**Acknowledgement**

I would like to express the deepest sense of gratitude and all sorts of praises to the Almighty Allah, the Omnipotent, Omnipresent and Omniscient, whose blessing have enabled me to complete this thesis.

I would like to express my gratefulness to my research supervisor, Professor Dr. Md. Masuduzzaman, Head, Department of Pathology and Parasitology, Chittagong Veterinary and Animal Sciences University (CVASU), for his sympathetic supervision, inspiration, constructive criticism, valuable suggestion and providing important information throughout the course work and research and towards preparation of the manuscript in time.

This research was largely funded by World bank sponsored UGC–HEQEP Round-II Sub-Project (CP:2180) and I am grateful to HEQEP project team members at University Grants commission (UGC) and the sub-project manager Prof. Dr. Mohammad Alamgir Hossain for providing grants and scholarship under this sub-project.

Special thanks to Prof. Dr. AMAM Zonaed Siddiki, Director (Research and Extension) for providing partial financial support from CVASU research fund (2012-2013 financial year).

I am also grateful to my Co-supervisor, Dr. Sharmin Chowdhury, Associate Professor, Department of Pathology and Parasitology, CVASU, for her encouragement and cooperation at every stage of this study from its inception to completion.

Special thanks to DR. Md. Shafiqul Islam, Lecturer, Department of Pathology and Parasitology, CVASU, for his technical support and active co-operation during the experimental period.

I feel great pleasure to express my best regards to Prof. Dr. A.K.M. Saifuddin, Head, Department of Physiology, Biochemistry and Pharmacology for providing technical support during the sample collection.

Thanks are also to DR. Inkeyas Uddin, Scientific officer, Poultry Research and Training Center (PRTC). MS students Salima Ferdous, Jagriti Chakma, Sharmin Sompa, Nasima Akter and all the faculty members of the Department of Pathology and Parasitology, CVASU.

Last but not the least; I would like to thanks all my well-wishers, kith and kin for their constant inspiration and blessings throughout the entire period of academic life.

**The Author**

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**LIST OF ABBREVIATIONS**

|  |  |
| --- | --- |
| **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.