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Sero-Surveillance of Infectious Laryngotracheitis in Layer Birds in Bangladesh

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A B S T R A C T

Infectious Laryngotracheitis (ILT) is an economically important respiratory disease primarily of chicken caused by Infectious Laryngotracheitis Virus (ILTV) belonging to the family Herpesviridae. After infecting its host, the virus induces immune response; antibodies are produced, which can be easily detected by several immunological or serological methods like Enzyme-Linked Immunosorbent Assay (ELISA). In the present study, the research work was conducted to reveal the prevalence of antibodies (IgG) against ILTV in layer birds in Bangladesh during the period of July 2010 to April 2011. A total of 324 blood samples were collected randomly from 30 layer farms located in 15 different districts in the country, and subsequently sera were prepared. Aiming to detect the antibody against ILTV, the sera samples were subjected to indirect ELISA using a commercial ILTV Antibody Test kit. Out of 324 sera samples examined, a total of 299 (92.28%) were positive. The results indicate that layers in the selected poultry farms were exposed to ILTV in Bangladesh and confirms the prevalence of this virus in a wider scale since the first report of its existence in 2010.
Keywords: Sero-survey, ILTV, Indirect ELISA, Layer birds, Bangladesh

Introduction

Infectious Laryngotracheitis virus (ILTV) belonging to the family Herpesviridae is an enveloped, non-segmented, linear double-stranded DNA virus. The disease (ILT), caused by this virus, mainly affects chickens and is characterized by conjunctivitis, ocular discharge, sneezing, rales, nasal exudates, swollen infra-orbital and nasal sinuses. In severe cases gasping, dyspnea and death through asphyxiation may occur (Jordan, 1993). The morbidity rate of ILT may be up to 100%, depending on the virulence of strain and immune status of the flock (Hanson and Bagust, 1997; and Hughes *et al.,* 1991a), and the mortality is highly variable, ranging from 0.1% to 70% (Hanson and Bagust, 1997). The disease is common in areas of intensive poultry production and its outbreaks result in high economic losses due to increased mortality, decreased growth rates, and lower egg production (Guy and Bagust, 2003; Humberd *et al.,* 2002).

Infection with ILTV was first described in 1925 (May and Thittsler, 1925), and subsequently it has been isolated in many countries in North America, South America, Europe, Asia and Australia. In 2010, the disease was first identified in Bangladesh (Islam *et al.,* 2010). However, the extent and severity of the disease were untouched. ILT is usually well controlled in layer flocks by the use of modified live virus vaccines.

Many laboratory diagnostic techniques such as cultural, histopathological and serological tests have been used for the detection of ILTV. Detection of antibodies by Enzyme-Linked I ELISA is one of the reliable methods to detect the presence or exposure of the virus in the body (Bauer *et al.,* 1999; Sander and Thayer, 1997). Although virus isolation is widely used and provides direct evidence of infection, virus isolation is time-consuming (Hughes *et al.,* 1988). Considering the above facts, the aim of the study was to study the sero-surveillance of ILT in layer populations and to determine the extent of the disease in Bangladesh.

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Materials and Methods

Farm and flock selection

Bangladesh is divided into seven divisions, 64 districts and 481 *upazilas* (sub-districts) for administrative purposes. The research work was conducted in six out of seven divisions as the layer farms are mostly located in these areas. The research work was done during the period from July 2010 to April 2011 at the Department of Microbiology and Hygiene, Bangladesh Agricultural University (BAU), Mymensingh and Phenix Agro Health Care Laboratory (PAHCL), Joydebpur, Gazipur. Thirty layer farms (Table 1) were selected randomly for this study. All these flocks had the history of clinical ILTV infection and the birds were not vaccinated against ILT.

Sample collection and serum preparation

A total of 324 blood samples were collected randomly and aseptically according to the method described by Islam *et al.* (2010). In brief, about 1 ml of blood was collected from a wing vein of each chicken using sterile 2-ml disposable plastic syringe without anticoagulant and were kept in flask containing ice pack and carried to the Department of Microbiology and Hygiene, BAU, and PAHCL,. Blood-containing syringe was kept in the refrigerator for 4-5 h. The serum was decanted in centrifuge tube and centrifuged at 2,500 rpm for 5 minutes under refrigerated temperature (4°C) to have clear serum. The serum was then collected in a sterile eppendorf tube and stored at -80°C until further study.

Indirect ELISA

The collected sera samples were subjected to indirect ELISA using commercial ILTV Antibody Test Kit (CK124, Biochek, Holland) per manufacturer's instructions. In brief, ILT antigen-coated plates were obtained and the sample position on a BioChek worksheet was recorded. A 100 µl of undiluted negative control was dispensed into ILT wells A1 and B1. Controls were ready to use; no dilution was required for each plate. A 100 µl of undiluted positive control was dispensed into ILT wells C1 and D1. Controls were ready to use; no dilution was required for each plate. After dispensing 100 µl of each diluted sample into the appropriate wells, it was then incubated at room temperature (20°C-25°C) for 30 minutes. The liquid contents of all wells were aspirated into the appropriate waste reservoir. Each well was washed with approximately 200 µl of phosphate buffered wash solution three times. The liquid contents of all wells were aspirated after each wash. Plate drying was avoided between plate washings and prior to the addition of conjugate reagent. Following the final wash fluid aspiration, residual wash fluid was tapped gently but firmly from each plate onto absorbent material. 100 µl of anti-chicken AP conjugate was dispensed into each well. Then it was incubated at room temperature for 30 minutes. Then liquid contents of all wells were aspirated into the appropriate waste reservoir. Each well was washed with approximately 200 µl of phosphate buffered wash solution three times. The liquid contents of all wells were aspirated after each wash. Plate drying was avoided between plate washings and prior to the addition of substrate solution. Following the final wash fluid aspiration, residual wash fluid was tapped gently but firmly from each plate onto absorbent material. 100 µl of substrate solution was dispensed into each test plate well. Then it was incubated at room temperature (20°C-25°C) for 15 minutes. 100 µl of stop solution was dispensed into each well of the test plate to stop the reaction. The intensity of color developed was measured by determination of OD (optical density) value of tested samples using an ELISA reader at 405 nm filter and the result was calculated by the following calculations.

Data analysis

The presence or absence of antibodies to ILTV was determined by comparison of unknown specimen test optical densities to the mean positive control optical density result of each assay (S/P ratio). The positive control has been standardized and represents a significant level of antibody to ILT antigen in chicken serum. The relative level of antibody to ILTV in the unknown sample is determined by calculating the sample to positive (S/P) ratio according to the methods provided by the manufacturer of the test kit. The presence or absence of antibody to ILTV is determined by calculating the S/P ratio for each sample.

Calculation of the Negative Control Mean OD (NC_x-) (well A1 and B1)

$$NC_x\text{-} = \frac{A1A\ (405)\ B1A\ (405)}{2}$$

Where, A1= OD value of negative control-1, B1= OD value of negative control-2

Calculation of the positive Control Mean OD (PC_x-) (wells C1 and D1)

$$PC_x\text{-} = \frac{A1A\ (405)\ B1A\ (405)}{2}$$

Where, C1= OD value of positive control-1, D1= OD value of positive control-2

Calculation of the (S/P) ratio of unknown samples

Interpretation of S/P Results

If the S/P ratio is less than 0.50, the sample is classified as negative for ILT antibodies.
If the S/P ratio is greater than or equal to 0.50, then the sample is classified as positive for ILT antibodies.

$$\frac{S}{P} = \frac{\text{OD Value of test sample (405)} - NC_x^-}{PC_x^- - NC_x^-}$$

Results and Discussion

The research work was conducted to investigate the sero-prevalence of ILTV in layer population of Bangladesh. Samples were collected randomly and aseptically from 324 layer chicken of 12 (twelve) farms of Dhaka Division, 4(four) farms of Chittagong Division, 6(six) farms of Rajshahi Division, 4(four) farms of Rangpur Division, 3(three) farms of Khulna Division and 1(one) farm of Barisal Division with the history of collection of DOC from different hatcheries of Bangladesh during the study period. Out of 320 sera samples collected from 110400 layer birds of 30 farms in the 15 districts of Bangladesh, 299(92.22%) sera samples were positive for anti-Infectious Laryngotracheitis IgG. Out of 164 sera samples of Dhaka Division examined, 144(87.80%) samples were found to be positive. Similarly, in the cases of 52, 32, 32, 28 and 16 sera samples of Rajshahi, Rangpur, Chittagong, Khulna and Barisal Divisions 52(100%), 27(84.37%), 32(100%), 28(100%) and 16(100%) were found to be positive, respectively. All the results have been illustrated in Table 1.

The study to detect seroprevalence of ILTV was conducted by the use of indirect ELISA. Such a test has been used for many a years by other investigators. Adair et al. (1985) compared four serological tests i.e. ELISA, SNT, FAT, AGID for the detection and titration of ILT chicken sera. These tests were compared using- (1) sera from experimentally inoculated birds bled regularly at intervals for 4 to 35 days post inoculation, (2) convalescent sera from a natural outbreak of ILTV, and (3) serial dilution of ILT positive serum. In all experiment, the ELISA test was of slightly greater sensitivity than SN and comparable to FAT test. In another study, Bauer et al. (1999) conducted an experiment regarding evaluation of two commercial ELISA kit for the detection of antibodies to avian ILTV, one for Australia (Trop-ELISA, Trop-Bio) and the other from the USA (Pro-Flock-ELISA, KPL) as well as to compare their performance with the conventional SNT in Chicken Embryo Liver cell. It was indicated that the sensitivity of ELISA was higher than that of SNT. In general, both ELISA were suitable alternatives to SNT with the convention that only negative/positive result was required. The present sero-surveillance study indicates that ILTV is present in Bangladesh and is the first report covering a broader geographical area..

The prevalence of antibodies against ILTV is reported from every poultry dense areas in Bangladesh. Every farm under this study was previously affected clinically and had no history of ILTV vaccination. In either case, the farms were affected by ILTV. Clinical cases of ILT was first reported in 2003 in Bangladesh. In 2004, ILT live vaccines are imported in Bangladesh for GP farms and the birds of these farms are applied with the vaccine following the vaccination regimen of the manufacturer. It is speculated that ILTV may have been introduced in Bangladesh. In such a situation when the Directorate of the Livestock Services (DLS) was informed, use of such vaccine was suspended. When the import of ILT vaccine was stopped there had been no reports of outbreaks. However, virus shedding is possible from earlier vaccinations which may have occasionally caused severe diseases. This being the situation, Breeder's Association of Bangladesh considered the disease (ILT) as second disaster disease in layer birds causing a colossal loss and the farmers by now have lost their assets. The present serological investigations indicated a high percentage (92.28%) of samples have had a seroconversion which may be due to the fact that the samples were collected from the birds reported to had been exposed to the

Table 1. Division-wise prevalence of antibodies (IgG) against ILTV in layer birds

Division	District	Farms (n)	Samples collected (n)	Positive Samples (%)	Positive samples division wise (%)
Dhaka	Gazipur	07	108	105 (97.22)	87.80
	Savar, Dhaka	01	16	11 (68.75)	
	Narsingdi	01	16	16 (100)	
	Mymensingh	03	24	12 (50)	
Rajshahi	Rajshahi	02	16	16 (100)	100
	Sirajganj	02	20	20 (100)	
	Joypurhat	02	16	16 (100)	
Rangpur	Rangpur	02	16	11 (68.75)	84.37
	Gaibanda	02	16	16 (100)	
Chittagong	Comilla	02	16	16 (100)	100
	Cox´s Bazar	02	16	16 (100)	
Khulna	Bagherhat	01	08	08 (100)	100
	Khulna	01	10	10 (100)	
	Satkhira	01	10	10 (100)	
Barisal	Pirojpur	01	16	16 (100)	100
Total	15	30	324	299 (92.28)	

virus earlier. If samples were randomly collected prevalence rate of ILT antibodies could be lower than the present study but it needs further studies and investigation including the isolation, identification and molecular characterization using other serological and molecular techniques.

Conclusion

In conclusion, it can be noted that the existence of ILTV in layer birds has been noticed for the first time in Bangladesh, which would attracts eyes of related Bangladeshi scientists to focus on the virus aiming to save the rising poultry industry. On other hand, it has been proved that the rapid test kit used in this research work can be applied for the rapid detection of antibodies against ILTV in field cases.

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