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ORIGINAL ARTICLE

Seroprevalence of major avian respiratory diseases in broiler and sonali chicken in selected areas of Bangladesh

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ABSTRACT

Objective: This study was conducted to investigate different respiratory diseases in broiler and sonali birds in some selected districts of Bangladesh.
Materials and Methods: We were collected a total of 460 blood samples from 46 farms with 36 broiler farms and 10 sonali farms (cross-breed) from 2015 to 2017. All the collected serum samples were tested for determining specific antibodies of avian rhinotracheitis (ART) virus, infectious laryngotracheitis (ILT) virus, infectious bronchitis (IBV) virus, and *Ornithobacterium rhinotracheale* (ORT) infection using commercially available enzyme-linked immunosorbent assay kits.
Results: The overall seropositivity was highest in ORT (45.9%), followed by IBV (37.6%), ART (2.6%), and ILT (0.4%). Out of 360 broiler samples, highest seropositivity was recorded in ORT (43.3%) and lowest in IBV (31.4%). Surprisingly, no broiler samples were found positive for ART and ILT. In case of sonali, the seropositivity was highest in IBV (60%) and lowest in ILT (2%). With respect to types of birds and age groups, the seropositive percentage of all four pathogens was found higher in sonali than broiler. Between two age groups of sonali, the seropositive percentage of ART (12%), ORT (55%), ILT (2%), and IBV (60%) was highest at 21–60 weeks of age compared to 5–20 weeks of age. However, based on location, the seropositive of ORT and IBV was highest in Jamalpur (63.3%) and Fulbariya and Trishal (50%) and lowest in Sreepur (16.7%) and Jamalpur (3.3%).
Conclusion: The four pathogens are ubiquitous in nature for the sonali chickens, and the prevalence of ORT and IBV was the most prevalent viruses in the study areas. This study indicates a need for improved surveillance and characterization of ORT and ART circulating in all types of poultry in Bangladesh.

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Introduction

The poultry industry is an important subsector of agriculture, has generated huge employment opportunity, increases the supply of good quality protein, ensured food security, involved in country's economic growth and reduced poverty level in both urban and rural areas of Bangladesh [1,2]. There are several constraints that hinder the development process in poultry sector; among them, disease is the major one. Various pathogens, such as bacteria, virus, fungus, parasite, etc., are responsible for causing diseases in poultry and they attack their different body systems [3]. Respiratory tract, an important part of poultry body system, frequently affected by pathogens causing respiratory diseases [4]. Several pathogens such as bacteria, viruses, fungi, and environmental factors initiate the respiratory diseases of chicken. Viral and bacterial pathogens are responsible for causing most of the respiratory diseases, namely, avian rhinotracheitis (ART), infectious laryngotracheitis (ILT),

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infectious bronchitis virus (IBV), *Ornithobacterium rhinotracheale* (ORT), etc., that lead to huge economic losses in poultry industry [5]. Bacterial pathogens colonize the respiratory system after primarily introducing of viral or environmental stress for pathogens [6].

ART virus is also known as avian pneumovirus (APV), important respiratory viral disease affecting both chickens and turkeys [7]. ART was first identified in Bangladesh at 2016 by Ali et al. [8] in broiler breeder, layer, and Sonali chicken (cross-breed between Rhode Island Red cocks and Fayoumi hens). Sneezing, depression, coughing tracheal rales, swollen infraorbital sinus, ocular and nasal discharges, and foamy conjunctivitis are the major signs associated with the disease [9]. This virus also causes swollen-head syndrome in broiler breeders and broiler [10] and dropped egg production in layers [11]. ORT, another important bacterial pathogen, belonging to the super family of RNA containing bacteria causes respiratory infections and affects air sac [12]. It has been reported throughout the world except Bangladesh and mainly affect in turkey and chickens but other species can often be infected with this pathogen [13]. This can act as primary or secondary agents depending on immune status, environmental factors, and pathogenicity of related strain and also the presence of other pathogens [14]. ILT virus, an important virus, causes respiratory infection in birds belonging to the family *Herpesviridae*. Thus, virus mainly affects the chickens and characterizes by sneezing, nasal discharge, swollen infraorbital, and nasal sinuses and sometime affects eye leads to conjunctivitis [15]. The disease more frequently occurs in the areas of intensive poultry production and outbreak causes' high economic loss as it increases mortality, reduces egg production, and declines growth rates [16]. Another important viral pathogens responsible for respiratory disease, namely, Avian IBV belonging to the genus *Gammacoronavirus* of the *Coronaviridae* family [17]. It can affect chickens of all ages, and primarily it replicates in respiratory tract, and later, it can move to epithelial cells gut, oviduct, and kidney, results decreased egg production and growth performance and sometimes attract other pathogens [18]. So far, it has been reported in chickens, turkey, pigeon, pheasant, Guinea fowl, and peafowl [17].

Despite the country with a large number of poultry farms, only a few reports are available in Bangladesh regarding respiratory infections. A few works have been done on IBV [19] and ILT [15,20,21], but the amount is quite scanty. In view of this, the present research work was conducted to perform a comparative serological study to check the presence of several viral and bacterial pathogens antibodies in chickens with special emphasis on ART, ILT, IBV, and ORT, as well as to determine the distribution of its specific antibody in respect of the types of birds (broiler and sonali), age groups, and locations of farms of different districts of Bangladesh.

Materials and Methods

Sample source

We were collected a total of 460 blood samples from broiler and sonali chickens located at four different districts, namely, Mymensingh, Gazipur, Jamalpur, and Bogura of Bangladesh. For broiler samples, six upazilas were selected where four upazilas (Muktagacha, Fulbariya, Trishal, and Valuka) from Mymensingh and one upazila from each Gazipur (Sreepur) and Jamalpur (Jamalpur sadar) districts, and a total of 360 blood samples comprising 60 from six farms of each upazilas were collected. All the sonali blood samples (100) were collected from ten farms of Bogura sadar upazilas of Bogura district, and each farm shared 10 samples. All the selected broiler and sonali farms had a history of respiratory problems and had not been vaccinated against the studied pathogens. During the sample collection, types (broiler and sonali), age of birds, and locations were recorded.

Sample collection, transportation and serum preparation

Approximately, 2–3 ml blood was collected from the wing vein of randomly selected birds showing respiratory signs for 2 weeks using 3 ml disposable syringe. The syringe containing blood was kept in standing position for clot formation, and serum was harvested by decanting method as described by Barberis et al. [22]. Later, the collected serum was taken to an eppendorf tube and transferred to laboratory maintaining cool chain and subjected to centrifuge at 3,000 rpm for 5 min for removing the remaining clots, insoluble materials, and red blood cells. Finally, the cleared serum samples were stored at −20°C until performing the test.

Serological test

Indirect enzyme-linked immunosorbent assay test (ELISA) was done to analyze the serum samples. Commercially available ELISA kits (BioChek®, Reeuwijk, Netherlands) of ART, ORT, ILT, and IBV were used to detect the antibodies. Serum samples were diluted at 1:50 dilution in dilution buffer, followed by 1:10 dilution, and final dilution of 1:500 was used as working samples for respective ELISA. 100 µl of negative and positive controls was added into antibody coated plate wells A1, B1 and C1, D1, respectively, remaining 92 wells were filled with samples. After that, plate incubated at room temperature for 30 min in case of IBV and 60 min in case of ART, ORT, and ILT. Meanwhile, conjugate and wash solutions were prepared according to manufacturer's instructions. After incubation, contents of wells were aspirated and washed four times with wash buffer (350 µl). Then, the plate was inverted and tapped firmly on absorbent paper to remove the moisture. Then, 100 µl conjugate reagents were added on each well. Again,

the plate was incubated for 30 min at room temperature in case of IBV and 60 min in case of ART, ORT, and ILT, respectively. After incubation, washed the plate with wash buffer following the procedure described previously. Then, the wells of microtiter plate were filled with substrate and incubated for 15 min at 22°C–27°C in case of IBV and 30 min for ART, ORT, and ILT. After incubation, the reaction was stopped by adding 100 µl stop solutions. Finally, the optical density value of each sample was measured at 405 nm within 15 min after adding stop solution, and the results were recorded by calculating sample to positive (S/P) ratio and antibody titer.

Interpretation of results

For each sample, S/P ratio and antibody titer were calculated using the following formulas.

For S/P ratio

$$\frac{S}{P} = \frac{OD\ of\ sample - OD\ of\ negative\ control}{OD\ of\ postive\ control - OD\ of\ negative\ control}$$

For antibody titer

ART: $\log_{10}(\text{titer}) = 1.0 \times \log_{10}(S/P) + 3.52, \text{titer} = 10^{\log_{10}}(\text{titer})$

ORT: $\log_{10}(\text{titer}) = 1.7 \times \log_{10}(S/P) + 3.16, \text{titer} = 10^{\log_{10}}(\text{titer})$

ILT: $\log_{10}(\text{titer}) = 1.1 \times \log_{10}(S/P) + 3.61, \text{titer} = 10^{\log_{10}}(\text{titer})$

IBV: $\log_{10}(\text{titer}) = 1.0 \times \log_{10}(S/P) + 3.62, \text{titer} = 10^{\log_{10}}(\text{titer})$

Statistical analysis

All the data were incorporated in SPSS (version 25) [23], and descriptive studies were performed for determining the association among different variable using Chi-square test.

Results

Overall seropositivity of four respiratory diseases according to type of birds

In this study, 460 serum samples collected from broiler and sonali were tested against three viruses and one bacterium using indirect ELISA. Among the pathogens, overall seropositivity was highest in ORT (45.9%) and lowest in ILT (0.4%) viruses. Out of 360 broiler samples, highest seropositivity was recorded in ORT (43.3%) followed by IBV (31.4%). Interestingly, no broiler samples were found positive for ART and ILT viruses. Among 100 sonali samples, highest number of seropositivity was found in IBV (60%) followed by ORT (55%), ART (12%), and ILT (2%), respectively. Compared to broiler, the seropositive percentage was higher in sonali. The seropositive difference among the four agents between broiler and sonali birds was found statistically significant ($p < 0.05$) (Table 1).

Age wise seropositivity of four respiratory diseases in broiler and sonali birds

In this study, serum samples were collected from two age groups, in case of broiler age was ranged from 3 to 4 weeks, and sonali age was varied from 5 to 40 weeks. The seropositive percentages regarding age groups were as similar as types of birds mentioned in Table 1. In case of sonali birds, samples were categorized into two age groups where 40 samples were collected from 5 to 20 weeks age group and 60 samples were from 21 to 40 weeks age group. The seropositive samples were significantly higher in 21–40 weeks age group compared to 5–20 weeks (Table 2).

Location wise seropositivity of four respiratory diseases in broiler and sonali birds

The serological results of different respiratory diseases based on locations are shown in Table 3. In broiler chicken, it was observed that ORT and IBV were highly prevalent in all study upazila areas, and the highest seropositive of ORT was observed in Jamalpur (63.3%) followed by Trishal (56.7%), Fulbariya (55%), Muktagacha (40%), Valuka (28.3%), and Sreepur (16.7%), respectively. Similarly IBV was observed highest in Fulbariya (50%) and Trishal (50%) followed by Muktagacha (31.7%), Valuka (30%), Sreepur (23.3%), and Jamalpur (2%), respectively. On the other hand, no broiler serum samples of all six study upazilas were found positive for ART and ILT viruses. The difference of ORT and IBV based on location was found statistically significant ($p < 0.05$). In case of sonali, they were

Table 1. Overall seropositivity of different respiratory diseases according to types of birds and age groups.

Types of birds	Age groups	Number of samples tested	Number of seropositive samples			
			ART	ORT	ILT	IBV
Broiler	3–4 weeks	360	0 (0%)	156 (43.3%)	0 (0%)	113 (31.4%)
Sonali	5–40 weeks	100	12 (12%)	55 (55%)	2 (2%)	60 (60%)
Total		460	12 (2.6%)	211 (45.9%)	2 (0.4%)	173 (37.6%)
p value			0.000	0.038	0.007	0.000

ART = avian rhainotracheitis, ORT = *Ornithobacterium rhinotracheale*, ILT = infectious laryngotracheitis, IBV = infectious bronchitis virus.

Table 2. Serological detection of different respiratory diseases in sonali according to different age groups.

Age groups	Number of samples tested	Number of seropositive samples			
		ART	ORT	ILT	IBV
5–20 weeks	60	0 (0%)	17 (28.3%)	0 (0%)	20 (33.3%)
21–40 weeks	40	12 (12%)	38 (95%)	2 (5%)	40 (100%)
Total	100	12 (12%)	55 (55%)	2 (2%)	60 (60%)
p value		0.000	0.000	0.080	0.000

ART = avian rhainotracheitis, ORT = *Ornithobacterium rhinotracheale*, ILT = infectious laryngotracheitis, IBV = infectious bronchitis virus.

Table 3. Serological detection of different respiratory diseases in broiler and sonali according to location of the farms.

Types of birds	Location of the farms	Number of samples tested	Number of seropositive samples			
			ART	ORT	ILT	IBV
Broiler	Muktagacha	60	0 (0%)	24 (40%)	0 (0%)	19 (31.7%)
	Fulbariya	60	0 (0%)	33 (55%)	0 (0%)	30 (50%)
	Trishal	60	0 (0%)	34 (56.7%)	0 (0%)	30 (50%)
	Valuka	60	0 (0%)	17 (28.3%)	0 (0%)	18 (30%)
	Sreepur	60	0 (0%)	10 (16.7%)	0 (0%)	14 (23.3%)
	Jamalpur	60	0 (0%)	38 (63.3%)	0 (0%)	2 (3.3%)
	p value			0.000		0.000
Sonali	Bogura	100	12 (12%)	55 (55%)	2 (2%)	60 (60%)
	p value		0.000	0.038	0.007	0.000

ART = avian rhainotracheitis, ORT = *Ornithobacterium rhinotracheale*, ILT = infectious laryngotracheitis, IBV = infectious bronchitis virus.

found to be seropositive for all the tested respiratory diseases with highest seroprevalence in IBV (60%) and lowest in ILT (2%).

Discussion

This study was conducted to investigate different respiratory diseases in broiler and layer through indirect ELISA. The results showed that overall seropositive percentage of ART, ORT, ILT, and IBV viruses was 2.6%, 45.9%, 0.4%, and 37.6%, respectively. Compared to broiler, the seropositive was found more in sonali chicken. Interestingly, no samples were found positive against ART virus in case of broiler but 12% were recorded in sonali birds. The past seroprevalence study of ART in Bangladesh found overall 53.29% chickens including 35.57% sonali and 50.85% layer chickens [8]. On the contrary, Sharma et al. [24] found the higher number of ART positive sample 31.7% in broiler flocks in Grenada, West Indies. A higher rate of seropositivity in the later study was observed as this area had the highest density of broiler flocks. Detection of ART antibodies, which is also known as APV, in broiler was negative compared to sonali birds (0% *vs.* 12%), this higher positive results in sonali to broiler might be due to the life span, that longer life span may allow the sonali birds to develop stronger immunity against the virus detected [24]. In Egypt, similar study was carried out by Nagy et al. [10] reported 21.7% ART seropositive broiler samples. Another reason of lower seropositive in this study may be due to the fact that infected chicken may not necessarily produce humoral antibodies or antibodies may be at very low levels at the time of the sampling [25].

In this study, the seropositive percentage of ORT was higher in both broiler and sonali birds (43.3% and 55%), which is in agreement with the results of Mehrabanpour et al. [13] who has reported 42.5% seropositivity of ORT in broiler chickens in Fars province, Iran and also in agreement with the findings of Ghanbarpour and Salehi [26] for large broiler flock who reported 42.9% seropositivity of ORT in Kermanshah province, west Iran. In addition, comparatively higher or lower seropositivity of ORT in broiler was reported in different parts of the world [12,27]. The difference among different studies might be due to variation in geo-climatic condition, age and exposure of weather which might contribute to variable prevalence of respiratory diseases [13].

In this study, entire 360 broiler serum samples were found negative for ILT virus but 2% sample was positive for sonali birds. It was previously reported that layer chickens are mostly associated with ILT infection in Bangladesh [15]. Another study in Gazipur district in Bangladesh reported 81.47% ILT seropositive in layer chicken [20]. The higher seropositivity in layer chicken might be that the disease is more prevalent in older age than young. Lower findings of ILT in this study might be due to the samples collected from broiler, and sonali had not been exposed by the virus previously [20].

A total of 460 serum samples were collected from sonali and broiler chickens. In this study, the overall seropositive of IBV was found to be 37.6%, of which 60% from sonali and 31.4% from broiler. Similar findings were previously reported where 71.83% and 23.82% of IBV from Sonali and broiler chickens in Bangladesh were found [19], while a higher seropositive of 54% of IBV from local chickens was recorded in Ghana [18]. Lower seropositive rates ranging from 17.52% to 21.2% of IBV from broiler and local chickens were reported from other geographical areas [28–31]. The differences in IBV seropositive might be linked with biosecurity, management practices, vaccination status, and environmental factors.

Age factor also has significance influence to the occurrence of respiratory diseases in birds. In this study, higher aged group chickens were most susceptible to respiratory diseases. ART specific antibodies were found higher in Sonali at 21–40 weeks of age, which supports the findings of Rahimi [32] who reported 48.1% and 93.2% of ART in broiler of 6–8 weeks of age and broiler breeder of 56–72 weeks of age, respectively. With respect to age, the seropositive of ORT in broiler and sonali was higher in later age. Allymehr [33] and Mousavi et al. [14] reported 44.2% and 55.6% of ORT seropositive in broiler flock at 40–45 days of age, which are in agreement with the current study. Similar findings were also observed in some other studies of different parts of the world [34–36]. In addition, ILT was recorded only in sonali chicken at 21–40 weeks of age, which was lower than several previous findings [27,37,38]. On the contrary, IBV was found in both broiler and sonali chickens but higher at later ages. The current findings are resembled with various previous results [39–41]. Maternally derived antibody remains up to 2 weeks of age, and subsequently, it decreases up to 7 weeks of age. But, during this period, chickens are susceptible to IBV if no vaccine is given against IBV to the broiler flock, and no biosecurity is maintained in the chicken farm.

Conclusion

From the above findings, it may be concluded that the percentage of ORT and IBV in broiler and sonali birds is higher than remaining two pathogens (ART and ILT). In Bangladesh, infectious bronchitis causes a great deal of economic losses through poor weight gain and production drop. High seropositive was found for ORT that indicates ubiquitous presence of the organism in study areas. As there is no vaccination practiced for ORT, it indicates that a larger portion of poultry flock is experiencing new field infection. Further investigation could be done to know the molecular characteristics and surveillance of the four respiratory diseases.

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Competing interests

The authors declare that they have no competing interests.

Authors' contributions

ZAB, MZA, ZUMK, and MG: Conceived and designed this study. ZAB, MZA, and MMM: Collected samples performed the experiments and laboratory analyses. MZA and MMM: Performed the data analyses. MMM and MAB: Drafted the manuscript. MZB, MZA, and NA: Revised the manuscript critically. All authors read and approved the final manuscript.

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