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Seroprevalence and Risk Factors of *Ornithobacterium rhinotracheale* in Poultry Farms in Bangladesh

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Abstract

Ornithobacterium rhinotracheale (ORT) is a bacterium that is causing respiratory problems, growth retardation, high mortality, and drop in egg productions in poultry. The aim of the present investigation was to determine the seroprevalence and potential risk factors associated with the ORT infection in commercial poultry flocks in selected areas of Bangladesh. A total of 1920 serum samples were collected from 270 poultry farms including broiler (n=397), broiler breeder (n=430), layer (n=738), and a local breed namely Sonali (n=355) in Bangladesh during 2017-2018 and the samples were tested for ORT antibodies using indirect Enzyme-Linked Immunosorbent Assay (iELISA). At individual level, the prevalence of ORT antibodies in examined samples was 39.05% (95% CI: 36.87-41.29) and at farm level, it was 50.74% (95% CI: 44.61-56.85). In multivariate Generalized Estimating Equation (GEE) analysis, sampling area, farm category, types of chicken biosecurity condition and types of drinkers used were identified as positively correlated ($p<0.05$) with the seroprevalence of ORT at poultry farms. Therefore, it could be concluded that ORT is prevalent among commercial poultry of Bangladesh. Proper biosecurity measures at farm level and vaccination of birds against the ORT are highly recommended.

KEYWORDS
Bangladesh; *Ornithobacterium rhinotracheale*; Poultry; Seroprevalence; Risk factors

INTRODUCTION

Ornithobacterium rhinotracheale (ORT) belongs to the family Flavobacteriaceae, a slow-growing, Gram-negative, highly pleomorphic, rod-shaped, non-motile bacteria, first discovered in 1991 by Jan DuPreez (Hafez and Vandamme, 2011). The bacterium has been isolated from wide range of poultry species worldwide including chickens, turkeys, ducks, quails, geese, guinea fowls, ostriches, and pigeons (Thieme *et al*., 2016, Hafez and Chin 2020). To date, a total of 18 serotypes (serotype A to R) of ORT have been identified from different poultry species and geographical regions, whereas the majority are either serotype A, B, D or E (Chansiripornchai *et al*., 2007; Thachil *et al*., 2007). There is a correlation between geographical origin of the ORT and its serotype. Serotype C is only isolated in South Africa and United States from chickens and turkeys, respectively. However, serotypes are not always host specific (Hafez and Chin, 2020). The disease spreads horizontally by direct and indirect contact. There is very low incidence of vertical transmission of ORT through eggs (hatching eggs and infertile eggs), reproductive organs, and dead embryos (El-Gohary, 1998).

The initial symptoms are coughing, sneezing and nasal discharge followed in some cases by severe respiratory distress, dyspnoea, prostration, and sinusitis. The symptoms are accompanied with a reduction in feed consumption and water intake, in layer are generally accompanied with a drop in egg production, decrease in egg size and poor eggshell quality. However, fertility and hatchability are unaffected in many cases. The common gross lesions are localised in the lungs and include oedema and uni- or bilateral consolidation of the lungs with fibropurulent exudate, pleuritis and airsacculitis peritonitis, pericarditis. The severity of clinical signs, duration of the disease and mortality are extremely variable and are influenced by many environmental factors such as poor management, inadequate ventilation, high stocking density, poor litter conditions, poor hygiene, high ammonia level, concurrent diseases, and the type of secondary infection. There are many reports showing synergism between ORT and Newcastle disease (ND), Infectious Bronchitis (IB), Turkey Rhinotracheitis (TRT), *Bordetella avium*, *Escherichia coli* as well as *Chlamydophila psittaci*, Hafez (2015). Haemophilus paragallinarum, *Mycoplasma* spp., Bordetella avium, avian influenza or other infectious agents in the immunocompromised bird's can results into increased mortality (Hoerr, 2010; Pan *et al*., 2012; Rahman *et al*., 2018).

Along with isolation and identification of bacteria, serological

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techniques are used for diagnosis of ORT in different countries or studies. There are several serological methods for detection of antibodies against ORT, such as agglutination test (Ozbey *et al*., 2004) and indirect enzyme-linked immunosorbent assay (iELISA), being used for flock screening. The efficacy of different ELISA kits for detecting antibody of several ORT serotypes were investigated by Hafez and Chin (2020). The results show that both used ELISAs are equally suitable for detecting antibodies in sera of experimentally infected specific pathogen free (SPF) chickens with different ORT serotypes.

Several countries have reported ORT infection in poultry (Bhuiyan *et al*., 2019a; Mayahi *et al*., 2016; Baksi *et al*., 2017; Szabó *et al*., 2017; Umar *et al*., 2017; Umali *et al*., 2018; ; Numee *et al*., 2018). In one study from Bangladesh has claimed the presence of antibody in Sonali poultry using ELISA test in a small study area they detected high seroprevalence (45.9%) in investigated samples indicated that ORT is relatively common pathogen causing respiratory problems in poultry (Bhuiyan *et al*., 2019b).

So, this study aimed to check the ORT seroprevalence in commercial chicken flocks in selected areas in Bangladesh during 2017 and 2018 and to identify possible associated risk factors.

MATERIALS AND METHODS

Study area and study design

A cross-sectional study was undertaken between 2017 and 2018 to study the seroprevalence of antibodies against *Ornithobacterium rhinotracheale* (ORT) in commercial chickens in selected areas of Bangladesh. Bangladesh is divided into 64 administrative districts, whereas five districts namely-Bogura, Rangpur, Gazipur, Mymensingh, and Dhaka have been selected based on the poultry population size and accessibility. The geographic locations of study areas is presented in Figure 1.

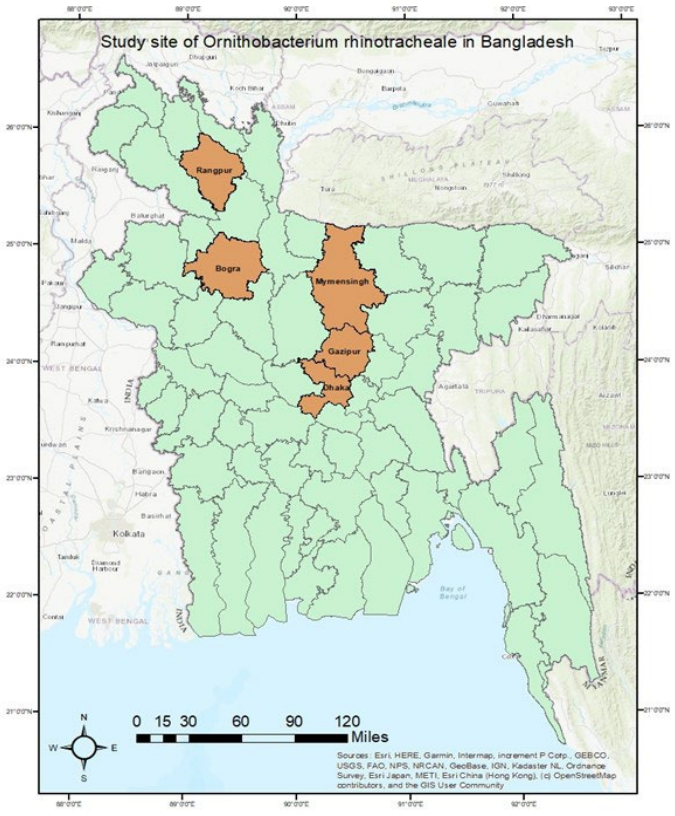

Fig. 1. Study sites of *Ornithobacterium rhinotracheale* in Bangladesh

Sampling technique

Multistage cluster sampling technique was applied for sample collection. The study comprised six sub-districts from each of the five districts, as well as three villages from each of the sub-districts, using the probability proportional to size technique. After that, from each village three (one large, one medium and one small) farms were sampled based on farm size i.e. number of poultry within the farm. In total, 270 farms from 30 sub-districts were surveyed. We divided the farms into three categories based on the number of chickens: small (≤1000 chickens), medium (1001-2000 chickens), and large (≥2001 chickens), as defined by the Department of Livestock Services, Bangladesh.

The animal study was reviewed and approved by The Animal Experimentation Ethics Committee (Dhaka, Bangladesh) approval number: BLRI0008.

Selection of different types of chicken farms

In this study four types of commercial chicken farms were included; broiler farms (Cobb 500); broiler breeder farms (Cobb 500); layer farms; and Sonali farms (a cross-bred between Rhode Island Red cocks and Fayoumi hens chicken strain of meat and egg purpose). Among 270 farms, 70 broiler, 60 broiler breeder, 80 layer and 60 Sonali farms were selected. Thus, 54 farms from each district were selected and sampled. The farm selection design is described as follows:

The required sample size for the study was calculated using the following formula (Thrusfield, 2018).

$n=(1.96^2\times Pexp \times(1-Pexp))/d^2$

Where, n=sample size, Pexp =expected prevalence, and d=absolute precision

The total number of calculated samples were 1920 (384 per district) considering 50% expected seroprevalence with 95% confidence level and 5% absolute precision level.

The number of chickens sampled varied depending on the size of the farm. As a result, from small, medium, and large chicken farms, we collected four, seven, and ten to eleven blood samples, respectively. Thus, the total samples for each type of chicken were 397, 430, 738, and 355 from broiler, broiler breeder, layer, and Sonali chicken, respectively.

Sample collection and processing

Individual chickens were randomly selected and sampled from each poultry farn. About 1.5 ml of blood from the wing vein was collected from each bird by using 3ml disposable plastic syringe. Serum was collected directly after clotting of the blood and stored at -20°C until used.

A structured and pre validated questionnaire was used for the collection of data related to demography of farm, flock size (number of poultry rearing in each poultry house), management and biosecurity practices.

Detection of antibodies to Ornithobacterium rhinotracheale (ORT)

All serum samples were examined for specific antibodies against ORT by indirect enzyme-linked immunosorbent assay (iELISA) test using commercially available ORT ELISA kit (Bio-Chek®, Netherland) according to the manufacturer protocols. The selected iELISA kit can detect antibodies to all seven serotypes of ORT namely types: A, B, C, D, E, F, and G. The optical density (OD) was measured at wavelength 405 nm by ELISA reader (Multiskan EX, ThermoFisher, USA). The samples with ≥0.5 cut-off

value (titer range 1432 or greater; S/P value 1.0 or greater) were considered ORT positive.

Statistical analysis

Questionnaire, animal, and farm/herd level data were entered in Microsoft Excel 2007 (MS Excel) spread sheet. The data were entered, cleaned, coded, and checked in MS Excel 2007. Epidemiological analysis was performed by using STATA-13 (StataCrop, 4905, Lakeway Drive, College station, Texas 77845, USA). Descriptive statistics were calculated to express the different types of poultry farm according to size, age wise distribution of samples, farm biosecurity practices etc. Associations involving potential risk factors (recoded into categorical variables) and ORT seropositive results were investigated using univariate logistic regression. All the factors, except age related variables in univariable analysis were forwarded for the multivariable analysis. As the age (lifetime) for broiler and other chickens are different, we omitted this for multivariable analysis. A multivariable logistic regression analysis was performed considering the significant variables ($P<0.05$) and the statistical significance of the contribution of each predictor was figured using Wald's test. We checked for confounding factors and collinearity by eliminating one of the variables and evaluating changes in the beta-estimate and its standard error (SE). The presence of confounding was considered based on change of coefficient more than 10%. The collinearity was checked based on changes in SE of a variable in the model increased significantly when entering a new variable in the model. In final model, relations between variables were tested for significance. The results for each predictor variable were shown as an odd ratio (OR) and 95% CI. For evaluation, we also ran the conditional logistic regression model at last. The goodness-of-fit using the Hosmer–Lemeshow test were used to assess the model and the receiver operating characteristic curve (ROC) was used to determine the predictive ability of the model (Dahoo *et al*., 2003). A map showing spatial distribution of sampling location was prepared by using ArcGIS-ArcMap version 10.2 (ESRI, USA).

RESULTS

Descriptive statistics

Of 270 poultry farms included in the study, there were 137 poultry farms (50.74%; 95% CI: 44.61-56.85) with at least one bird tested positive based on iELISA test. On the other hand, 39.05% (n=1920; 95%CI: 36.87-41.29) seroprevalence was found at the individual level (Table 1).

In terms of age group, 34 (32.35%) broiler farms were rearing 1 to 15 days old birds and 35 farms rearing 16 to 35 days old birds. In contrast, 54 layers and breeder farms rearing chickens 54 flocks aged 15-21 weeks, 52 aged 22-30 weeks, and 51 aged between 31-40 weeks and 44 farms reared birds older than 40 weeks o (Table 1).

The biosecurity level was good at 83 farms, maintaining moderate on 100 farms and poor biosecurity practice waere found on 87 farms (Table 1).

Univariate analysis

The results revealed a significantly higher seroprevalence for large size poultry farms (73%), layer poultry (66.25%), farm practicing poor biosecurity (71.26%), use ball (71.975) for drinking ($p<0.05$). Broiler aged 16-35 days (68.57%) and in case of layer, 40 weeks aged (77.27%) were significantly associated with the

Table 1. Univariable and multivariable logistic regression analysis of potential risk factors for ORT seropositivity in poultry farms of Bangladesh.

Variables	Category	No. of farms tested	No. of samples positive (%)	Univariable analysis		Multivariable analysis	
				Odds ratio	95% CI	Odds ratio	95% CI
Districts	Dhaka	54	21 (38.89)	ref	ref	ref	ref
	Bogura	54	30 (55.56)	1.16	0.86-1.55	7.31*	02.24-23.85
	Gazipur	54	33 (61.11)	1.78	1.34-2.38	5.94*	01.85-19.11
	Mymensingh	54	31 (57.41)	1.21	0.90-1.62	4.00*	01.32-12.04
	Rangpur	54	22 (40.74)	0.81	0.59-1.08	3.34*	01.06-10.53
Farm category	Small	90	20 (22.22)	ref	ref	ref	ref
	Medium	91	52 (47.14)	2.86	2.04-4.01	13.01*	04.65-36.42
	Large	89	65 (73.03)	6.68	4.85-9.21	65.67*	16.27-265.12
Chicken type	Sonali	60	20 (33.33)	ref	ref	ref	ref
	Layer	80	53 (66.25)	2.98	2.25-3.97	1.34	00.40-04.48
	Broiler	70	36 (51.43)	2.57	1.88-3.52	5.43*	01.85-15.95
	Broiler breeder	60	28 (46.67)	1.38	1.00-1.89	3.55*	01.10-11.45
Biosecurity	Good	83	18 (21.69)	ref	ref	ref	ref
	Moderate	100	57 (57.00)	8.59	6.01-12.29	06.62*	02.62-16.78
	Poor	87	62 (71.26)	37.05	25.59-53.65	26.56*	08.12-86.87
Drinker	Nipple	132	42 (30.43)	ref	ref	ref	ref
	Ball	138	95 (71.97)	10.06	8.05-12.57	7.48*	03.22-17.40
Broiler age (Days)	15-May	34	11 (32.35)	ref	ref	-	-
	16-35	35	24 (68.57)	4.1	2.69-6.24	-	-
Others chicken age (Weeks)	15-21	54	13 (24.07)	ref	ref	-	-
	22-30	52	18 (34.62)	4.14	2.51-6.82	-	-
	31-40	51	37 (72.55)	16.9	10.52-27.14	-	-
	>40	44	34 (77.27)	52.63	32.18-86.06	-	-

*Statistically significant risk factor for seropositivity

higher seroprevalence of ORT (Table 1). The present prevalence with reference to biosecurity categories: good (21.69%), moderate (57.00%) and poor (71.26%) was also significant (P=0.001). The following drinker types had an impact: ball (71.97%) and nipple (30.43%) (P=0.001). Effect of age: broiler age grouped 5-15 days (32.35%) and 16-35 days (68.57%) (p=0.003). Among other types of chicken those are age grouped more than 40 weeks (77.27%), 31-40 weeks (72.55%), 22-30 weeks (34.62%), and 15-21 weeks (24.07%) (P=0.001) (Table 1). There was no significant difference in seroprevalence among the study districts (Table 1).

Multivariable logistic regression analysis

In multivariable risk factor analysis, there were five factors recognized as potential risk factors for the seroprevalence of ORT in poultry farms by adapting the result of the factors with each other including sampling area, farm groups, types of chicken reared, biosecurity condition and types of drinkers used. The receiver operating characteristic curve (ROC) broaden well into the upper left-hand bend and the area under the curve was 0.91 (Figure 2).

Farms that located in Bogura, Gazipur, Mymensingh and Rangpur were 7.31, 5.94, 4.00, and 3.34 times more likely to have tested positive for ORT antibodies, respectively, than the farms located in Dhaka. The seroprevalence of ORT was 65.67 times higher in large poultry farms and 13.01 times higher in medium farms than the small poultry farms. Farms rearing broilers only were 5.43 times, broiler breeder 3.55 times and layer 1.34 times more likely to have ORT seroprevalence than Sonali poultry rearing. Farms having poor and moderate biosecurity were 26.56 and 6.62 times more likely to be seropositive than farm having good biosecurity practices. Farms where drinking water was supplied by ball was 7.48 times higher for ORT seropositivity compared to farms providing drinking water with nipple drinker (Table 1). District and management system did not act as confounders of the effects of the above variables, as their rejection resulted in <20% change to coefficients.

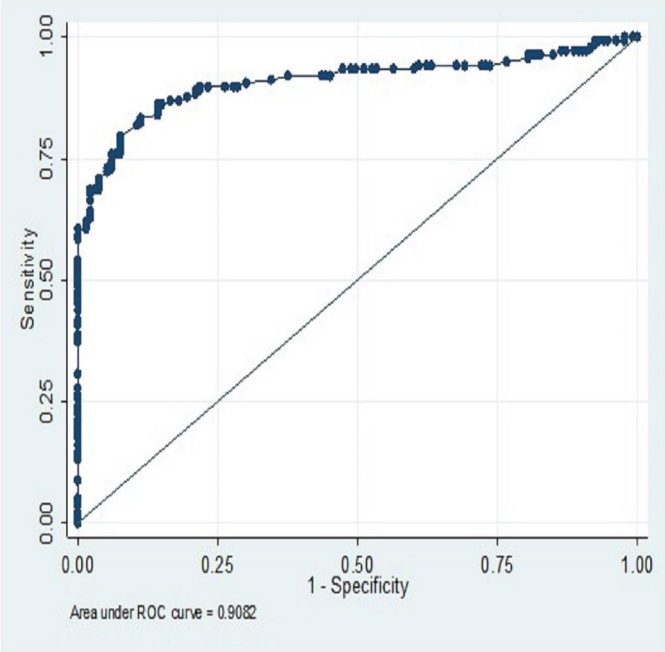

Fig. 2. Plot of sensitivity versus 1-specificity for a receiver operating characteristic curve (ROC) of various parameters of the logistic model of seroprevalence of ORT in commercial poultry farms of Bangladesh. The point of the curve which is most towards the upper left of the figure indicates the maximum accuracy of the model.

DISCUSSION

The aim of this study was to estimate the seroprevalence and potential risk factors associated with the ORT seroprevalence in commercial poultry farms located in five districts in Bangladesh using systematic epidemiological approach. This study used structured epidemiological procedures and tools to describe the ORT in farms and the association with management practices. We found that the overall seroprevalence of ORT was 50.74%. To the best of our knowledge, this is the first published report of the seroprevalence, and risk factors associated with ORT in different types of poultry in Bangladesh.

In Bangladesh, until now ORT vaccine is not used in the poultry farms until now, however, we detected antibodies in all tested flocks. At farm level seroprevalence was found 50.74% (95% CI: 44.61-56.85). In Thailand Chansiripornchai *et al*. (2007) found out the seroprevalence was lower than the 100% broiler breeder using ELISA test. In Germany, 96.6% of serum samples collected from broiler breeder flocks were positive for ORT and in commercial broiler flocks were (26%) (Ali *et al*., 2019a; Ali *et al*., 2019b; Bhuiyan *et al*., 2019a). Similar results were also found in turkey flocks, 91% in Slovenia (Canal *et al*., 2003). In a study from West Azerbaijan (north-west of Iran), examined 82% sera from 50 broiler flocks were positive for ORT (Allymehr, 2006). Also, in south east of Iran reported 81% broiler flocks of 21 examined were positive in the present investigation the seroprevalence at individual level was (39.05%; 95%CI: 36.87-41.29). These agree with the studies from Mehrabanpour *et al*. (2017) from Fars province, Iran, (42.5%), Bhuiyan *et al*. (2019) from Bangladesh (45.9%) and Sakai *et al*. (2000) from Japan (41.9%). Higher seroprevalence has been reported by Seifi (2012) from Mazandaran Province, North of Iran, where 71.1% seroprevalence was found in broilers. Lower prevalence of ORT has been reported by Chansiripornchai *et al*. (2007) from Thailand they examined 17 broiler farms (19 flocks) and the sera analysis on individual 280 samples from broiler showed that 19.60% positive antibody response. Ghanbarpour and Salehi (2009) found 31.90% (n=420) seroprevalence of ORT in broiler located in the southeast of Iran. However, the difference in seroprevalence could be due to the different species tested, rearing form, the time of the infections, and time of sample collection, age, as well as the environmental factors, and the involved strains of ORT (Canal *et al*., 2005; Chansiripornchai *et al*., 2007; Mehrabanpour *et al*., 2017).

In this study, seroprevalence was examined in relation to different locations, farm management, disease prevalence, and other factors impacting growth, production, reproduction, and health of birds. This agrees with publication from Baksi *et al*. (2017). The farms that located in Bogura and Gazipur districts were shown higher prevalence of ORT that might be for the highest density of poultry farms over the country were located in that regions (Huque *et al*., 2016).

The results of iELISA test revealed that layer flocks had higher seroprevalence than other types of tested poultry and broiler breeders showed higher seroprevalence of ORT than broiler. Similar findings were described by Hafez and Sting (1999) in Germany, as well as by Canal *et al*. (2003) in Brazil, (Chansiripornchai *et al*., 2007) in Thailand. Further publication from layer flocks in India found also higher seroprevalence (96.04%) (Sumitha *et al*., 2015). The longer life span of layer, broiler breeders may be contributing to get higher exposure to ORT and moreover, the higher prevalence. Strikingly, researchers demonstrated ORT detection in the broiler breeders may lead to the vertical transmission of the bacteria to broilers through the eggs (Chansiripornchai *et al*., 2007; Sumitha *et al*., 2015). Some reports have shown that vertical transmission is suspected of the isolation of ORT at a very low incidence from reproductive systems and from infertile eggs, hatching eggs, and dead embryos also (El-Gohary, 1998). Van Veen *et al*. (2004), observed that specific pathogen-free (SPF) broiler chickens that were hatched in incubators at a commercial turkey hatchery during hatch found respiratory tract lesions at post-mortem examination that were positive for ORT by both

bacteriological and immunohistological examination. Nevertheless, when ORT was inoculated with embryonating chicken eggs, the embryos were killed by the 9th day and ORT was not isolated from the eggs, proof that it is not transmitted via eggs during hatching (Varga *et al*., 2001).

In case of broiler and others type of poultry, we found higher prevalence among older age groups compared to others. Research from other countries also reported similar findings. Research conducted by Hafez (1996) and Turan and Ak (2002) demonstrated that ORT infections are more prevalent in broilers of age group between 3 to 4 weeks, where, in broiler breeders it was more common before laying and during peak of laying age (between 24 to 52 weeks of age).

The farm size, type of birds, methods of water supply and biosecurity practices were statistically significantly differed among ORT testing farms. One study from southern Brazil conducted by Canal *et al*. (2003), they tested serum samples collected at the slaughterhouses and found out that prevalence of ORT antibodies in broiler flocks was 63.83%, but in each individual flock only 6.52% of the birds were positive. Instead of, in broiler breeder flocks the prevalence was 100%, and in each individual flock 94.62% of the birds were positive. Moreover, statistical difference between flock size, stocking density of poultry in the sheds, and rearing different types of poultry in the same farm, improper cleaning and disinfection of the equipment's, contaminated environment, aggravated by the reuse of the litter for several cycles are the common source of infection and may facilitate horizontal transmission (Canal *et al*., 2003; van Empel and van den Bosch, 1998; Ali *et al*., 2020). In general, ORT appears to be highly contagious so that strict farm biosecurity measures should be followed to prevent its transmission into a flock. However, after a farm is infected, ORT becomes widespread, especially in multiple-age farms and in areas with densely poultry populated and intensive poultry production flocks (Hafez and Chin, 2020).

CONCLUSION

Based on the above study it could be concluded that seropositivity of ORT is prevalent among different types of commercial poultry of Bangladesh and it could be controlled or minimized by strict biosecurity measures, proper farming practices along with proper vaccination.

According to our findings, the ORT is found in all types of commercial chickens studied and is higher prevalent in layer flocks in the areas investigated. Improper farming practices, particularly the size of the farm, higher stocking density, water supply, and biosecurity controls, are the major factors contributing to the prevalence of ORT. Improvements in farming procedures, sanitation, and immunization of birds against the disease are all highly recommended for controlling or minimizing ORT infection in poultry.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest in this paper.

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