Faculty of Veterinary Medicine

Department of Virology

Isolation, Molecular Characterization and Pathogenicity of IBDV associated with Mortalities in Broiler Chickens

A Thesis presented by

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بسم الله الرحمن الرحيم

﴿وَعَلَّمَكَ مَا لَمْ تَكُنْ تَعْلَمُ

وَكَانَ فَضْلُ اللَّهِ عَلَيْكَ

عَظِيمًا﴾

صدق الله العظيم

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Abstract

The present study was performed to detect, isolate and identify the causative agent associated with mortalities in broiler flocks in Beheira Governorate in EGYPT. Field samples were collected from broiler chickens demonstrating mortalities. The samples were inoculated in one-day old SPF chicks via intranasal and intraocular route. Chicks were kept in isolators with daily observation for five weeks post infection. Blood samples were collected and tested for infectious bursal disease virus (IBDV), chicken anemia virus (CAV), infectious bronchitis virus (IBV), Newcastle disease virus (NDV) and Avian influenza H5 and H9 subtypes by ELISA and HI tests. The results indicated that the samples were positive for IBDV. Histopathological examination of the collected bursae and kidneys, four and seven days post inoculation (DPI) revealed characteristic lesions for IBDV including inter-follicular connective tissue proliferation, compressed follicles, depleted lymphocytes in the bursae, congested blood vessels and hemorrhage in the kidneys. Conventional Reverse transcriptase-polymerase chain reaction (RT-PCR) and Real-time RT-PCR (rtRT-PCR) assays for VP2 gene of IBDV confirmed the presence of IBDV. Sequence analysis of amplified PCR product indicated continuous circulation of virulent IBDV strains in Egypt and it was closely related to previously isolated very virulent strain Giza 2008. Respective nucleotide sequence was submitted to NCBI GenBank with the access number KY200662. The pathogenic effect of the isolated (Egypt/ IBDV/Behera 2011) strain was studied. One- day old SPF chicks were inoculated by intra ocular and intra nasal route with the bursal homogenate of the infected farm (10^3ID_{50}/ml). At 3^{rd}, 7^{th}, 10^{th}, 14^{th}, 21^{th} and 28^{th} days post inoculation (DPI), 10 chicks were randomly selected, weighted, bled, humanely euthanized and then necropsied. Bursa, spleen, and kidney were taken out and weighted. Isolated IBD strain Pathogenicity was evaluated by mortality percent, bursa, spleen, kidney /body weight ratio. Five bursae were collected at 24 hours interval up to 96 hours, then at 7^{th} and 10^{th} DPI. Collected bursae were either fixed in 10% formol saline or in a mixture of 2.5% glutaraldehyde for histopathology and transmission electron microscope (TEM) respectively. Histopathological lesions were scored from 0 to 4 based on the presence of degenerative alterations, necrosis, follicular atrophy in the bursa of Fabricius and the percentage of altered lymphoid follicles. IBD antigen demonstrated by Agar gel diffusion test (AGDT). Antibodies against IBDV were measured by Enzyme-linked Immunosorbent assay (ELISA). Twenty infected chicks died (25%), mortalities started 3 DPI and ceased 7 DPI with the highest mortality of 3 chicks at 5 DPI (7.5%) compared with negative control which showed no deaths at all over experimental time. The highest bursa/ body weight ratio was observed at 3^{rd}, 7^{th}, 10^{th} DPI then decreased at 14^{th}, 21^{th}, 28^{th} DPI; respectively. Histopathologically, severe tissue reaction in the bursa of Fabricius of inoculated chicks, and the degree of damage varies including hyperemia, hemorrhage and edema, degeneration, necrosis. The Mean Severity Index (MSI) ranged from 0.5 to 4. IBD viral particles were visualized by Transmission Electron microscope (TEM). Agar gel diffusion test (AGDT) confirmed the presence of IBDV. ELISA were positive for IBDV antibodies from 7 DPI, increased at 21 DPI then declined at 28 DPI. Indeed the study reports the isolation, molecular characterization and pathogenicity of IBDV strain (Egypt/ IBDV/Behera 2011).

Key Words: Infectious Bursal Disease Virus (IBDV); Vp2 Gene Hypervariable Region; Real-time RT-PCR; SPF chickens; immunosuppression; pathogenicity.

DEDICATION

To my beloved Father whom I carry his name proudly,

My great Mother the secret of my life and success,

My twin soul Hatem for his encouragement and support,

My husband Mostafa for faithful efforts & encouragement

My lovely children JANA & ASSER, I see optimism in their eyes &happiness in their laughter.

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List of abbreviations

AGPT	Agar gel precipitation test
bp	Base pair
BF	Bursa of Fabricius
CMI	Cell-mediated immunity
CLVB	Central Laboratory for Evaluation of Veterinary Biologics
CIAV	chicken infectious anemia virus
CAM	Chorioallantoic membrane
DPI	day post infection
DNA	Deoxy-ribonucleic acid
EU	ELISA unit
ECE	Embryonated chicken egg
ELISA	Enzyme linked immunosorbant assay
GALT	Gut-associated lymphoid tissues
HI	Heamagglutination inhibition
HA	Hemagglutination
HVR	Hyper Variable Region
IBA	Infectious bursal agent
IBDV	Infectious bursal disease virus

µl	microliter
NDV	Newcastle disease virus
NO	Nitric oxide
OIE	Office of international Epizootics
ORF	open reading frame
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
PM	Postmortem
pH	Power of hydrogen
rtRT-PCR	Real time Reverse transcriptase polymerase chain reaction
RBCs	Red Blood Corpuscles
RT-PCR	Reverse transcriptase polymerase chain reaction
RNA	ribonucleic Acid
SPF	Specific pathogen free
sIgM	surface immunoglobulin M
TEM	Transmission Electron microscope
VNT	virus neutralization test
V	Volume
Wt	Weight

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