negative, P: Positive, [a]Pearson's chi-square test; [b]After continuity correction; [c]Fisher exact tests; significant at 1% (P<0.01), Significant at 5% (P<0.05); N=Number.

Table 2. Frequencies of ND in broiler according to broiler farm characteristics.

Variables	Category Level	RSF		ND Status n=116		%		P-Value
		(n)	%	N	P	N	P	
Farming System	Conventional	79	68.10	60	19	75.95	24.05	0.096[a]
	Modern	37	31.90	33	4	89.19	10.81	
Housing Floor Condition	Mud Poultry House	5	4.31	3	2	60.00	40.00	
	Concrete	34	29.31	29	5	85.29	14.71	0.326[c]
	Bamboo/Wood	77	66.38	61	16	79.22	20.78	
Broiler Shed Location	Inside	32	27.59	17	15	53.13	46.88	0.000[a]
	Outside	84	72.41	76	8	90.48	9.52	
Ventilation	Insufficient	42	36.21	29	13	69.05	30.95	0.024[a]
	Sufficient	74	63.79	64	10	86.49	13.51	

RSF: Number of respondent farmers, N: Negative, P: Positive, [a]Pearson's chi-square test; [b]After continuity correction; [c]Fisher exact tests; Significant at 1% (P<0.01), Significant at 5% (P<0.05); n=Number.

Table 3. Potential Risk Factor for ND Status Associated with Broiler Farm.

Variables	Category Level	RSF		ND Status n=116		%		P-Value
		(n)	%	Negative	Positive	Negative	Positive	
Vaccination status	No	27	23.28	10	17	37.04	62.96	
	Only ND Vaccine	26	22.41	23	3	88.46	11.54	0.000[a]
	ND + Other Vaccine	63	54.31	60	3	95.24	4.76	
Types of Feed	Ready Feed	105	90.52	86	19	81.90	18.10	
	Handmix	4	3.45	3	1	75.00	25.00	0.186[c]
	Local	7	6.03	4	3	57.14	42.86	
Use of Antibiotics	No	33	28.45	26	7	78.79	21.21	
	Yes	75	64.66	60	15	80.00	20.00	0.856[a]
	Others	8	6.90	7	1	87.50	12.50	
Seasonal Influence	Summer	49	42.24	43	6	87.76	12.24	
	Rainy	31	26.72	20	11	64.52	35.48	0.034[a]
	Winter	36	31.03	30	6	83.33	16.67	
Age of Birds (Days)	≤10	26	22.41	23	3	88.46	11.54	
	11 To ≤20	32	27.59	27	5	84.38	15.63	
	21 To ≤30	38	32.76	29	9	76.32	23.68	0.222[a]
	31 To ≤40	12	10.34	7	5	58.33	41.67	
	>40	8	6.90	7	1	87.50	12.50	

RSF: Number of respondent farmers, N: Negative, P: Positive, [a]Pearson's chi-square test; [b]After continuity correction; [c]Fisher exact tests; Significant at 1% (P<0.01), Significant at 5% (P<0.05); n=Number.

According to Table 4, some of the common biosecurity practices in broiler farms were encountered as study variables. In this accordance, our study transpired that visitor's access, pet in farm areas, feeder cleaning frequency, waterer cleaning frequency, and lastly, the seasonal variation had a significant relation with ND occurrence by P<0.5. Likewise, Table 4 shows, ND positive farm of 24.14% had access to visitors and 6.9% no access to visitors. Alsahami et al. (2018) stated that free entry of visitors significantly (P<0.01) associated with higher seroprevalence to ND, agreed with our findings. Accordingly, pets on the farm or in the same premises are one of the important factors for assessing biosecurity. Access of pets or presence in farm areas also breaches the farm biosecurity which may imply the ND occurrence. In fact, good biosecurity could protect the poultry flocks from the ND as reported by the other authors (Yune and Abdela, 2017). On other side, farms practice the cleaning of waterer and feeder weekly had more trends to ND positive case by 38.46% and 35.14% respectively. Belgrad et al. (2018) reported that ND positive cases were higher for those who clean the waterer once per three or four weeks and feeder once in a week, these findings also suggested our result.

Additionally, cleaning is the second most effective element of biosecurity followed by disinfection (FAO 2008). The only regular cleaning and disinfecting activities by spraying inside and outside of the poultry shed was observed by Rimi et al. (2017). As Table 4 shows, the daily use of disinfectant reduced the ND positive case indicated by 5.41%, while the farm never uses disinfectant placed 42.86%. Likewise, Guan et al. (2014) suggested that disinfectant especially surface decontamination foam were more effective for the inactivation of Newcastle disease virus.

According to the Table 5, Binary logistic regression was performed to assess the impact of several factors on the likelihood that the broiler farm had an outbreak of Newcastle disease in the Barishal district of Bangladesh. The model contained ten independent variables (Farmers Training, Broiler Shed Location, Ventilation, Vaccination Status, Feeder Cleaning Frequency, Waterer Cleaning Frequency, Access of Visitors, Pets in The Farm or in Same Premises, Using Disinfectant and Seasonal Influence).

Table 4. Biosecurity Practices in broiler farm Associated with ND Status

Variables	Category Level	RSF		ND Status				P-Value
				n=116		%		
		(n)	%	N	P	N	P	
Access Of Visitors	No	29	25.00	27	2	93.10	6.90	0.044[a]
	Yes	87	75.00	66	21	75.86	24.14	
Pets in the Farm or in Same Premises	No	81	69.83	70	11	86.42	13.58	0.010[a]
	Yes	35	30.17	23	12	65.71	34.29	
Wild Birds Have Contact	No	24	20.69	20	4	83.33	16.67	0.882[b]
	Yes	92	79.31	73	19	79.35	20.65	
Farm Animals in Same Premises	No	74	63.79	62	12	83.78	16.22	0.195[a]
	Yes	42	36.21	31	11	73.81	26.19	
Feeder Cleaning Frequency	Weekly	37	31.90	24	13	64.86	35.14	0.018[a]
	Daily	53	45.69	46	7	86.79	13.21	
	Every Time After Use	26	22.41	23	3	88.46	11.54	
Waterer Cleaning Frequency	Weekly	26	22.41	16	10	61.54	38.46	0.022[a]
	Daily	48	41.38	40	8	83.33	16.67	
	Every Time After Use	42	36.21	37	5	88.10	11.90	
Use of Disinfectant	Not at All	28	24.14	16	12	57.14	42.86	0.001[a]
	Often	51	43.97	42	9	82.35	17.65	
	Daily	37	31.90	35	2	94.59	5.41	
Disposal Of Dead Birds	Thrown in Bush	38	32.76	28	10	73.68	26.32	0.616[c]
	Thrown in Water	21	18.10	17	4	80.95	19.05	
	Bury	54	46.55	45	9	83.33	16.67	
	Burn	3	2.59	3	0	100.0	0.00	
Disposal Of Litter	Compost	30	25.86	27	3	90.00	10.00	0.106[a]
	Thrown in Bush/Road Side	49	42.24	35	14	71.43	28.57	
	Thrown in Water	37	31.90	31	6	83.78	16.22	

RSF: Number of respondent farmers, N: Negative, P: Positive, [a] Pearson's chi-square test; [b] After continuity correction; [c] Fisher exact tests; Significant at 1% (P<0.01), Significant at 5% (P<0.05), n=Number.

Table 5. Regression model of factors associated with broiler farms leads to Newcastle diseases occurrences.

Variables	Category Level	B	Wald	P-Value	O.R.	95% C.I. for O.R.	
						Lower	Upper
Farmers Training	No		Ref.				
	Yes	-3.688	5.758	0.016	0.025	0.001	0.509
poultry shed location	Inside		Ref.				
	Outside	-3.338	7.445	0.006	0.035	0.003	0.390
ventilation	Insufficient		Ref.				
	Sufficient	-0.091	0.009	0.923	0.913	0.145	5.737
Vaccination status	No		Ref.				
	Only ND	-4.088	6.057	0.014	0.017	0.001	0.435
	ND+Others	-4.468	9.317	0.002	0.011	0.001	0.202
Feeder cleaning frequency	Weekly		Ref.				
	Daily	-0.413	0.110	0.741	0.662	0.058	7.609
	Every Time After Use	-2.015	1.898	0.168	0.133	0.008	2.344
Waterer cleaning frequency	Weekly		Ref.				
	Daily	1.310	0.598	0.439	3.707	0.134	102.576
	Every Time After Use	1.909	1.306	0.253	6.745	0.255	178.213
Access of visitors	No		Ref.				
	Yes	2.068	2.066	0.151	7.908	0.471	132.677
Pets in the farm or in same premises	No		Ref.				
	Yes	0.487	0.188	0.665	1.627	0.180	14.683
Using Disinfectant	Not At All		Ref.				
	Often	-2.374	3.669	0.055	0.093	0.008	1.057
	Daily	-4.473	7.281	0.007	0.011	0.000	0.294
Seasonal Influence	Summer		Ref.				
	Rainy	1.164	1.098	0.295	3.204	0.363	28.270
	Winter	-0.243	0.044	0.833	0.784	0.082	7.496
Constant		3.323	1.909	0.167	27.734		
R^2= 0.477 (Cox & Snell R Square) , 0.756 (Nagelkerke R Square)							

Hosmer and Lemeshow Test P value: 0.921; Ref.= Reference category; Significant at 1% (P<0.01), Significant at 5% (P<0.05). C.I.= Confidence Interval; O.R. = Odd Ratio.

The full model containing all predictors was statistically significant, $\chi2$ (10, $N = 116$) = 75.109, P<0.001, indicating that the model was able to distinguish between farmers whose farm was positive and negative for Newcastle disease outbreak. The model as a whole explained between 47.7% (Cox and Snell R square) and 75.6% (Nagelkerke R squared) of the variance in Newcastle disease status, and correctly classified 90.2% of cases. As shown in Table 5, only four (Farmers training, Broiler shed location, Vaccination status, and Using disinfectant) of the independent variables made a unique statistically significant contribution to the model and all the four predictors had approximately similar strength on reporting Newcastle disease outbreak. Among the four significantly independent variables, the vaccination status (ND+ another vaccine) had had to record a lower odd ratio of 0.011 which are less than one, indicating that for every additional farm practices vaccine against ND with other diseases were 0.011 times less likely to the outbreak of ND in broiler. Additionally, this is also similar for them who using disinfectant daily in their broiler farm, controlling for other factors in the model. Hence, the p-value (P>0.05) of Hosmer and Lemeshow test indicate the final model is fit. Although a total of 116 broiler farms in Barishal district in Bangladesh were included in the study, the sample size and geographic location of broiler farms might be the most likely reason for some of the very wide confidence intervals in the inferential analysis of several factors and subsequent disease outcome.

Conclusion

The present study implicated that poultry shed located inside the home yard having insufficient ventilation with inappropriate farm biosecurity measures relating irregular cleaning of feeder and waterer equipment, unfamiliar with the use of disinfectant regularly, visitor accession and pets or other animals in farm area were more recumbent to exposure of NDV. Likewise, the rainy season had an influence on ND occurrence. Another important factor vaccination, even though the prevalence of ND hindered by practicing only against NDV, but practicing with others vaccine against all possible pathogen are more prone to decreasing the chance of ND occurrence. However, the findings of this study implies that NDV infection can be greatly reduced in broiler farms by improving the level of common biosecurity practices, minimizing the potential risk factors and improving the farm management practices through training of farmers, awareness build-up, and enforcement of these practices on the farms. This study also identified areas in which further research could be on understanding the seroprevalence of ND with a larger sample sizes in layer birds as well as broiler. Therefore, updated epidemiological data on poultry diseases is essential that could be used to design prevention and control strategies.

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Conflict of interest

Authors declare that there is no conflict of interest.

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