|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **CHAPTER** | **TOPICS** | | | **PAGE NO** |
|  | Acknowledgement  Statement of author  Contents  List of tables  List of figure  Acronyms used  Summary | | | V  VII  VIII  XI  XII  XIV  XVI |
| I | **INTRODUCTION** | | | 1-4 |
| II | **REVIEW OF LITERATURE** | | | 5-44 |
|  | 2.1 | Overview and structure of Herpes viruses | | 5 |
|  |  | 2.1.1 | Classification | 5 |
|  |  | 2.1.2 | General Properties | 7 |
|  | 2.2 | History of ILT Virus | | 9-10 |
|  |  | 2.2.1 | Early identification of Laryngo tracheitis | 9 |
|  |  | 2.2.2 | Early attempts of isolation and identification | 10 |
|  | 2.3 | Overview of ILT | | 11-15 |
|  |  | 2.3.1. | Classification | 11 |
|  |  | 2.3.2 | Structure | 11 |
|  |  | 2.3.3 | Viral replication | 14 |
|  |  | 2.3.4 | Chemical and physical viral resistance | 15 |
|  | 2.4 | Epidemiology | | 15-18 |
|  |  | 2.4.1 | Sources of ILTV | 15 |
|  |  | 2.4.2 | Method of spread | 16 |
|  | 2.5 | Antigenicity and virulence of ILTV strains | | 18 |
|  | 2.6 | Latency of ILTV | | 19 |
|  | 2.7 | In vitro cultivation | | 20 |
|  | 2.8 | Sequencing of ILTV | | 21-26 |
|  | 2.9 | Pathogenesis | | 26-28 |
|  |  | 2.9.1 | Transmision | 26 |
|  |  | 2.9.2 | Clinical Signs | 27 |
|  | 2.10 | Pathology | | 28-30 |
|  |  | 2.10.1 | Gross lesions | 28 |
|  |  | 2.10.2 | Microscopic lesions | 29 |
|  | 2.11 | Economic Importance of ILT | | 30 |
|  | 2.12 | Diagnosis of ILT | | 31-38 |
|  |  | 2.12.1 | Histological examination of the trachea | 31 |
|  |  | 2.12.2 | Detection of virus | 33 |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **CHAPTER** | **TOPICS** | | | **PAGE NO** |
|  |  | 2.12.3 | Detection of antibodies | 34 |
|  |  | 2.12.4 | Molecular techniques | 34-38 |
|  | 2.13 | Differential Diagnosis | | 38 |
|  | 2.14 | Prevalence in Bangladesh | | 39-40 |
|  | 2.15 | Prevention and control of ILT | | 41-44 |
|  |  | 2.15.1 | Management procedures for prevention and control | 41 |
|  |  | 2.15.2 | Immunization for prevention and control | 41 |
|  |  | 2.15.3 | Traditional live attenuated ILT vaccines | 42 |
|  |  | 2.15.4 | Recombinant subunit vaccines | 43 |
|  |  | 2.15.5 | Construction of live attenuated recombinant ILT vaccines | 43 |
|  |  | 2.15.6 | Novel vaccines approaches | 43 |
|  |  | 2.15.7 | Eradication | 44 |
| III | **MATERIALS AND METHOD** | | | 45-62 |
|  | 3.1 | Study areas and period | | 45 |
|  | 3.2 | Sampling Strategy | | 45 |
|  | 3.3 | Sample Size | | 46 |
|  | 3.4 | Source of chicken eggs | | 46 |
|  | 3.5 | Preparation of PBS & Viral Transport Media (VTM) | | 48 |
|  | 3.6 | Storage and preservation of samples | | 48 |
|  | 3.7 | Virus cultivation | | 48-49 |
|  |  | 3.7.1 | Viral Inoculum Preparation | 48 |
|  |  | 3.7.2 | Cultivation of ILTV in CAM route | 48 |
|  | 3.8 | Preparation of positive CAM samples for viral DNA/RNA extraction | | 49 |
|  | 3.9 | Molecular Detection | | 50-60 |
|  |  | 3.9.1 | Equipments and Reagents | 51 |
|  |  | 3.9.2 | Extraction of Nucleotide (DNA and RNA ) | 51 |
|  |  | 3.9.3 | Multiplex PCR (mRT-PCR) amplification of respiratory viruses | 52 |
|  |  | 3.9.4 | PCR amplification of ICP4 gene of ILTV | 56 |
|  |  | 3.9.5 | Sequencing of amplified PCR products | 60 |
|  |  | 3.9.6 | Assessment of nucleotide bases | 60 |
|  |  | 3.9.7 | Determination of GC and AT content of obtained sequences | 60 |
|  |  | 3.9.8 | Sequence Submission to Genbank | 60 |
|  |  | 3.9.9 | Comparing obtained sequences with the previously reported ILT Isolate | 61 |
|  |  | 3.9.10 | Determination of genetic interrelationship of obtained ICP4 gene sequences | 61 |
|  |  | 3.9.11 | Construction of phylogenetic tree | 61 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **CHAPTER** | | **TOPICS** | | | **PAGE NO** | |
| IV | **RESULTS** | | | | | 63-84 |
|  | 4.1 | | Isolation of field virus | | | 63 |
|  | 4.2 | | Screening of Respiratory viral pathogens using multiplex RT-PCR assay | | | 64 |
|  | 4.3 | | Amplification of ICP4 gene of ILTV by PCR | | | 65 |
|  | 4.4 | | Overall distribution of the ILTV infection identified in the present Study | | | 67 |
|  | 4.5 | | Identification of nucleotide bases according to chromatogram peak | | | 68 |
|  | 4.6 | | GC content of obtained sequences | | | 69 |
|  | 4.7 | | Genbank accession number of submitted sequences | | | 69 |
|  | 4.8 | | NCBI BLAST analysis | | | 71 |
|  |  | | 4.8.1 | NCBI BLAST analysis of vaccine isolates (Cvac) | | 71 |
|  |  | | 4.8. 2 | NCBI BLASTN analysis of ILTV field isolate | | 76 |
|  | 4.9 | | Evaluation of genetic variations of Bangladeshi field isolate (KC576525) with Vaccine isolates (KC576526) and closely related sequences | | | 80 |
|  | 4.10. | | Genetic interrelationship of obtained ICP4 gene sequences | | | 84 |
| V | **DISCUSSION** | | | | | 85-90 |
| VI | **CONCLUSION** | | | | | 91 |
| VII | **REFERENCES** | | | | | 92-116 |
|  | **ANNEX** | | | | | 117-22 |