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Article

Seroprevalence study of infectious laryngotracheitis virus antibody of commercial layer in Gazipur Districts of Bangladesh

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Abstract: This research work was conducted to detect the prevalence of Infectious Laryngotracheitis (ILT) Virus-specific antibody in chickens from Gazipur district in Bangladesh at Department of Microbiology, Hajee Mohammad Danesh Science & Technology University, Dinajpur and Poultry Care Lab, Garzipur from January to June 2012. A total number of 232 sera sample of commercial layer chicken were collected from 17 different commercial layer farms at different ages. The layers prognosed for sampling had not been previously vaccinated against ILTV. The indirect enzyme linked immunosorbent assay (iELISA) was performed to estimate the Infectious Laryngotracheitis (ILT) Virus-specific antibody. Out of 232 samples, 189 (81.47%) samples were found to positive for Infectious Laryngotracheitis (ILT) Virus-specific antibody. In 17 different commercial farms prevalence based on age were 75%, 87.5%, 87.5%, 90%, 81.25%, 80%, 100%, 70%, 81.25%, 81.25%, 90%, 93.08%, 87.5%, 75%, 75%, 68.75% and 75% in the age limit 08, 09, 11, 12, 13, 14, 15, 16, 17, 17, 19, 21, 25, 31, 35, 44 and 51 weeks respectively and farms showed high level of ILT virus specific antibodies (IgG). This result showed that in 15 weeks of age prevalence was highest position i.e; 100%. The result of this study indicate that there were a high prevalence of Infectious Laryngotracheitis (ILT) Virus circulating at Gazipur district in Bangladesh.

Keywords: seroprevalence; ILTV; indirect ELISA; commercial layer

1. Introduction

Commercial poultry farming has been growing rapidly since 1990 and at present nearly 12 million commercial poultry farms in Bangladesh. There are about 320.63 million poultry population in Bangladesh and producing around 120 million egg per day and 1363 tons meat per day (Livestock Economy, DLS. BD, 2016-17 and FAOSTAT, 2016). Infectious laryngotracheitis (ILT) is caused by gallid herpes virus-1 of the family herpesviridae, subfamily alphaherpesvirinae, genus Iltovirus. The virus is an envelope, non segmented, linear dsDNA virus (Bagust *et al*., 1986). Infection with ILTV was first described in 1925 (May and Thittsler) and it has been described in many countries in which ILT remains as a serious disease mainly in areas of intensive

production and large concentrations of chicken such as America, Europe, China, Asia and Australia. In 2010, the disease was first identified in Bangladesh (Islam *et al*., 2010). ILTV strains are antigenically homogenous, naturally vary in virulence, from highly virulent strains, causing high morbidity and mortality and low virulence that produce mild-to-unapparent infection (Bauer *et al*., 1999). Chickens are infected for ILTV through the upper respiratory and ocular routes (Goodwin *et al*., 1991). Clinical sings include gasping, depression, nasal discharge, conjunctivitis and expectoration of body mucus. In postmortem examination of the trachea, characteristic severe hemorrhage and mucus plugs are observed (Cover and Benton, 1958). The morbidity rate of ILT may be upto 100%, depending on the virulence of strain and immune status of the flock (Shibley *et al*., 1962). This 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 (Davison and Miller, 1988). ILT is usually well controlled in layer flocks by the use of modified live virus vaccines. Live attenuated ILT vaccines provide immunity when apply via infra –orbital sinuses, intra nasal instillation (Benton *et al*., 1958), eye drop (Sinkovic, 1968) and orally through drinking water (Samberg *et al*., 1971). However, application of ILT vaccines by eye drop method appears to be more protective than application by water or spray (Fulton *et al*., 2000). Serious disease outbreaks continue to occur periodically whenever ILT virus strains can move from persistently infected flocks to non-vaccinated birds. Many laboratory diagnostic techniques such as cultural, histopathological and serological tests have been used for the detection of ILTV. Detection of antibodies by serum neutralization or ELISA are useful although serological tests do not provide a timely diagnosis (Bauer *et al*., 1999). In Bangladesh, breeders as well as commercial farms follow vaccination regularly. Considering the prevalence of the disease and losses to poultry raisers, it is felt that there is a national need to identify the disease quickly and to suggest protective measures of the disease. In Bangladesh, for controlling ILT, both the commercial and breeder poultry raisers are using ILT vaccine imported from abroad without any concern of the local isolates/serotypes of ILTV. Considering the above facts, the present study was undertaken to study the seroprevalence of Infectious Laryngotracheitis (ILT) virus antibody in commercial layer populations.

2. Materials and Methods

2.1. Study design

The research work was conducted to detect the prevalence of Infectious Laryngotracheitis (ILT) Virus-specific antibody in chickens from Gazipur district of Bangladesh. A total number of 232 commercial layer sera were collected from 17 commercial layer chicken farms of Gazipur district in Bangladesh. The studied commercial layer had not been previously vaccinated at different ages.

2.2. Samples collection

Blood samples were collected randomly and aseptically from a total number of 232 commercial layer sera sample from 17 commercial layer farms. About 1 ml of blood sample was taken from each chicken from wing vein using sterile 3 ml disposable plastic syringe without anticoagulant and were kept in flask containing ice pack. After collection of blood sample were carried to the Department of Microbiology, HSTU and Poultry Care Lab, Garzipur, Bangladesh.

2.3. Separation of serum from blood samples

Blood containing syringe were kept in the refrigerator for 4-5 hrs. The serum (liquid portion) was decanted in centrifuge tube and centrifuge at 2,500 rpm for 5 minutes under refrigerated temperature (4°C) to have clear serum .The serum was then collected in sterile eppendorf tube and then preserved at -20° C until further processing for the serological study.

2.4. Indirect ELISA

The ILT ELISA kit was used to measure the amount of antibody to ILT in the serum of chickens. Microtitre plates was pre-coated with inactivated ILT antigen. 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. A yellow color was developed in positive case of anti-ILT antibody in the sample. 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.

2.5. Calculations

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

$$NCx^- = \frac{A1(405) + B1(405)}{2}$$

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

Calculation of the positive control mean OD (PCx) (wells C1 and D1)

$$PCx^- = \frac{C1(405) + D1(405)}{2}$$

Here, C1=OD value of positive control -1, B1=OD value of positive control-2 Calculation of the (S/P) ratio of unknown blood serum samples

$$\frac{S}{P} = \frac{\text{OD value of test sample}\,(405 - BCx^-)}{PCx^- - NCx^-}$$

2.6. Interpretation of S/P Results

If the S/P ratio was less than 0.50, the sample was classified as negative for ILT antibodies. If the S/P ratio was greater than or equal to 0.50, then the sample was classified as positive for ILT antibodies. For example, Sample calculation: unknown OD (405nm) = 0.658

NCx^- = 0.125

PCx^- = 0.52

$$\frac{S}{P} = \frac{0.658 - 0.125}{0.52 - 0.125} = \frac{0.533}{0.395} = 1.34$$

This sample was positive for ILT antibodies because 1.34 is greater than 0.5

2.7. Validity Specifications

The test result was valid the mean negative control absorbance was read below 0.30 and the difference between the mean positive control and the mean negative control optical densities (OD) was greater or equal to 0.150 according to the Biochek ILTV Antibody Test kit-CK 124 protocol.

3. Results and Discussion

This research work was conducted to detect the prevalence of Infectious Laryngotracheitis (ILT) Virus-specific antibody in chickens from Gazipur district in Bangladesh. There were 232 serum sample was collected

randomly and aseptically from 17 different commercial layer farm of Gazipur district. Out of 232 blood serum samples from 17 different commercial layer farms the result of indirect ELISA shown that, 189 samples were found to be positive for ILT antibodies (IgG) and 43 samples were found to be negative for ILT antibodies (IgG) i.e. prevalence of ILT virus specific antibody was 81.47 % that was shown in table: 01. In 17 different commercial farms prevalence based on age were 75%, 87.5%, 87.5%, 90%, 81.25%, 80%, 100%, 70%, 81.25%, 81.25%, 90%, 93.08%,87.5%, 75%, 75%, 68.75% and 75% in the age limit 08, 09, 11, 12, 13, 14, 15, 16, 17, 17, 19, 21, 25, 31, 35, 44 and 51 weeks respectively and farms showed high level of ILT virus specific antibodies (IgG) that is shown in Figure 1. This result shown that in 15 weeks of age prevalence was highest in position i.e, 100%. ELISA in ILT virus specific antibody procedure prognosed to positive confirmation was shown in Figures 2, 3, 4 and 5. Such a test had been used for many years by several researchers. Mallinson *et al*., 1981 compared four serological tests i.e. ELISA, SNT, FAT, AGID for the detection and titration of ILT chicken sera. The prevalence of Infectious Laryngotracheitis (ILT) Virus-specific antibody in commercial chickens farm at Gazipur district of Bangladesh was 81.47 %, which was more or less similar to Jahan *et al*., 2012 where prevalence was 92.28%. Guy *et al.*, 1992 conducted an experiment regarding evaluation of two commercial ELISA kit for the detection of antibodies to avian ILT virus, one for Australia (TROP-ELISA, Trop-Bio) and the other from USA (Pro-Flock-ELISA, KPL) as well as to compare their performance with the conventional SNT in chicken Embryo Liver cell. This seroprevalence study indicates that ILTV is present in Bangladesh.

Table 1. Prevalence of ILT virus specific antibody.

Total Number of Serum Sample	Total number of positive sample	Total number of negative sample	Prevalence of ILT virus specific antibody
232	189	43	81.47 %

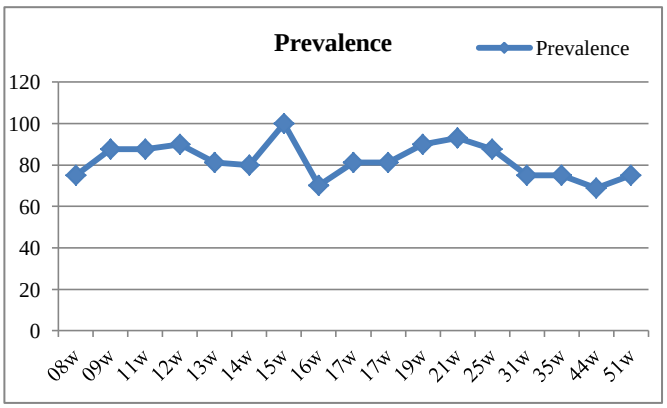

Figure 1. Showing that prevalence of ILT virus specific antibody based on age.

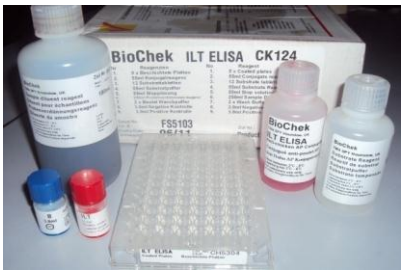

Figure 2. ILT antigen coated plate containing chemicals and reagents used for ELISA.

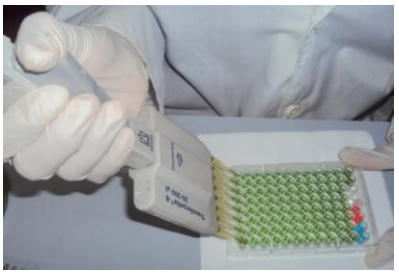

Figure 3. Diluted serum was taken on ILT antigen coated plate.

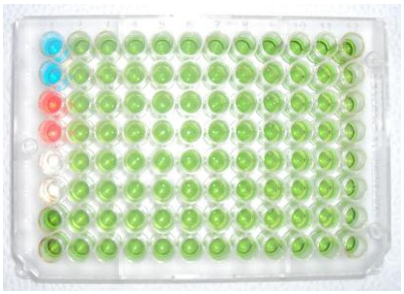

Figure 4. Diluted serum containing ILT antigen coated plate with negative, positive and reference control.

Figure 5. Yellow color was developed in positive case.

4. Conclusions

This research work was shown that the prevalence of ILT virus specific antibody at Gazipur district in Bangladesh was 81.47% in different commercial layer chicken farm belonging 8 to 51 weeks of age. It was showing that, the ILTV was circulating in this area at high level of significance, which is one of the most risk factor for outbreaks of ILTV in poultry sector. Hence there is an urgent need for the development of prevention and control policies against ILT in poultry sector of Bangladesh.

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Conflict of interest

None to declare.

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