SEROPREVALENCE STUDY OF INFECTIOUS LARYNGOTRACHEITIS VIRUS ANTIBODY OF COMMERCIAL LAYER IN GAZIPUR DISTRICT OF BANGLADESH

A THESIS

BY

MD. MIJANUR RAHMAN
REGISTRATION NO. 1105018

SESSION: 2011

SEMESTER: JANUARY- JUNE, 2012

MASTER OF SCIENCE (M. S.)
IN
MICROBIOLOGY

DEPARTMENT OF MICROBIOLOGY
FACULTY OF VETERINARY AND ANIMAL SCIENCE

HAJEE MOHAMMED DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR

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Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur in Partial Fulfilment of the Requirements for the Degree of

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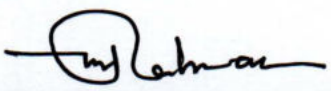

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JUNE, 2012

DEDICATED
TO MY
BELOVED PARENTS

ACKNOWLEDGEMENTS

All praises are due to the almighty Allah, the sovereign Lord of the universe, who enabled the author to materialize his dream to complete the thesis timely.

With great pleasure, I would like to express my deepest sense of gratitude, sincere appreciation, indebtedness and profound regards to my reverend teacher and research supervisor ***Prof. Dr. Md. Mostafizer Rahman****, Chairman, Department of Microbiology, Faculty of Veterinary & Animal Science (VAS), Hajee Mohammad Danesh Science & Technology University (HSTU), Dinajpur for his sincere guidance and supervision, constant encouragement ingenious suggestions and constructive criticisms throughout the research work and in writing up the thesis.*

I would also like to express my gratefulness and indebtedness to my respected teacher and research co-supervisor ***Dr. Mir Rowshan Akter****, Assistant Professor. Department of Microbiology, VAS, HSTU, for his constructive criticism, valuable suggestions and advice throughout the period of the research work.*

It is my pleasure to take the opportunity to express my deepest sense of gratitude and sincere appreciation to ***Dr. Md. Fakhruzzaman****, Assistant Professor Department of Microbiology, VAS, HSTU.*

I am also grateful to all the office staffs of Central Laboratory, Department of Microbiology, HSTU as well as Poultry Care Lab, Gazipur for their kind co-operation towards the completion of the research work smoothly.

Finally but immensely, I wish to express my appreciations and sincere indebtedness to my parents, brothers, and other relatives and well-wishers for their heartiest blessings, affectionate feelings and all sorts of sacrifices to achieve my higher education and successful completion of this work.

The Author *June, 2012*

ABSTRACT

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 sera of commercial layer were collected from 17 commercial layer farms. The studied commercial layers had not been previously vaccinated and were different ages. 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 be positive. The result of this study indicate a high Infectious Laryngotracheitis (ILT) Virus activity in Gazipur district of Bangladesh hence there is an urgent need for the development of prevention and control policies against ILT in Bangladesh poultry farms and a national control programme for Infectious Laryngotracheitis Virus infection should be planned.

CONTENT

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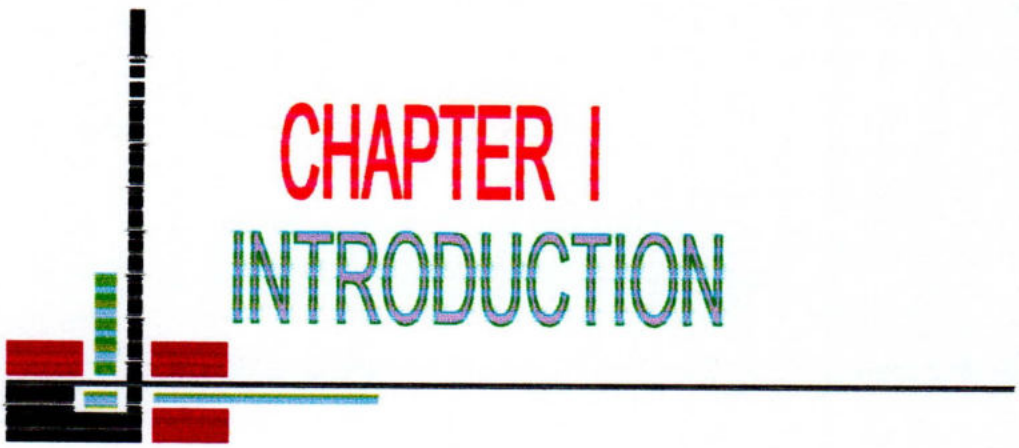
LIST OF ABBREVIATION AND SYMBOLS

AGID	= Agar Gel Immunodiffusion
CAM	= Chorioallantoic membrane
CC	=Cell Culture
CEO	= Embryonating egg
CEL	=Chicken Embryo Liver
CK	= Chicken Kidney
dsDNA	= double stranded Deoxyribo Nucleic Acid
DW	= Distilled Water
ELISA	= Enzyme Linked Immunosorbent Assay
iELISA	= indirect Enzyme Linked Immunosorbent Assay
ECE	= Embryonated chicken egg
g/ml	= Gram per mililitre
HSTU	= Hajee Mohammad Danesh Science and Technology University
IFA	=Indirect Fluorescent Antibody
ILT	= Infectious Laryngotracheitis
ILTV	= Infectious Laryngotracheitis Virus
Ig	= Immunoglobulin
Kb	=Killo Base
NC	= Negative control
NC $^{-}$	= Negative Control Mean
nm	= Nanometre
OD	= Optical Densities
PC	= Positive Control
PCR	= Polymer Chain Reaction
p^{NNP}	= p-Nitrophenyl Phosphate
p.i	= Post infection
PCx^{-}	=positive control mean
PCL	= Poultry Care Lab
rpm	= Rotation per minute

S/P	= Sample to Positive
TCO	= Tissue Culture
TRG	= Trigeminal ganglion
VAS	= Veterinary and Animal Science
VN	= Virus Nutralization
w/v	= Weight by Volume
µl	=Micro liter
°C	= Degree centigrade



CHAPTER 1
INTRODUCTION

CHAPTER I

INTRODUCTION

Respiratory diseases of poultry result in great production losses for the industry due to the severity of clinical signs. Infectious laryngotracheitis virus (ILTV) is included among the respiratory pathogens that can infect chickens and cause important economic losses. The disease can be present in two epizootic forms. The severe form is characterized by high morbidity, and moderate to high mortality, whereas the mild form commonly present now a days in the developed poultry industries, is responsible for the presence of clinical signs including, tracheitis, sinusitis, conjunctivitis, general depression, watery eyes, and low mortality. Although recognized as the mild presentation of the disease it produces weight loss and decreased egg production and has had a significant economic impact for the industry in the past decade.

Infectious laryngotracheitis is caused by gallid herpes virus-1 of the family herpesviridae, subfamily alphaherpesvirinae, genus *Iltovirus*. The virus is an enveloped, non segmented, linear dsDNA virus (Bagust *et al.*, 1986). The morbidity rate of ILT may be upto 100%, depending on the virulence of strain and immune status of the flock (Shibleyet *et al.*, 1962).

Although ILTV strains are antigenically homogenous, ILTV strains naturally vary in virulence from highly virulent strains, causing high morbidity and mortality, the strains with low virulence that produce mild-to-unapparent infection (Bauer *et al* 1999). Differentiation of ILTV strains varying in virulence, particularly mild type and modified live vaccine virus is an important practical problems. Clinical sings associated with the severe form of the disease include gasping, depression, nasal discharge, conjunctivitis and expectoration of body mucus. Upon gross examination of the trachea, characteristic severe haemorrhage and mucus plugs are observed (Dobson, 1935). The clinical signs associated with less severe forms of

the disease include conjunctivitis, swelling of the infra-orbital sinuses, closed eyes, persistent nasal discharge and mild tracheitis (Fahey *et al.*, 1984).

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 (Davidson *et al.*, 1988). Chickens are infected for ILTV through the upper respiratory and ocular routes (Goodwin *et al.*, 1991). Ingestion could be another way of infection but after that, exposure of nasal epithelium must occur (Hilbink, 1987).

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. Chicken flocks which are endemically infected with ILTV may occur only in some regions of countries or even in particular multiple-age production sites. However, 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).

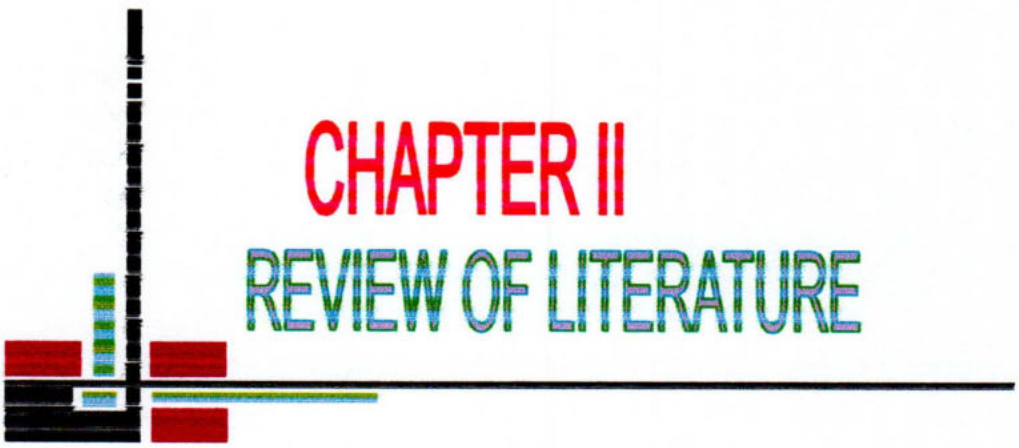
In Bangladesh, Reports of isolation of ILTV have not been published but the disease is rampant and breeder as well as commercial farms follow vaccination regularly. ILT is usually well controlled in layer flocks by the use of modified live virus vaccines. Live attenuated ILT vaccines provide immunity 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).

Several investigators studied the disease problems of ILT, its prevalence and incidence, pathology, immunity, propagation of its etiological agent and diagnosis of disease in various parts of the world (Bagust *et al.,* 1995).

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 with the following objectives:

1. To study the seroprevalence of Infectious Laryngotracheitis (ILT) virus antibody in commercial layer populations.



CHAPTER II

REVIEW OF LITERATURE

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REVIEW OF LITERATURE

This section of the thesis deals with the ILTV, its clinical manifestations and such other properties relevant to the piece of work under taken. The review of literature contains the following:

2.1 Etiological agent

Murphy *et al.*, 2000 recognized that infectious laryngotracheitis is caused by gallid herpes virus-1 of the family herpesviridae, subfamily alphaherpesvirinae, genus *Iltovirus*. The virus is an enveloped, non segmented, linear dsDNA virus. The morbidity rate of ILT may be upto 100% depending on the virulence of strain and immune status of the flock.

2.2 Composition Morphology and chemical

Bagust and Johnson, 1995 described that the glycoproteins of the virus, like other herpesviruses, are responsible for stimulating humoral and cell-mediated inmune responses. Five major envelope glycoproteins with molecular weights of 205, 160, 115, 90, and 60 kD have been reported.

Plummer *et al.*, 1969 reported that the nucleic acid of ILTV is comprised of DNA having a buoyant density of 1.704 g/ml, similar to other herpesviruses. Laryngotracheitis virus DNA has been reported to have a guanine plus cytosine ratio of 45%. The DNA genome consist of a linear 155-kb double-stranded molecule comprised of unique long and short segments flanked by inverted repeats.

Cruickshank *et al.*, 1963 described the hexagonal nucleocapsid of ILTV to be 80-100 nm in diameter. The nucleocapsid have icosahedral symmetry and are composed of 162 elongated hollow capsomeres. The complete virus particle including an irregular envelope surrounding the nucleocapsid has a diameter of

195- 250 nm. The envelope contains fine projections representing viral glycoprotein spikes on its surface.

Watrach *et al.*, 1961 reported that electron micrographs of ILTV-infected chicken embryo cell cultures demonstrate the presence of icosahedral viral particles similar in morphology to herpes simplex virus.

2.3 Viral replication

Guo *et al.*, 1993 reported that the virus initiates infection by attachment to cell receptors followed by fusion of the envelope with the host cell plasma membrane. The nucleocapsid is released into the cytoplasm and transported to the nuclear membrane, viral DNA is released from the nucleocapsid and migrates into the nucleus through nuclear pores. Transcription and replication of viral DNA occur within the nucleus. Transcription of ILTV DNA occurs in a highly regulated, sequentially ordered cascade similar to that of other alphaherpesviruses. Approximately 70 virus-coded proteins are produced; several are enzymes and DNA-binding proteins that regulate viral DNA replication, but most are viral structural proteins.

Pulsford and Stokes, 1953 found that viral DNA replication occurs by a rolling circle mechanism with the formation of concatemers which are cleaved into monomeric units and packaged into preformed nucleocapsids within the nucleus. DNA-filled nucleocapsids acquire an envelopeby migration through the inner lamellae of the nuclear membrane. Enveloped particles then migrate through the endoplasmic reticulum and accumulate within vacuoles in the cytoplasm.

2.4 Chemical and physical viral resistance

Neighbour *et al.*, 1994 found that ILTV has been inactivated in less than 1 minute under a 3% cresol or 1% solution action. Laboratory bench surfaces can be readily decontaminated with commercial iodophors or halogen-detergent mixtures. The complete inactivation of ILTV infectivity was obtained with a 5% hydrogen peroxide mist as a fumigant for poultry house equipment.

Meulemans and Halen, 1978a observed that ILT virus infectivity survives for several months when stored at 4° C in diluents like glycerol or nutrient broth.

Jordan, 1966 mentioning that LTV is able to survive in tracheal exudates and chicken carcasses for periods of 10-100 days at ambient temperatures of 13-23 °C.

Fitzgerald and Hanson, 1965 found that Enveloped ILTV infectivity is affected by organic solvents (lipolytic agents) such as chloroform and ether.

Jordan, 1963 found that ILTV infectivity has been rapidly inactivated by heat when exposed to 55°C for 15 minutes or 38 °C for 48 hrs.

Webster, 1959 found that 1% of the infectivity of a Belgian strain was retained after 1 hr at 56 °C.

Cover and Benton 1958 reported that ILTV is destroyed in 44 hrs at 37 °C in tracheal tissues within chicken carcasses or in chorioallantoic membranes (CAMs) after 5 hr at 25 °C.

2.5 Antigenicity and virulence of LTV strains

Lieb *et al.*, 1987 found that restriction endonuclease analysis of LTV DNA has been used extensively in epidemiologic studies of field outbreaks to differentiate wild-type and modified live-vaccine viruses. Reciprocal DNA: DNA hybridization using cloned DNA fragments also has been shown to discriminate ILTV strains.

Izuchi and Hasagawa, 1982 found that differentiation of ILTV strains of varying virulence, particulary wild-type and modified live-vaccine viruses, is an important practical problem. Several methods for differentiating ILTV viruses have been studied including analysis of virulence for chicken embryos, restriction endonuclease analyses of viral DNA and DNA hybridization assays.

Kotiw *et al.*, 1980 reported that Assessment of mortality patterns in embryonated chicken eggs was proposed as a biologic system for differentiating ILTV strains

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and mortality patterns correlated closely with virulence. Restriction endonuclease cleavage of viral DNA and electrophoretic separation of DNA fragments has been shown to distinguish different ILTV strains.

Ide, 1978 reported that Laryngotracheitis virus strains appear to be antigenically homogenous based on virus-neutralization, inmunofluorescence tests, and cross-protection studie. However minor antigenic variation among strains has been suggested by the finding that some strains are neutralized poorly by heterologous antisera.

Cover and Benton, 1958 reported that naturally occurring LTV strains vary in virulence from highly virulent strains that produce high morbidity and mortality in exposed chickens to strains of low virulence that produce mild to inapparent infections .

2.6 Latency of ILTV

Williams *et al.*, 1992 demonstrated that mature laying chicken inoculated intratracheally with a field strain of ILTV showed viral DNA by PCR in trigeminal ganglia at 31, 46 and 61 days post inoculation. The re-excretion of ILTV virus from latently infected chicks following the stress of re-housing and the onset of reproduction.

Bagust, 1986 established latent infections, which have been demonstrated by the re-isolation of virus from the seventh week after infection by repeated tracheal swabbings, and at 2 months after infection in tracheal organ cultures.

Kaleta *et al.*, 1984 showed that trigeminal ganglion (TRG) is the main site of latency of ILT virus. The TRG provides the main sensory innervation to the tissues of the upper respiratory tract, and then neural viral migration is strongly inferred.

2.7 Transmission

Williams *et al.*, 1994 found that the virus may remain at very low levels up to 10 days p.i., no clear evidence exist for a viremic phase of infections. Clinically inapparent ILTV infection of the respiratory tract is a major feature of ILT persistence. Collecting laryngeal and tracheal swabs from recovered infected birds and then inoculating susceptible chickens, indicated a "field" carrier rate of approximately 2% up to 16 months after a disease outbreak.

Robertson and Egerton, 1981 observed that transmission occurs from acutely infected birds. Transmission through contact with clinically recovered carrier birds is more difficult to occur. ILTV infections of the upper respiratory tract of susceptible chickens is followed by intense viral replication. Infectious virus usually is present in tracheal tissues and secretions for 6-8 days p.i.

Turner, 1972 founded that mechanical transmission can occur by use of contaminated equipment and litter.

Jungherr, 1958 studied with tracheal organ cultures explanted from chickens experimentally infected with Australian wild-type ILTV and vaccine strains have been showed latent tracheal infections for similar periods in 50% or more of infected chickens.

Beaudette, 1937 found that chickens are infected for ILTV through the upper respiratory and ocular routes. Ingestion could be another way of infection but after that, exposure of nasal epithelium must occurs.

2.8 Clinical Signs

Seddon and Hart, 1935 reported that clinical signs generally appear 6-12 days following natural exposure.

Hinshaw *et al.*, 1933 observed clinical signs associated with mild enzootic forms include unthriftiness, reduction in egg production, eye secretion, conjunctivitis, swelling of infra orbital sinuses, persistent nasal discharge, and hemorrhagic conjunctivitis. The course of the infection varies with the severity of lesions. Generally, most chickens recover in 10-14 days, but extremes of 1-4 week have been reported.

Kernohan, 1931a reported that characteristic clinical signs include nasal discharge and moist rales followed by coughing, gasping, sneezing, depression and conjunctivitis.

Beach, 1926 founded that severe epizootic forms of the disease occur, signs also include labored breathing and expectoration of blood-stained mucus; and upon gross examination of the trachea, severe hemorrhages and mucus plugs are characteristics.

2.9 Gross lesions

Davidson *et al.*, 1988 described gross lesions are most consistently observed in the larynx and trachea, even though the conjunctiva and other respiratory tissues could be also be affected. Tissue changes in tracheal and laryngeal tissues may be mild, with only excessive amount of mucus, conjunctivitis, sinusitis, and mucoid or severe, with hemorrhage and/or diphtheric changes. In severe forms, degeneration, necrosis, and hemorrhage occur in later stages. Mucoid secretions extended along the entire length of the trachea may be present. In other cases, severe hemorrhage into the tracheal lumen may result in blood clots, or blood may be mixed with mucus and necrotic tissue. Inflammation may extend down the bronchi into the lungs and air sacs. Edema and congestion of the epithelium of the conjunctiva and infraorbital sinuses may the only gross lesion observed in mild forms of ILT.

2.10 Microscopic lesions

Guy *et al.*, 1992 reported that early microscopic changes in tracheal mucosa include the loss of goblet cell and infiltration of mucosa with inflammatory cells.

As the viral infection progresses, cell enlarge, lose cilia, and become edematous. Multinucleated cells (syncytia) are formed and lymphocytes, histocytes, and plasma cells migrate into the mucosa and submucosa after 2-3 days. Later, cell destruction and desquamation result in a mucosal surface either covered by a thin layer of basal cells or lacking any epithelial covering; blood vessels within the lamina propia may protrude into the tracheal lumen. Hemorrhage may occur in cases of severe epithelial destruction and desquamation with exposure and rupture of blood capillaries. Intranuclear inclusion bodies are found in epithelial cells by 3 days p.i. Inclusion bodies generally are present only in the early stages of infection (1-5 days).

2.11 Immunity

Fahey and York, 1990 showed that the principal mediator of ILT resistance is the local cell-mediated immune response in the trachea. Maternal antibodies to ILTV do not protect offspring against infection or interfere with vaccination.

York *et al.*, 1989 observed secretary antibodies, including IgA, are important to confer resistance to infection at mucosal surfaces, such as respiratory tract.

Jordan, 1981 described several types of immune responses are involved after ILTV infection. Virus-neutralization antibodies can be detected within 5 – 7 days p.i., with peak at 21 days and then antibody waned to be detected to low levels over a year.

Waldman and Ganguly, 1974 described that mucosal IgA antibody responses are also known to be elicited more efficiently by local rather systemic administration of antigen.

Benton *et al.*, 1958 founded that the susceptibility of chickens to ILTV declined with age and meat-type males are more susceptible than meat-type females. It has been also demonstrated that high environmental temperatures (35° C) cause higher mortality from ILTV infection in heavy adult breeds than in light adult breeds.

Hitchner *et al.*, 1957 founded that total specific antibody against ILTV was detected in tracheal washings from day 5 p.i., IgA antibody appeared at day 6 p.i., but neutralizing antibody could not be detected until day 14. In ILTV vaccinated chickens there was a substantial increase in the number of IgA and IgG synthesizing cells in the trachea by day 3 p.i. with a marked increase in the numbers of IgA positive cells at day 7 p.i.

2.12 Epidemiology

Guy and Bagust, 2003 described that ILT is a major viral respiratory disease included within List E of the Office International des Epizooties. The chicken is the only significant primary host species for ILTV, and no other reservoir species have been recognized, even though pheasants and peafowls can sometimes be naturally infected by contact with chickens actively shedding ILTV .

Linares *et al.*, 1994 described the evidences on some outbreaks of mild enzootic forms of ILT in some countries in South America, such as Chile, Peru, Bolivia, Argentina and Brasil. Sporadic cases occur in all classes of birds, including hobby/show/game chickens, broilers, heavy breeders, and commercial leghorns. In the case of heavy breeders and leghorns, which are typically vaccinated against ILT, sporadic cases are often related to errors in vaccine application and to biosecurity failures: commercial table egg producers may desire to avoid the expense associated with eye drop administration of ILTV vaccine, and change to mass application. This change may result in inadequate protection. Since multiple age layer complexes are common, and inadequately-vaccinated flock may be exposed to ILTV later, when a younger, vaccinated flock is moved into the complex and sheds the back passaged vaccine virus, resulting in disease signs in the older flock. Cases in molted flocks that were not re-vaccinated, and the use of recently vaccinated "spiking males" in a poorly vaccinated, older breeder flock are other classic examples. Virus shed after the latent period is another source of virus capable of causing disease in susceptible birds.

Hilbink *et al.*, 1987 founded that the sources of ILTV are: (a) clinically affected chickens, (b) chickens which are latent carriers of infection, and (c) fomites and poultry farm personnel contaminated with ILTV. No matter the portals of entry for ILTV (nasal, oral, conjunctiva, or even experimentally intraorbital sinus) the epithelium of the trachea and larynx is always affected by ILTV, and the most active viral replication will occur within the trachea. In its acute form, ILT is characterized by signs of respiratory distress in birds, accompanied by gasping and expectoration of bloody exudates. In addition, the mucous membranes of the trachea become swollen and hemorrhagic. These events last for approximately 7 to 10 days with large amount of ILTV production. This is most important period for virus shedding.

Robertson and Egerton, 1981 investigated that application of ILT vaccines by spray may result in adverse reactions as a result of insufficient attenuation of vaccine virus, deep penetration of respiratory tract due to small droplets size of spray or excessive dose. Laryngotracheitis vaccine viruses have been shown to spread readily from vaccinated to nonvaccinated chickens.

Clarke *et al.*, 1980 reported that ILT is a slowly spreading, controllable disease. If a diagnosis of ILT is obtained early in an outbreak, vaccination of unaffected birds may induce adequate protection before they become exposed. Administration of modified live-ILT vaccines in drinking water or by spray are desirable methods for rapid, mass application of these vaccines; however, the administration of ILT vaccines by the drinking water routes results in a high proportion of chickens that fail to develop protective immunity.

Shibleyet and Helmboldt, 1962 described the epizootic form of the disease spreads rapidly and although severe forms of the disease cause high morbidity (90 – 100%), and mortality varies from 5% to 70% and averages 10-20%. Severe epizootic forms of ILT were commonly described in earlier years. However, it is also possible to found mild enzootic forms of ILT observed in the intensive poultry producing areas of Europe, Australia, New Zealand and the United States.

2.13 Diagnosis of ILT

McCracken, 1978a demonstrated that CEL and CK cells were more efficient for ILTV isolation and propagation, with CEK cells, chicken embryo lung cells, and CAM inoculation of embryonated chicken eggs being less sensitive. Viral cytopathic effects may be observed in cell culture as early as 4-6 hr p.i. with a high multiplicity of infection. It could be found increased refractiveness and swelling of cells, chromatin displacement, and rounding of the nucleoli. Cytoplasmic fusion results in formation of multinucleated giant cells. Intranuclear inclusion bodies can be detected as early as 12 hr post-infection. Large cytoplasmic vesicles develop in the multinucleated cells and become more basophilic as cells degenerate.

Chang *et al.*, 1977 reported that macrophage cultures were as susceptible to ILTV infection as CK cells, but replication of most ILTV strains examined was restricted. Both cell genotype and virus genotype influenced the extent of restriction of virus replication. Histopathology examination remains the standard method for the rapid diagnosis of ILT. Characteristic lesions of ILT include syncytial cell formation of the tracheal epithelial cells with the development of pathognomonic intranuclear inclusion bodies, necrosis, and hemorrhage.

Reynolds, 1968 founded that ILTV has been also replicated in avian leukocyte cultures derived from chicken buffy coat.

Keller and Hebel, 1962 showed that inclusion bodies could be detected in 57% of 60 specimens, while virus was isolated from 72% of the same specimens.

Pirozok *et al.*, 1957 founded that inclusion bodies are usually present in the early stages of infection 1 to 5 days p.i., and disappear as infection progresses as a result of necrosis and desquamation of epithelial cells.

Brandly, 1937 reported that ILT infections must be differentiated from others respiratory diseases which present similar clinical signs and lesions. In these cases ILT diagnosis must be assisted by laboratory methods ILTV isolation.

Laryngotracheitis virus may be propagated in embryonated chicken eggs (ECE) and a variety of avian cell cultures. In embryonated chicken eggs the virus causes formation of opaque plaques on the CAM resulting from necrosis and proliferative tissue reactions. Plaques are observed as early as 2 days p.i. and embryo deaths occur 2-12 days later. Survival time of inoculated embryos decreases with additional egg passages.

Burnet, 1934 reported that ILTV has been propagated in a variety of avian cell cultures (CC) including chicken embryo liver (CEL), chicken embryo lung, chicken embryo kidney (CEK), and chicken kidney (CK) cell cultures.

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2.14 ILTV identification by immunoprobes

Goodwin *et al.*, 1991 studied rapid assays for identification of ILTV utilize immunoprobes to detect viral antigens. Fluorescent-labeled polyclonal antibodies are commonly used as immunoprobes to detect ILTV in tracheal and conjunctival smears.

Guy *et al.*, 1990 reported that monoclonal antibodies have also been used to detect viral antigens in suspensions of tracheal scraping by enzyme-linked immunosorbent assay (ELISA).

Braune and Gentry, 1965 founded that immunoperoxidase-labeled monoclonal antibodies have been used to detect viral antigens from frozen tissue sections.

2.15 ILTV DNA detection.

Keam *et al.*, 1991 described the techniques for detection of ILTV DNA utilizing dot-blot hybridization assay and cloned ILTV DNA fragments labeled with digoxigenin. These procedures were shown to be highly sensitive for detection of ILTV in acutely infected chickens, as well as convalescent chickens, when detection was no longer possible using virus isolation and ELISA. These assays

also were shown to provide rapid methods for detection of chickens latently infected with ILTV.

2.16 Serology

Adair *et al.*, 1985 reported the demonstration of ILTV antibodies in serum can be done through different tests: agar gel immunodiffusion (AGID), virus neutralization (VN) in ECE or CC, indirect fluorescent antibody (IFA) test, and ELISA. Actually, ELISA offers ease of testing for large number of sera. This method has been demonstrated to be more sensitive than VN and of comparable sensitivity of IFA, with AGID being the least sensitive.

2.17 Management procedures for prevention and control

Kingsbury and Jungherr, 1958 described for intensive broiler production, the short growth cycle and high level of bio-security measures on farms can reduce the need for prophylactic vaccination. The application of bio-security measures will avoid exposing susceptible chickens via contaminated fomites. The importance of site quarantine and hygiene in preventing the movement of potentially contaminated personnel, feed, equipment, and birds is central to successful prevention and control of ILT. Cooperative control of ILT outbreaks by collaboration between government and industry is most desirable. Where outbreaks have been contained, recovered flocks should be moved for processing under quarantine as soon as possible. Experience with ILT outbreaks in Pennsylvania indicates that this interval can be as short as 2 wk after the last clinical signs of ILT are observed on a farm. For control of an ILT outbreak, the most effective approach is a coordinated effort to obtain a rapid diagnosis, to establish a vaccination program, and prevent further virus spread. Vaccination in the face of an outbreak will both limit virus spread and shorten duration of the disease. Spread of ILTV between farms can be prevented by appropriated bio-security measures. Laryngotracheitis virus infectivity is readily inactivated outside

the host chicken by disinfectants and warm temperatures, thus carryover between successive flocks in a house can be prevented by adequate cleanup.

2.18 Immunization for prevention and control

Keeler *et al.*, 1993 described ILTV infections are usually limited to the upper respiratory tract and viremia is rarely observed. Furthermore, the humoral immune response, including secretory and maternal antibodies, and levels of neutralizing antibodies do not correlate well with protection. Instead, protection seems to be mediated primarily by the cellular immune response. These points are important to consider in the development of a vaccination strategy. Any effective vaccine will have to elicit an effective mucosal, cell-mediated, protective immune response Vaccination for ILT has generally been used only in areas where the disease is endemic, since vaccination can result in the occurrence of long-term "carrier" birds due to the virus' ability to enter a latent state in the sensory ganglia. Furthermore, current vaccines are themselves mildly pathogenic, with a resulting economic "cost". There is justifiable concern over the negative performance (growth, mortality, feed conversion) associated with current ILT vaccines. Modified live ILT vaccines increase in virulence by mutation during bird-to-bird passage in the field. This has led to an additional reluctance to vaccinate for ILT unless a region is faced with an active outbreak of the disease. Other groups contend that vaccine strains of ILTV are genetically stable . However, by spreading into flocks that may contain birds of different ages and with a different immune status, the incompletely attenuated modified live vaccine strains of ILTV may manifest a clinically more severe disease.

2.19 Live attenuated ILT vaccines

Fulton *et al.*, 2000 reported that most vaccines when given by eye drop method had lower mean microscopic lesion scores and higher ELISA titers after one vaccination. In contrast to other mass application methods, eye drop vaccination in flock situations when applied correctly ensures that all birds in that flock have

received vaccine. Careful attention must be given to procedures of vaccine administration to ensure adequate immunization.

Samberg *et al.*, 1971 reported traditionally there have been two sources of live attenuated ILT vaccines. Vaccines attenuated by multiple passages in embryonating eggs (CEO) , are higher effective. However, in many cases their use can result in lower performance and higher condemnation rates. Broilers are generally vaccinated with CEO vaccine by drinking water only in the face of an outbreak. Furthermore, CEO-derived vaccine strains of ILTV are generally indistinguishable from true field isolates of ILTV providing diagnosticians and regulators with additional challenges. ILT vaccines generated by multiple passages in tissue culture (TCO) generally offer less protection as they are more highly attenuated and less immunogenic. TCO vaccines are commonly used in layer breeders and layers.

CHAPTER III
MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

The present research work was conducted during the period from January/2012 to June/2012 in the department of Microbiology, Hajee Mohammad Danesh Science and Technology University (HSTU), and Poultry care Lab, Gazipur. The materials and Methods which were used to perform this research work is described below:

3.1 Materials

3.1.1 Samples

A total number of 232 commercial layer sera were collected from 17 commercial layer farms of Gazipur district in Bangladesh. The studied commercial layer had not been previously vaccinated and were different ages. All these flocks had history of infection clinically with ILTV.

Table 1: Enlist of selected commercial layer farms

Sl. No	Name of farm	Address	Age	Flock size (bird)	Number of collection of sample
01	Zahid Poultry	Mirer Bazar, Gazipur	14w	1500	10
02	Akhi Poultry	Mirer Bazar, Gazipur	13w	3000	16
03	Helal Poultry	Pubile, Gazipur	21w	2500	16
04	Malak Poultry	Pubile, Gazipur	19w	1600	10
05	Chanmia Poultry	Kaliakor, Gazipur	09w	2500	16
06	Rashed Poultry	Kaliakor, Gazipur	16w	1400	10
07	Tamanna Poultry	Harinal, Gazipur	51w	2500	16
08	Zillu Poultry	Harinal, Gazipur	12w	1000	10
09	Kader Poultry	Zolarpar, Gazipur	11w	1000	8
10	Billal Poultry	Zolarpar, Gazipur	31w	2500	16
11	Masud Poultry	Zolarpar, Gazipur	17w	3000	16
12	Deen Poultry	Chabagan, Gazipur	25w	2700	16
13	Sahin Poultry	Chabagan, Gazipur	08w	3000	16
14	Sorif Poultry	Chabagan, Gazipur	15w	1200	8
15	Asraf Poultry	Kapasia, Gazipur	44w	2300	16
16	Siful Poultry	Kapasia, Gazipur	17w	2000	16
17	Manik Poultry	Kaligonj, Gazipur	35w	2500	16

3.1.2 Materials used for the collection and transportation of blood samples

- Data sheet
- Gloves
- Ice carrier with ice packs
- Disposable plastic glass
- Sterile cotton
- Permanent marker pen
- Disposable plastic syringe(3ml)
- Hexisol solution

3.1.3 Materials used to obtain serum

- Gloves
- Plastic rack
- Centrifuge tube
- Centrifuge machine
- Marker pen
- Disposable pipette tips
- Single channel pipette
- Multi-channel pipette
- Cryovials
- Ice carrier with ice
- Eppendorf tube

3.1.4 Equipments And appliances not provided with the kit

- Single channel pipette (delivery range: 0.5µl to 10 µl) fordiluting and transferring specimens.
- Single channel pipette (delivery range: 100 µl to 1000 µl) for sample diluent.

- Multi-channel pipette (50 µl -300 µl delivery volume; 8 channels) for diluting specimen and reagent handling.
- Disposable pipette tips
- Graduated cylinders for wash solution preparation (500 to 2000ml)
- Dilution tubes for specimen preparation
- Absorbance paper
- Microtitre plate reader with 405 nm filter

3.1.5 Reagents Provided with the kit

- **ILT Coated Plate**: Inactivated viral antigen on microtitre plates.
- **Conjugate Reagent**: Anti-Chicken, Alkaline Phosphatase in This buffer with protein stabilizers, inert red dye and sodium azide preservative (0.1%w/v).
- **Substrate Tablets**: PNNP (p-Nitrophenyl Phosphate) tablets to dissolve with Substrate buffer.
- **Substrate Buffer Reagent**: Diethanolamine buffer with enzyme co-facrors.
- **Stop Solution**: Sodium Hydroxide in Diethanolamine buffer
- **Sample Diluent Reagent**: Phosphate buffer with protein stabilizers and sodium azide preservative (0.1%w/v).
- **Wash Buffer Sachets**: Powdered Phosphate Buffered saline with Tween
- **Negative Control**: Specific pathogen free serum in phosphate buffer with protein stabilizers and sodium azide preservative (0.1%w/v).
- **Positive Control**: Antibodies specific to ILT in phosphate buffer protein stabilizers and sodium azide preservative (0.1%w/v).

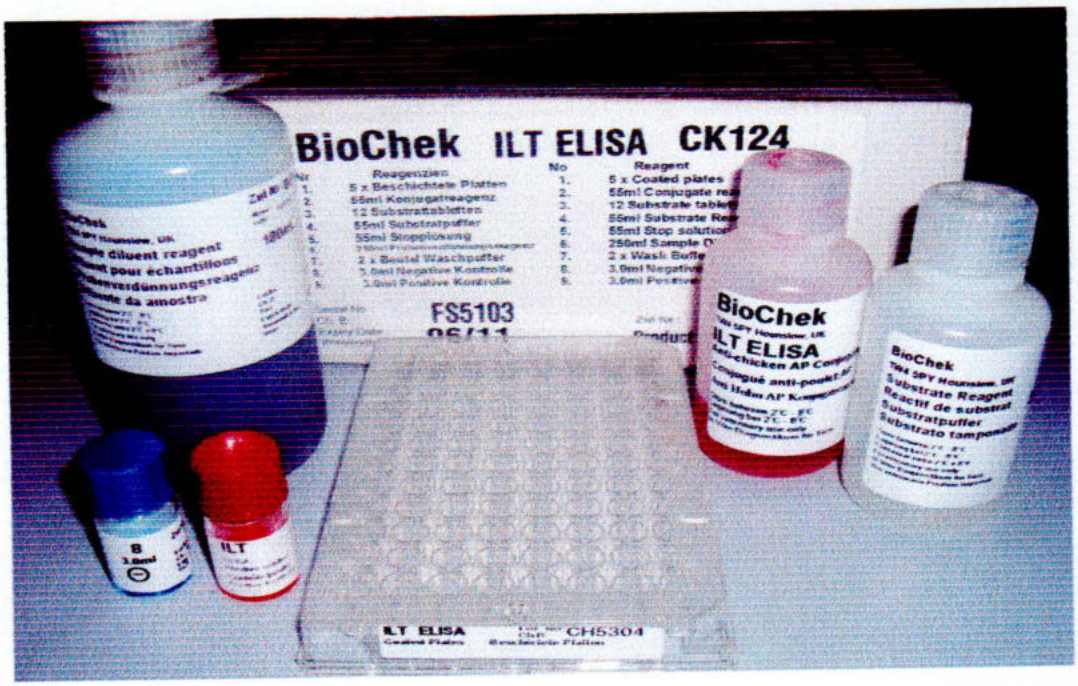


Fig. 1: ILT antigen coated plate, chemicals and reagents used for ELISA.

3.2 Methods

3.2.1 Experimental Design

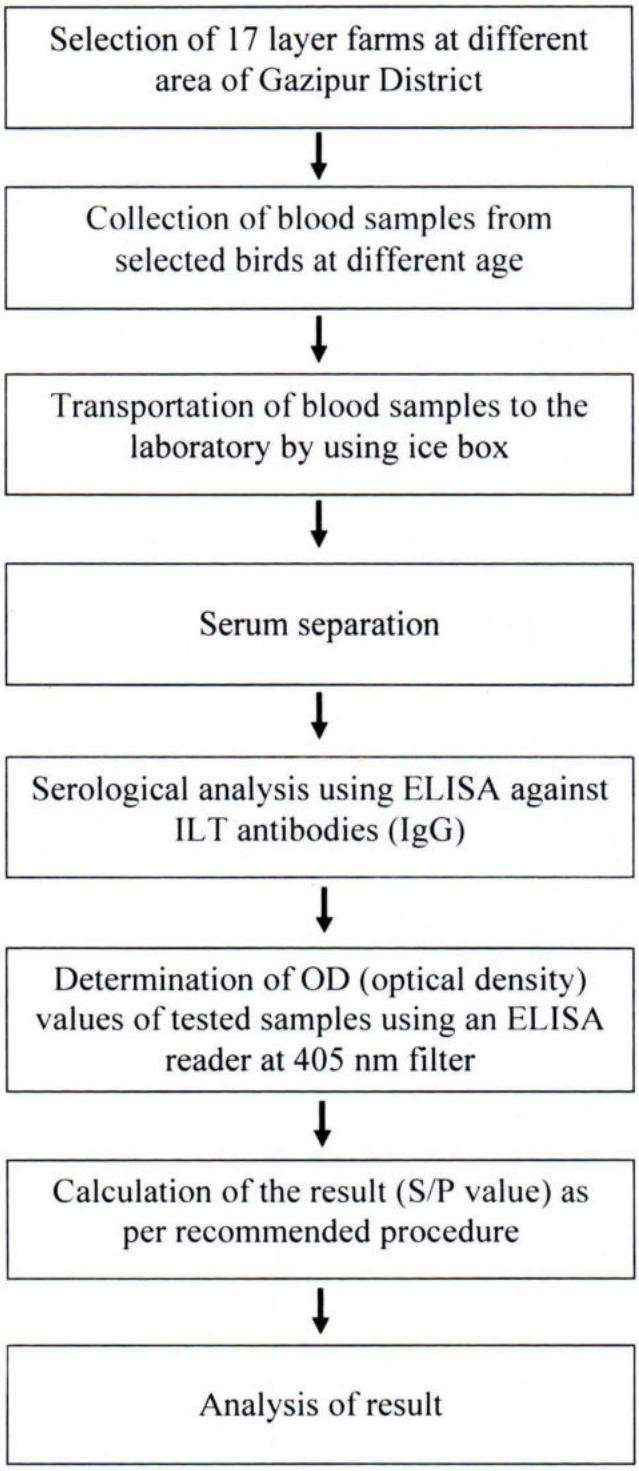


Fig. 2 : Schematic illustration of the experimental design

3.2.4.2 Wash solution preparation

The concentrated wash solution wash allowed to reach at room temperature (20° C-25°C) prior to preparation .The concentrate was mixed in the bottle by inversion to dissolve any precipitated salts and to ensure a homogenous solution. The wash concentrate was diluted with distilled water (1 sachet in 1000 ml DW) according to the methods provided by BioChek Infectious Laryngotracheitis Virus Antibody Test Kit-CK124 using a graduated cylinder 25 ml of diluted wash were needed for each plate tested i.e. for 3 plates; 75 ml of wash concentrate was required.

3.2.4.3 Preparation of Substrate Reagent.

To make substrate, add 1 tablet to 5.5-6 ml of substrate buffer and allow to mix until fully dissolved (+/- 10 minutes). The prepared reagent should be made on day of use but will be stable for one week if kept in dark at +4° C. Drop tablets into clean container and add appropriate volume of substrate buffer.

3.2.5 Principles of Indirect ELISA Test

The ILT ELISA kit will measure the amount of antibody to ILT in the serum of chickens. Microtitre plates have been pre-coated with inactivated ILT antigen. Chicken serum samples are diluted and added to the microtitre wells where any anti-ILT antibodies present will bind and form an antigen antibody complex. Non specific antibodies and other serum proteins are then washed away. Anti chicken IgG labelled with the enzyme alkaline phosphatase is then added to the wells and binds to any chicken anti ILT antibodies bound to the antigen. After another wash to remove unreacted conjugate, substrate is added in the form of p^{NNP} chromogen. A yellow colour is developed if anti-ILT antibody is present and the intensity is directly related to the amount of anti-ILT antibody present in the sample.

3.2.6 Procedure of Indirect ELISA Test

- ILT coated plate was removed from sealed bag and record location of samples on template.
- 100 µl of negative control was added into wells A1 and B1
- 100 µl of positive control was added into wells C1 and D1.
- 100 µl of diluted samples were added into the appropriate wells. Cover plate with lid and incubated at room temperature (22-27°C) for 30 minutes.
- Contents of wells were aspirated and washed with 4 times by buffer (350 µl per well). Invert plate and tap firmly on absorbent paper until no moisture were visible.
- 100 µl of Conjugate reagent was added into the appropriate wells. Cover plate with lid and incubated at room temperature (22-27° C) for 30 minutes.
- The plate was washed repeat like procedure as in 5.
- 100 µl of Substrate reagent was added into the appropriate wells. Cover plate with lid and incubated at room temperature (22-27° C) for 30 minutes.
- 100 µl of Stop solution was added to appropriate wells to stop reaction.
- Lastly, the plate placed on ELISA Reader and recorded the absorbance of controls and the samples by reading at 405 nm.

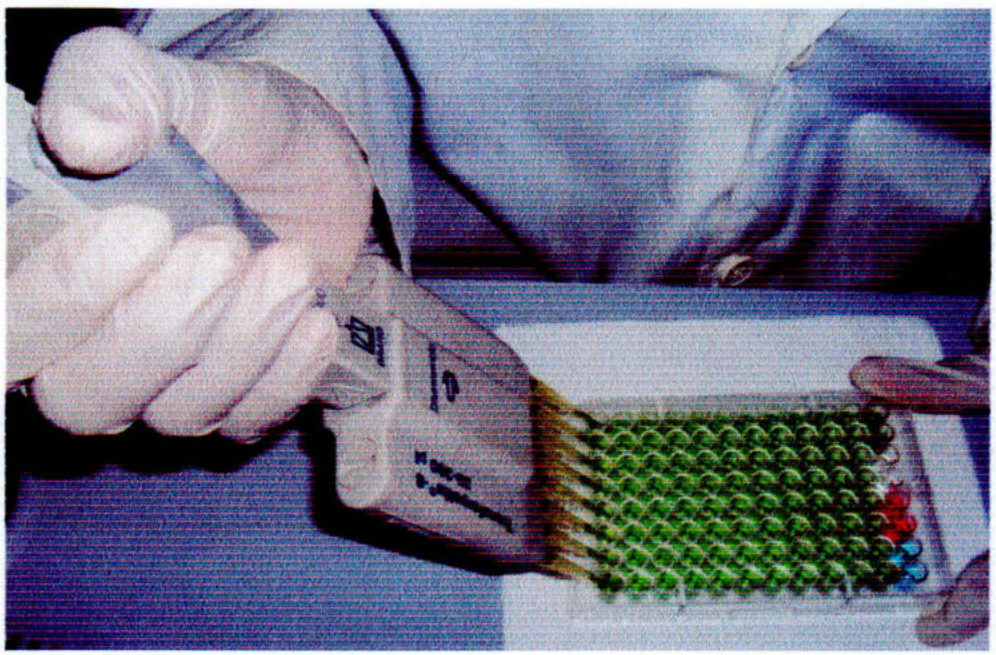


Fig. 3: Diluted serum taken on ILT antigen coated plate.

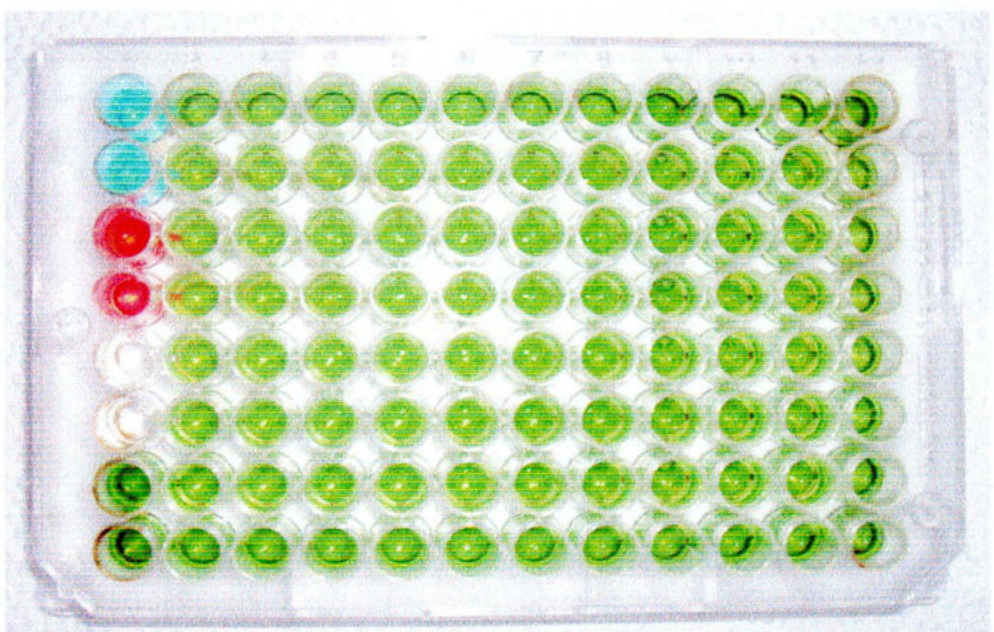


Fig. 4: Diluted serum containing ILT antigen coated plate with negative, positive and reference control.

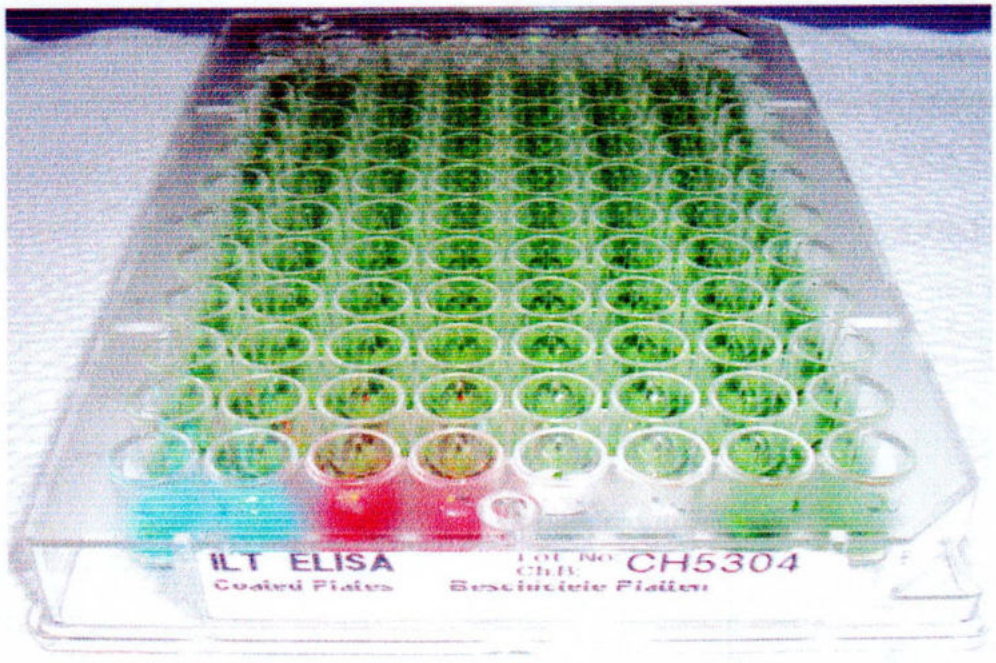


Fig. 5: Diluted serum containing ILT antigen coated plate.

Fig. 6: Conjugate Reagent containing ILT antigen coated plate.

Fig. 7: Substrate reagent containing ILT antigen coated plate.

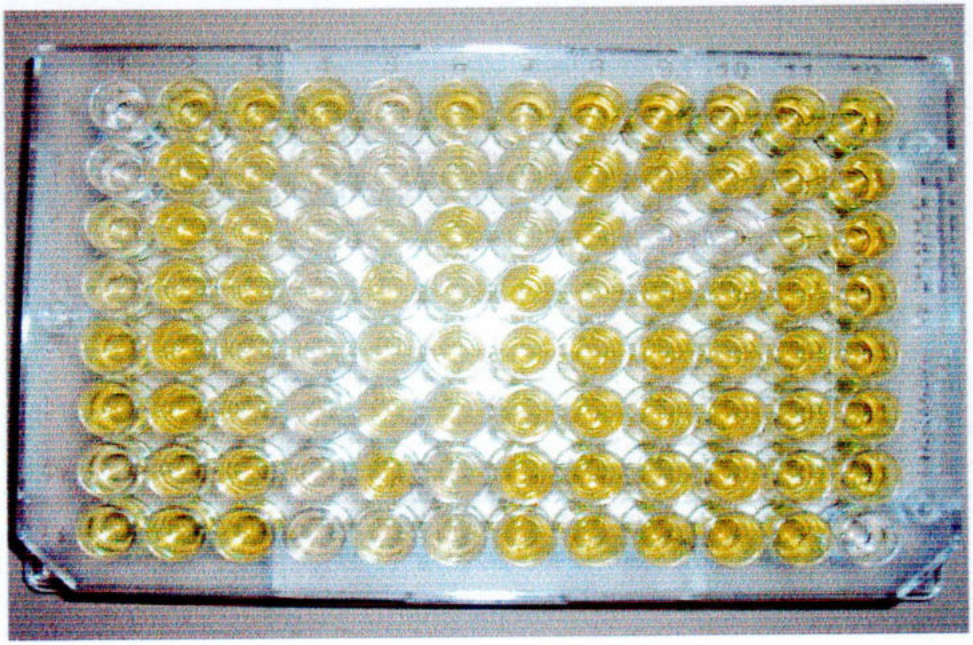


Fig. 8: Yellow colour develop in positive case.

3.2.7 Calculations

3.2.7.1 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

3.2.7.2 Calculation of the positive control mean OD (PCx) 9 wells C1 and D1)

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

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

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

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

3.2.7.4 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.

For example,

Sample calculation: unknown OD (405nm)= 0.658

$NCx^- = 0.125$

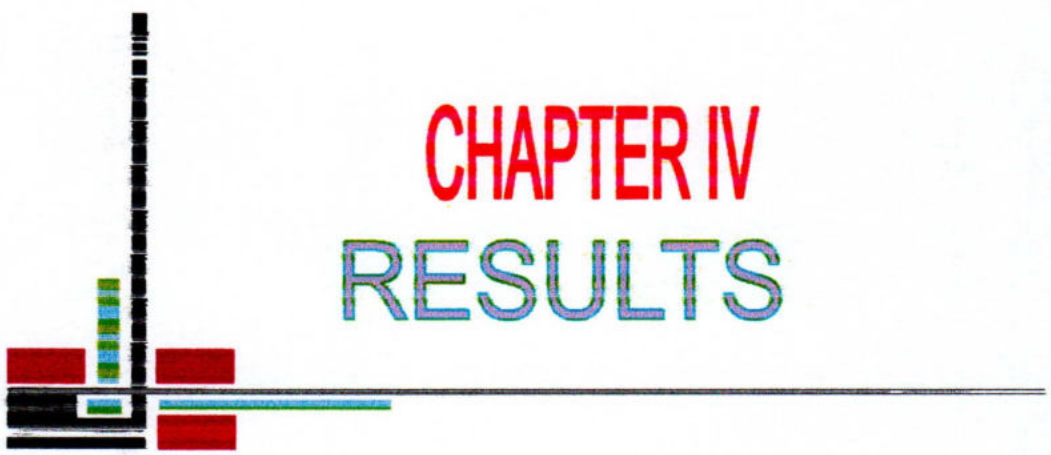
$PCx^- = 0.52$

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

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

3.2.7.5 Validity Specifications

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



CHAPTER IV
RESULTS

CHAPTER IV

RESULTS

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 farms of Gazipur district in Bangladesh. The studied commercial layer had not been previously vaccinated and were different ages. Blood samples were then carried to the department of Microbiology, HSTU and Poultry Care Lab, Garzipur. The serum samples were collected by centrifuging the liquid portion of the clotted blood at 2,500 rpm for 5 minutes. These were then subjected to indirect ELISA using Biochek ILTV virus Antibody Test Kit-CK124.

The result of indirect ELISA using biochek ILTV virus Antibody Test Kit against ILT antibody (IgG) from different blood samples of different farms are shown in table: 02-18. Out of 232 samples examined and 189 samples were found to be positive for ILT antibodies (IgG) i.e. 81.47 % samples were found to be positive.

The positively of unknown sera for the antibodies against ILT was determined by calculating the sample to positive (S/P) ratio on the basis of the OD values of positive controls , negative controls and samples using an ELISA reader at 405nm filter .The representative OD values of ELISA plates are shown in the table ,02-18.

OD values of indirect ELISA (Table 02 -08; plate 01)

Negative control mean OD value, $NCx^{-} = \frac{0.120 + 0.131}{2} = 0.125$

Positive control mean OD value, $PCx^{-} = \frac{0.527 + 0.513}{2} = 0.52$

Difference between positive and negative control OD value,

$$PCx^{-} - NCx^{-} = 0.52 - 0.125 = 0.395$$

Table 2: Determination of ILTV antibody of Zahid poultry farm

Sample No	Positive Control mean	Negative Control mean	OD Values at 405 nm	S/P Values	Result	No. and % of Positive samples
1.	0.125	0.52	0.304	0.452	NEG -	8(80)
2.			0.844	1.821	POS +	
3.			0.873	1.895	POS +	
4.			0.613	1.236	POS +	
5.			0.462	0.853	POS +	
6.			0.249	0.313	NEG -	
7.			0.575	1.139	POS +	
8.			0.581	1.155	POS +	
9.			0.498	.944	POS +	
10.			0.745	1.570	POS +	

Table 2 Showed that out of 10 sera samples tested by ELISA, S/P values of 8 (80%) samples were greater than 0.5 which indicated positive results where as S/P values of 02(20%) sera samples less than 0.5 indicated negative result.

Table 3: Determination of ILTV antibody of Akhi Poultry farm

Sample No	Positive Control mean	Negative Control mean	OD Values at 405 nm	S/P Values	Result	No. and % of Positive samples
01	0.125	0.52	0.550	1.076	POS +	13(81.25)
02			0.745	1.570	POS +	
03			0.250	0.316	NEG -	
04			0.906	1.978	POS +	
05			0.647	1.322	POS +	
06			0.477	0.891	POS +	
07			0.579	1.150	POS +	
08			0.301	0.445	NEG -	
09			0.621	1.256	POS +	
10			0.670	1.380	POS +	
11			0.575	1.139	POS +	
12			0.608	1.223	POS +	
13			0.213	.222	NEG -	
14			0.627	1.271	POS +	
15			0.542	1.056	POS +	
16			0.674	1.390	POS +	

Table 3 Showed that out of 16 sera samples tested by ELISA, S/P values of 13 (81.25%) samples were greater than 0.5 which indicated positive results where as S/P values of 03(18.75%) sera samples less than 0.5 indicated negative result.

Table 4: Determination of ILTV antibody of Helal Poultry farm

Sample No	Positive Control mean	Negative Control mean	OD Values at 405 nm	S/P Values	Result	No. and % of Positive samples
01	0.125	0.52	0.747	1.575	POS +	15(93.8)
02			0.875	1.900	POS +	
03			0.901	1.966	POS +	
04			0.719	1.504	POS +	
05			0.604	1.213	POS +	
06			0.593	1.185	POS +	
07			0.992	2.196	POS +	
08			0.624	1.264	POS +	
09			0.389	0.668	POS +	
10			0.453	0.830	POS +	
11			0.549	1.074	POS +	
12			0.832	1.791	POS +	
13			0.224	0.250	NEG -	
14			0.910	1.989	POS +	
15			0.732	1.537	POS +	
16			0.642	1.309	POS +	

Table 4 Showed that out of 16 sera samples tested by ELISA, S/P values of 15 (93.8%) samples were greater than 0.5 which indicated positive results where as S/P values of 01(6.2%) sera samples less than 0.5 indicated negative result.

Table 5: Determination of ILTV antibody of Malak Poultry farm

Sample No	Positive Control mean	Negative Control mean	OD Values at 405 nm	S/P Values	Result	No. and % of Positive samples
01	0.125	0.52	0.979	2.163	POS +	9(90%)
02			0.766	1.624	POS +	
03			0.939	2.062	POS +	
04			0.301	0.445	NEG -	
05			0.596	1.193	POS +	
06			0.662	1.360	POS +	
07			0.619	1.251	POS +	
08			0.573	1.134	POS +	
09			0.837	1.804	POS +	
10			0.482	0.904	POS +	

Table 5 Showed that out of 10 sera samples tested by ELISA, S/P values of 9(90%) samples were greater than 0.5 which indicated positive results where as S/P values of 01(10%) sera samples less than 0.5 indicated negative result.

Table 6: Determination of ILTV antibody of Chanmia Poultry farm

Sample No	Positive Control mean	Negative Control mean	OD Values at 405 nm	S/P Values	Result	No. and % of Positive samples
01	0.125	0.52	0.675	1.393	POS +	14(87.5)
02			0.636	1.294	POS +	
03			0.481	0.901	POS +	
04			0.653	1.337	POS +	
05			0.549	1.074	POS +	
06			0.189	0.162	NEG -	
07			0.803	1.717	POS +	
08			0.787	1.677	POS +	
09			0.419	0.744	POS +	
10			0.791	1.687	POS +	
11			0.675	1.393	POS +	
12			0.636	1.294	POS +	
13			0.875	1.900	POS +	
14			0.742	1.563	POS +	
15			0.529	1.023	POS +	
16			0.267	0.359	NEG -	

Table 6 Showed that out of 16 sera samples tested by ELISA, S/P values of 14 (87.5%) samples were greater than 0.5 which indicated positive results where as S/P values of 02(12.5%) sera samples less than 0.5 indicated negative result.

Table 7: Determination of ILTV antibody of Rashed Poultry farm

Sample No	Positive Control mean	Negative Control mean	OD Values at 405 nm	S/P Values	Result	No. and % of Positive samples
01	0.125	0.52	0.743	1.565	POS +	7(70)
02			0.224	0.250	NEG -	
03			0.570	1.127	POS +	
04			0.656	1.345	POS +	
05			0.682	1.411	POS +	
06			0.250	0.316	NEG -	
07			0.807	1.728	POS +	
08			0.687	1.423	POS +	
09			0.304	0.452	NEG -	
10			0.701	1.459	POS +	

Table 7 Showed that out of 10 sera samples tested by ELISA, S/P values of 7(70%) samples were greater than 0.5 which indicated positive results whereas S/P values of 03(30%) serum sample less than 0.5 indicated negative result.

Table 8: Determination of ILTV antibody of Tamanna Poultry farm

Sample No	Positive Control mean	Negative Control mean	OD Values at 405 nm	S/P Values	Result	No. and % of Positive samples
01	0.125	0.52	0.860	1.862	POS +	12(75)
02			0.653	1.337	POS +	
03			0.568	1.122	POS +	
04			0.265	0.354	NEG -	
05			0.829	1.783	POS +	
06			0.511	0.977	POS +	
07			0.406	0.711	POS +	
08			0.764	1.619	POS +	
09			0.276	0.381	NEG -	
10			0.808	1.730	POS +	
11			0.310	0.468	NEG -	
12			0.529	1.023	POS +	
13			0.431	0.774	POS +	
14			0.710	1.482	POS +	
15			0.911	1.991	POS +	
16			0.232	0.270	NEG -	

Table 8 Showed that out of 16 sera samples tested by ELISA, S/P values of 12(75%) samples were greater than 0.5 which indicated positive results whereas S/P values of 04(25%) serum sample less than 0.5 indicated negative result.

OD values of indirect ELISA (Table 09 -15; plate 02)

Negative control mean OD value, $NCx^{-} = \frac{0.121 + 0.139}{2} = 0.13$

Positive control mean OD value, $PCx^{-} = \frac{0.618 + 0.622}{2} = 0.62$

Difference between positive and negative control OD value,

$PCx^{-} - NCx^{-} = 0.62 - 0.13 = 0.49$

Table 9: Determination of ILTV antibody of Zillu Poultry farm

Sample No	Positive Control mean	Negative Control mean	OD Values at 405 nm	S/P Values	Result	No. and % of Positive samples
01	0.13	0.62	1.041	1.859	POS +	9(90)
02			0.598	0.955	POS +	
03			0.912	1.596	POS +	
04			0.683	1.129	POS +	
05			0.204	0.151	NEG -	
06			0.795	1.357	POS +	
07			0.572	0.902	POS +	
08			0.598	0.955	POS +	
09			0.683	1.129	POS +	
10			0.589	0.937	POS +	

Table 9 Showed that out of 10 sera samples tested by ELISA, S/P values of 9 (90%) samples were greater than 0.5 which indicated positive results whereas S/P values of 01(10%) serum sample less than 0.5 indicated negative result.

Table 10: Determination of ILTV antibody of Kader Poultry farm

Sample No	Positive Control mean	Negative Control mean	OD Values at 405 nm	S/P Values	Result	No. and % of Positive samples
01	0.13	0.62	0.694	1.151	POS +	7(87.5)
02			0.705	1.173	POS +	
03			0.540	0.837	POS +	
04			0.193	0.129	NEG -	
05			0.648	1.057	POS +	
06			0.662	1.086	POS +	
07			0.713	1.190	POS +	
08			0.739	1.243	POS +	

Table 10 Showed that out of 08 sera samples tested by ELISA, S/P values of 7 (87.5%) samples were greater than 0.5 which indicated positive results whereas S/P values of 01(12.5%) serum sample less than 0.5 indicated negative result.

Table 11: Determination of ILTV antibody of Billal Poultry farm

Sample No	Positive Control mean	Negative Control mean	OD Values at 405 nm	S/P Values	Result	No. and % of Positive samples
01	0.13	0.62	0.622	1.004	POS +	12(75)
02			1.456	2.706	POS +	
03			0.307	0.361	NEG -	
04			0.823	1.414	POS +	
05			0.989	1.753	POS +	
06			0.775	1.316	POS +	
07			0.942	1.657	POS +	
08			0.507	0.769	POS +	
09			0.239	0.222	NEG -	
10			1.568	2.935	POS +	
11			0.427	0.606	POS +	
12			0.928	1.629	POS +	
13			0.293	0.333	NEG -	
14			0.854	1.478	POS +	
15			0.594	0.947	POS +	
16			0.198	0.139	NEG -	

Table 11 Showed that out of 16 sera samples tested by ELISA, S/P values of 12 (75%) samples were greater than 0.5 which indicated positive results whereas S/P values of 04(25%) serum sample less than 0.5 indicated negative result.

Table 12: Determination of ILTV antibody of Masud Poultry farm

Sample No	Positive Control mean	Negative Control mean	OD Values at 405 nm	S/P Values	Result	No. and % of Positive samples
01	0.13	0.62	0.827	1.422	POS +	13(81.25)
02			0.651	1.063	POS +	
03			0.638	1.037	POS +	
04			0.301	0.349	NEG -	
05			0.549	0.855	POS +	
06			0.680	1.122	POS +	
07			0.254	0.253	NEG -	
08			1.022	1.820	POS +	
09			0.672	1.106	POS +	
10			1.213	2.210	POS +	
11			0.805	1.378	POS +	
12			0.605	0.969	POS +	
13			1.048	1.873	POS +	
14			0.488	0.731	POS +	
15			0.193	0.129	NEG -	
16			0.547	0.851	POS +	

Table 12 Showed that out of 16 sera samples tested by ELISA, S/P values of 13(81.25%) samples were greater than 0.5 which indicated positive results whereas S/P values of 03(18.75%) serum sample less than 0.5 indicated negative result.

Table 13: Determination of ILTV antibody of Deen Poultry farm

Sample No	Positive Control mean	Negative Control mean	OD Values at 405 nm	S/P Values	Result	No. and % of Positive samples
01	0.13	0.62	0.997	1.769	POS +	14(87.5)
02			0.677	1.116	POS +	
03			0.682	1.127	POS +	
04			0.709	1.182	POS +	
05			1.075	1.929	POS +	
06			1.177	2.137	POS +	
07			0.963	1.700	POS +	
08			0.516	0.788	POS +	
09			0.278	0.302	NEG -	
10			1.177	2.137	POS +	
11			0.685	1.133	POS +	
12			0.821	1.410	POS +	
13			0.197	0.137	NEG -	
14			0.685	1.133	POS +	
15			0.874	1.518	POS +	
16			0.682	1.127	POS +	

Table 13 Showed that out of 16 sera samples tested by ELISA, S/P values of 14(87.5%) samples were greater than 0.5 which indicated positive results whereas S/P values of 02(12.5%) serum sample less than 0.5 indicated negative result.

Table 14: Determination of ILTV antibody of Sahin Poultry farm

Sample No	Positive Control mean	Negative Control mean	OD Values at 405 nm	S/P Values	Result	No. and % of Positive samples
01	0.13	0.62	0.904	1.580	POS +	12(75)
02			0.871	1.512	POS +	
03			0.563	0.884	POS +	
04			0.278	0.302	NEG -	
05			0.849	1.467	POS +	
06			0.756	1.278	POS +	
07			0.312	0.371	NEG -	
08			0.642	1.045	POS +	
09			0.659	1.080	POS +	
10			0.218	0.180	NEG -	
11			0.801	1.369	POS +	
12			0.301	0.349	NEG -	
13			0.781	1.329	POS +	
14			0.552	0.861	POS +	
15			0.687	1.137	POS +	
16			0.638	1.037	POS +	

Table 14 Showed that out of 16 sera samples tested by ELISA, S/P values of 12 (75)%) samples were greater than 0.5 which indicated positive results whereas S/P values of 04 serum sample less than 0.5 indicated negative result.

Table 15: Determination of ILTV antibody of Sorif Poultry farm

Sample No	Positive Control mean	Negative Control mean	OD Values at 405 nm	S/P Values	Result	No. and % of Positive samples
01	0.13	0.62	0.781	1.329	POS +	8(100)
02			1.271	2.329	POS +	
03			0.948	1.669	POS +	
04			1.008	1.792	POS +	
05			0.502	0.759	POS +	
06			0.781	1.329	POS +	
07			0.576	0.910	POS +	
08			0.746	1.257	POS +	

Table 15 showed that out of 08 sera samples tested by ELISA, S/P values of 8(100%) samples were greater than 0.5 which indicated positive results result.

OD values of indirect ELISA (Table 16 -18)

Negative control mean OD value, $NCx^{-} = \frac{0.157 + 0.166}{2} = 0.162$

Positive control mean OD value, $PCx^{-} = \frac{0.585 + 0.580}{2} = 0.583$

Difference between positive and negative control OD value,

$PCx^{-} - NCx^{-} = 0.583 - 0.162 = 0.421$

Table 16: Determination of ILTV antibody of Asraf Poultry farm

Sample No	Positive Control mean	Negative Control mean	OD Values at 405 nm	S/P Values	Result	No. and % of Positive samples
01	0.162	0.583	0.548	0.918	POS +	11(68.75)
02			0.303	0.336	NEG -	
03			0.668	1.203	POS +	
04			0.243	0.194	NEG -	
05			0.474	0.742	POS +	
06			0.264	0.243	NEG -	
07			0.705	1.291	POS +	
08			0.458	0.704	POS +	
09			0.329	0.398	NEG -	
10			0.814	1.550	POS +	
11			0.218	0.134	NEG -	
12			0.673	1.215	POS +	
13			0.837	1.605	POS +	
14			0.728	1.346	POS +	
15			0.417	0.607	POS +	
16			0.596	1.032	POS +	

Table 16 Showed that out of 16 sera samples tested by ELISA, S/P values of 11(68.75%) samples were greater than 0.5 which indicated positive results whereas S/P values of 04(31.25%) serum sample less than 0.5 indicated negative result.

Table 17: Determination of ILTV antibody of Siful Poultry farm

Sample No	Positive Control mean	Negative Control mean	OD Values at 405 nm	S/P Values	Result	No. and % of Positive samples
01	0.162	0.583	0.310	0.353	NEG -	13(81.25)
02			0.575	0.982	POS +	
03			0.627	1.106	POS +	
04			0.569	0.968	POS +	
05			0.470	0.733	POS +	
06			0.556	0.937	POS +	
07			0.390	0.543	POS +	
08			0.669	1.205	POS +	
09			0.404	0.576	POS +	
10			0.367	0.488	NEG -	
11			0.971	1.923	POS +	
12			0.245	0.198	NEG -	
13			0.624	1.099	POS +	
14			0.659	1.182	POS +	
15			0.391	0.545	POS +	
16			0.569	0.968	POS +	

Table 17 Showed that out of 16 sera samples tested by ELISA, S/P values of 13(81.25%) samples were greater than 0.5 which indicated positive results whereas S/P values of 03(18.75%) serum sample less than 0.5 indicated negative result.

Table 18: Determination of ILTV antibody of Manik Poultry farm

Sample No	Positive Control mean	Negative Control mean	OD Values at 405 nm	S/P Values	Result	No. and % of Positive samples
01	0.162	0.583	0.515	0.840	POS +	12(75)
02			0.492	0.785	POS +	
03			0.625	1.101	POS +	
04			0.451	0.688	POS +	
05			0.498	0.799	POS +	
06			0.221	0.141	NEG -	
07			0.576	0.985	POS +	
08			0.199	0.089	NEG -	
09			0.471	0.735	POS +	
10			0.492	0.785	POS +	
11			0.427	0.631	POS +	
12			0.195	0.080	NEG -	
13			0.532	0.880	POS +	
14			0.212	0.120	NEG -	
15			0.604	1.051	POS +	
16			0.529	0.873	POS +	

Table 18 showed that out of 16 sera samples tested by ELISA, S/P values of 13 (81.25%) samples were greater than 0.5 which indicated positive results whereas S/P values of 03(18.75%) serum sample less than 0.5 indicated negative result.

Table 19 : Percentage of ILTV antibody of all selected farms (At a glance)

Sl. No	Name of farm	Age	Number of sample collected	Number of positive samples and %	Total % of positive samples
01	Zahid Poultry	14w	10	8(80%)	189(81.47%)
02	Akhi Poultry	13w	16	13(81.25%)	
03	Helal Poultry	21w	16	15(93.8%)	
04	Malak Poultry	19w	10	9(90%)	
05	Chanmia Poultry	09w	16	14(87.5%)	
06	Rashed Poultry	16w	10	7(70%)	
07	Tamanna Poultry	51w	16	12(75%)	
08	Zillu Poultry	12w	10	9(00%)	
09	Kader Poultry	11w	8	7(87.5%)	
10	Billal Poultry	31w	16	12(75%)	
11	Masud Poultry	17w	16	13(81.25%)	
12	DeenPoultry	25w	16	14(87.5%)	
13	Sahin Poultry	08w	16	12(75%)	
14	Sorif Poultry	15w	8	8(100%)	
15	Asraf Poultry	44w	16	11(68.75%)	
16	Siful Poultry	17w	16	13(81.25%)	
17	Manik Poultry	35w	16	12(75%)	
Total			232	189(81.47%)	

Table 19 showed that out of 232 samples, 189 (81.47%) samples were found to be positive.

CHAPTER V

DISCUSSION

The research work was conducted to detect the seroprevalence of Infectious Laryngotracheitis (ILT) Virus-specific antibody in chickens from Gazipur district of Bangladesh. A total number of 232 sera of commercial layer were collected from 17 commercial layer farms. The studied commercial layers had not been previously vaccinated and were different ages. 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 be positive. The result of this study indicate a high Infectious Laryngotracheitis (ILT) Virus activity in Gazipur district of Bangladesh.

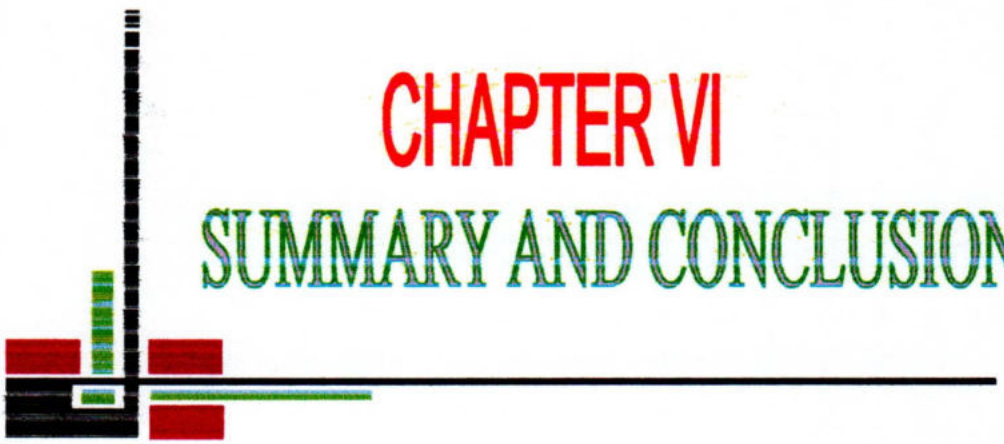
The study to detect seroprevalence of ILTV was conducted by the use of indirect ELISA. Such a test has been used for many a years by several authors. Thus (Mallinson *et al.*, 1981) compared four serological tests i.e. ELISA and AGID for the detection and titration of ILT chicken sera. The present seroprevalence study indicates that ILTV is present in Bangladesh. However this is not the case to be totally dependent upon. It needs further investigation such as isolation, identification and molecular characterization using other serological and molecular techniques.

Every farm which was selected for the collection of samples were previously affected clinically and had no historical background of vaccination. Before 2003 ILT was not found clinically but following which the disease (ILT) were observed with clinical sign and symptoms in layer birds .From the year 2004 ,ILT live vaccines are imported in Bangladesh for GP farms and the birds of these farms are applied with the vaccine following a vaccination schedule suggested by specialist of the manufacturing foreign farms. In this way ILTV might have entered in our country.

The present serological investigations indicated a high percentage (81.47%) of samples have had a seroconvertion which may be due to the fact that the samples were collected from the birds reported to had been exposed to the virus earlier.

However, further study in connection with this research work might be warranted as:

1. Prevention and control policies against ILT in Bangladesh
2. Isolation, identification and characterization of ILTV from layer birds.
3. Moleculer detection of ILTV.
4. Sero-surveillance study in vaccinated and non-vaccinated birds
5. Preparation of vaccine against ILTV using field samples.



CHAPTER VI

SUMMARY AND CONCLUSION

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SUMMARY AND CONCLUSION

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 sera of commercial layer were collected from 17 commercial layer farms. The studied commercial layers had not been previously vaccinated and were different ages. 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 be positive.

The OD (optical density) values of possive controls , negative controls and samples taken at 405 nm filter were also found valid according to the direction of the kit authority, Biochek infectious Laryngotracheitis Virus Antibody Test kit –CK124 and the difference between the possive control mean and the negative control mean optical density was greater than or equal to 0.150 . In addition, the negative control mean optical density was less than 0.3

From the findings of this study it may be concluded that:

1. Antibodies against infectious laryngotracheitis (ILT) virus were successfully detected by using Biochek infectious laryngotracheitis (ILT)virus Antibody Test Kit, Biochek, brug Bracklaan 57, 2811 BP Reeuwijk , Holland .
2. It was indicated that the ILTV were present in layer birds of farms from which the blood samples were obtained.
3. There was an urgent need for the development of prevention and control policies against ILT in Bangladesh poultry farms.
4. A national control programme for Infectious Laryngotracheitis Virus infection should be planned and regular ILT vaccination program should be maintained in commercial layer farms.


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