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**CONTENTS**

|  |  |  |
| --- | --- | --- |
| **CHAPTER** | **TOPICS** | **PAGE** |
|  | ACKNOWLEDGEMENT | i |
|  | **CONTENTS** | **ii-v** |
|  | **LIST OF TABLES** | **vi** |
|  | LIST OF FIGURES | vii |
|  | LIST OF ABBREVIATIONS | viii-x |
|  | ABSTRACT | xi |
| **I** | **INTRODUCTION** | 1-3 |
| **II** | **REVIEW OF LITERATURE**  | **4-32** |
|  | **2.1** | **AVIAN INFLUENZA** | **6-12** |
|  |  | 2.1.1 | History | 6 |
|  |  | 2.1.2 | Economic importance | 6 |
|  |  | 2.1.3 | Etiology | 7 |
|  |  | 2.1.4 | Virus replication  | 7 |
|  |  | 2.1.5 | Epidemiology  | 8 |
|  |  | 2.1.6 | Chemical and physical viral resistance | 8-9 |
|  |  | 2.1.7 | Pathogenesis  | 9 |
|  |  | 2.1.8 | Clinical signs | 9-10 |
|  |  | 2.1.9 | Pathology | 10 |
|  |  | 2.1.9.1 | Gross lesions | 10-11 |
|  |  | 2.1.9.2 | Microscopic lesions | 11 |
|  |  | 2.1.10 | Diagnosis | 11 |
|  |  | 2.1.11 | Prevention and control | 12 |
|  | **2.2** | **NEWCASTLE DISEASE** | **13-25** |
|  |  | 2.2.1 | History | 13 |
|  |  | 2.2.2 | Economic importance | 13 |
|  |  | 2.2.3 | Etiology | 13-14 |
|  |  | 2.2.4 | Virus replication  | 14 |

|  |  |  |
| --- | --- | --- |
| **CHAPTER** | **TOPICS** | **PAGE** |
|  |  | 2.2.5 | Epidemiology  | 15 |
|  |  | 2.2.6 | Chemical and physical viral resistance | 15 |
|  |  | 2.2.7 | Pathogenesis  | 15-16 |
|  |  | 2.2.8 | Clinical signs | 16-17 |
|  |  | 2.2.9 | Pathology | 17 |
|  |  | 2.2.9.1 | Gross lesions | 17 |
|  |  | 2.2.9.2 | Microscopic lesions | 17-18 |
|  |  | 2.2.10 | Diagnosis | 18 |
|  |  | 2.2.11 | Prevention and control | 18-19 |
|  | **2.3** | **INFECTIOUS BRONCHITIS** | **19-25** |
|  |  | 2.3.1 | History | 19 |
|  |  | 2.3.2 | Economic importance | 20 |
|  |  | 2.3.3 | Etiology | 20 |
|  |  | 2.3.4 | Virus replication  | 21 |
|  |  | 2.3.5 | Epidemiology  | 21-22 |
|  |  | 2.3.6 | Chemical and physical viral resistance | 22 |
|  |  | 2.3.7 | Pathogenesis  | 22-23 |
|  |  | 2.3.8 | Clinical signs | 23 |
|  |  | 2.3.9 | Pathology | 24 |
|  |  | 2.3.9.1 | Gross lesions | 24 |
|  |  | 2.3.9.2 | Microscopic lesions | 24 |
|  |  | 2.3.10 | Diagnosis | 25 |
|  |  | 2.3.11 | Prevention and control | 25 |
|  | **2.4** | **INFECTIOUS LARYNGOTRACHEITIS** | **25-32** |
|  |  | 2.4.1 | History | 26 |
|  |  | 2.4.2 | Economic importance | 26 |
|  |  | 2.4.3 | Etiology | 27 |
|  |  | 2.4.4 | Virus replication  | 27 |

|  |  |  |
| --- | --- | --- |
| **CHAPTER** | **TOPICS** | **PAGE** |
|  |  | 2.4.5 | Epidemiology  | 27-28 |
|  |  | 2.4.6 | Resistance to chemical and physical agents  | 28 |
|  |  | 2.4.7 | Pathogenesis  | 28-29 |
|  |  | 2.4.8 | Clinical signs | 29-30 |
|  |  | 2.4.9 | Pathology | 30 |
|  |  | 2.4.9.1 | Gross lesions | 30 |
|  |  | 2.4.9.2 | Microscopic lesions | 30-31 |
|  |  | 2.4.10 | Diagnosis | 31-32 |
|  |  | 2.4.11 | Prevention and control | 32 |
| **III** | **METHODOLOGY** | **33-47** |
|  | 3.1 | Study period | 33 |
|  | 3.2 | Study area | 33 |
|  | 3.3 | Sample collection and preservation  | 35 |
|  | 3.4 | Histopathological study | 35 |
|  | 3.4.1 | Equipment and appliances for histopathology | 35 |
|  | 3.4.2 | Collection of samples and processing | 36 |
|  | 3.4.3 | Routine hematoxylin and eosin staining procedure | 37 |
|  | 3.5 | Optimization of multiplex PCR protocol for detection of respiratory viruses | 38 |
|  | 3.5.1 | Viral nuclic acid extraction | 38 |
|  | 3.5.2 | Extraction protocol | 39 |
|  | 3.5.2.1 | cDNA synthesis of template RNA | 40 |
|  | 3.5.3 | PCR amplification of viral nuclic acid | 40 |
|  | 3.5.4 | Other instrument and chemicals used for PCR | 42 |
|  | 3.5.5 | Optimization of multiplex PCR Protocol | 42-43 |
|  | 3.5.6 | PCR assay programming in the thermal cycler | 44-45 |
|  | 3.5.7 | Agar gel electrophoresis | 45 |
|  | 3.5.7.1 | Materials and Reagents | 46 |
|  | 3.5.7.2 | Procedure of agar gel electrophoresis | 46 |
|  | 3.5.8 | Precautions followed in the PCR laboratory | 46 |
|  | 3.5.9 | Schematic outline of the study | 47 |

|  |  |  |
| --- | --- | --- |
| **CHAPTER** | **TOPICS** | **PAGE** |
| **IV** | **RESULTS** | **48-55** |
|  | 4.1 | Isolation of field’s sample | 48 |
|  | 4.2 | Diagnosis of the respiratory viral infections based on gross lesions  | 48-51 |
|  | 4.3 | Screening of viral respiratory pathogens by performing PCR from cDNA of the viral nucleic acid and RT-PCR assay | 51-52 |
|  | 4.4 | Amplification of the PCR product in agarose gel electrophoresis | 52 |
|  | 4.5 | Optimization of Multiplex PCR | 54 |
|  | 4.6 | Histopathological examination  | 54-55 |
| **V** | **DISCUSSION** | **56-58** |
| **VI** | **CONCULSION** | **59** |
| **VII** | **RECOMMENDATIONS AND FUTURE PERSPECTIVES** | **60** |
| **VIII** | **REFERENCES** | **61-78** |
|  | **APPENDIX** | **79-81** |
|  | **BRIEF BIO-DATA OF THE STUDENT** | **82** |

**LIST OF TABLES**

|  |  |  |
| --- | --- | --- |
| **Table** | **Title** | **Page** |
| Table 1 | Livestock and poultry population in Bangladesh (2004-2011) | 4 |
| Table 2 |  Materials provided with the extraction kit Viral Gene-spin™ Viral DNA/RNA Extraction Kit  | 38 |
| Table 3 | cDNA synthesis reaction | 40 |
| Table 4 |  Details of the primers used for PCR | 41 |
| Table 5 | PCR Master mix solution | 42 |
| Table 6 | Composition of reaction mixture for multiplex PCR | 43 |
| Table 7 | Steps and conditions of thermal cycling for PCR | 44 |
| Table 8 | Overall Characteristics gross lesion of the suspected diseases  | 50 |
| Table 9 | Overall percentages of tentatively diagnosed diseases | 51 |
| Table 10 | Comparison of accuracy of tentative diagnosis with Molecular identification of Respiratory viral diseases | 52 |
| Table 11 | Overall percentage of the diseases confirmed by PCR | 52 |
| Table 12 | An over view of 48 chickens data investigated for viral respiratory diseases in commercial poultry | 80-81 |

**LIST OF FIGURES**

|  |  |  |
| --- | --- | --- |
| **Figure** | **Title** | **Page** |
| Figure-1 | Map showing the location of the study areas. | 34 |
| Figure-2 | Figure shows the characteristic postmortem lesions these similar findings are found in many respiratory disease | 49 |
| Figure-3 | Amplification of Matrix (M) gene of AIV by single step PCR or RT-PCR | 53 |
| Figure-4 | Amplification of fusion protein gene of NDV by single step PCR or RT-PCR | 53 |
| Figure-5 | Amplification of Nucleoprotein gene of IBV by single step PCR or RT-PCR | 53 |
| Figure-6 | Optimized mPCR assay of different sets of amplification. | 54 |
| Figure-7 | Histopathological changes in some organ showing similar lesion in respiratory infection | 551 |

**LIST OF ABBREVIATIONS**

|  |  |
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| **Sign** | **Abbreviations** |
| °C | Degree celsius |
| A | Adenine |
| AGID | Agar gel immunodiffusion |
| AGPT | Agar gel precipitation test |
| AI | Avian influenza |
| AIV | Avian influenza virus |
| AIV-M- F | Avian influenza virus forward primer |
| AIV-M- R | Avian influenza virus reverse primer |
| APMV | Avian paramyxovirus |
| BBS | Bangladesh Bureau of Statistics |
| bp | Base pair |
| C | Cytosine |
| CC | Cell cultures |
| cDNA | Complementary Deoxyribo Nucleic Acid |
| CEK | Chicken embryo kidney |
| CEL | Chicken embryo liver |
| CK | Chicken kidney |
| CVASU | Chittagong Veterinary and Animal Sciences University |
| DNA | Deoxyribo Nucleic Acid |
| dNTPs | Deoxy nucleotide phosphates |
| dsDNA | Double stranded Deoxyribo Nucleic Acid |
| e.g. | Example |
| ECE | Embryonated chicken eggs |
| ELISA | Enzyme-linked immunosorbent assay |
| F0 | Fusion protein |
| G | Guanine |

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| **Sign** | **Abbreviations** |
| GDP | Gross domestic products |
| HA | Haemagglutinin |
| HI | Haemagglutination inhibition |
| HPAI | Highly pathogenic avian influenza |
| IB | Infectious bronchitis |
| IBV | Infectious bronchitis virus |
| IBV-F | Infectious bronchitis virus forward primer |
| IBV-R | Infectious bronchitis virus reverse primer |
| IFA | Immunofluorescence assay |
| ILT | Infectious laryngotracheitis |
| ILTV | Infectious laryngotracheitis virus |
| ILTV-F | Infectious laryngotracheitis virus forward primer |
| ILTV-R | Infectious laryngotracheitis virus reverse primer |
| IPA | Immunoperoxidase assay |
| M | Matrix proteins |
| mA | mili Ampere |
| MAbs | Monoclonal antibodies |
| ml | Mili litre |
| mm | mili meter |
| mRT-PCR | Multiplex Reverse Trascription-polymerase chain reaction |
| N | Nucleoproteins |
| NA | Neuraminidase |
| ND | Newcastle disease |
| NDV | Newcastle disease virus |
| NDV-F | Newcastle disease virus forward primer |
| NDV-R | Newcastle disease virus reverse primer |
| OIE | Office International des Epizooties |
| PB | Polymerase basic |
| PCR | Polymerase chain reaction |

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| **Sign** | **Abbreviations** |
| PRTC | Poultry Research and Training Centre |
| QACs | Quaternary ammonium compounds |
| RNA | Ribo nucleic acid |
| rpm | Rotation per minute |
| rRNA | Ribosomal RNA |
| RT | Reverse trascriptase |
| RT-PCR | Reverse Trascription-polymerase chain reaction |
| SAR | Special administration region |
| s RT-PCR | Separated Reverse Transcription polymerase chain reaction |
| T | Thiamine |
| TAE | Tris Acetate EDTA |
| Temp. | Temperature |
| U | Unit |
| UTR | Untranslated region |
| UV | Ultra violet |
| UV | Ultra violet |
| V | Volt |
| VN | Virus neutralization |
| VNT | Virus neutralization test  |
| VVND | Very virulent newcastle disease |
| WHO | World Health Organization |
| μl | Micro litre |

**ABSTRACT**

The present work was to optimize a multiplex PCR protocol for rapid and accurate diagnosis of avian respiratory viral diseases. Four sets of specific oligonucleotide primers were used in this study that amplified products of predicted sizes from each virus in the sRT-PCR as well as in the mPCR assays (1023, 320, 647, 149 bp for AIV, NDV, ILTV, and IBV respectively) with the use of 1% agarose gel electrophoresis for PCR amplified products. A total of 48 specimen samples included trachea, lungs, liver, spleen, proventriculus etc. were collected from different dead birds that were tentatively diagnosed as respiratory viral infection on the basis of anamnesis and postmortem lesions by the expert veterinary clinician. Samples were preserved in 10% buffered formalin for histopathological study and in 100% alcohol with -80oC refrizaration for molecular study like RNA/DNA extraction for PCR or RT-PCR.A questionnaire was used during the period from August 2013 to March 2014 to record additional information and history of the cases such as age and type of birds, litter type, vaccination history, farm category, sex, pathological lesions, histopathology etc. Out of 48 cases 35.42% was tentatively diagnosed as AI, 33.33% was ND and 12.25% was IB where visceral gout was most prominent lesion. No ILT was diagnosed tentatively through necropsy. Mixed infection is common in commercial chicken flock, among the cases 4.17% was diagnosed as AI+ND, 12.25% and 2.08% was diagnosed as AI/ ND and IB/ND respectively. Among 48 test samples both the PCR screening process a total of 33(68.75%) samples showed positive band in 1% agarose gel electrophoresis where 15(31.25%), 16(33.33%) and 2(4.16%) samples were found positive for AI, ND and IB respectively. No ILT was found in this screening. Among the diseases AI was diagnosed accurately in 70.58% cases through necropsy and 81.25% and 33.33% for ND and IB respectively. ILT was not diagnosed tentatively which was also proved by molecular diagnosis that indicates the 100% accuracy of the tentative diagnosis. Microscopically congestion and hemorrhage in trachea, lungs, liver and spleen were found commonly in most of the samples, in addition with exudates in trachea and hemorrhagic kidney with urate crystal in renal tubules in IB infected sample. Hemorrhage in proventriculus is also found in only AI and ND infected cases.

**Key words:** Commercial chicken, viral respiratory infection, tentative diagnosis, multiplex PCR, histopathology.