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List of Abbreviation

AA	:	Amino Acid
AGPT	:	Agar Gel Precipitation Test
avIBDV	:	antigenic variant IBDV
B/B	:	Bursal body weight ratio
bp	:	base pair
CAM	:	Chorioallantoic membrane
CEF	:	Chicken embryo fibroblasts
CPE	:	Cytopathic Effect
cvIBDV	:	classical virulent IBDV
DNA	:	Deoxyribo Nucleic Acid
dsRNA	:	Double strand RNA
ECE	:	Embryonated Chicken Eggs
ELISA	:	Enzyme Linked Immunosorbent Assay
IBA	:	Infectious Bursal Agent
IBD	:	Infectious Bursal Disease
IBDV	:	Infectious Bursal Disease Virus
HVR	:	Highly Variable Region
Kb	:	Kilobase
ORF	:	Open reading frame
PCR	:	Polymerase Chain Reaction
PEG	:	Polyethylene Glycol 6000
p.i.	:	post inoculation
RNA	:	Ribonucleic Acid
RT-PCR	:	Reverse Transcriptase- Polymerase Chain Reaction
SNT	:	Serum neutralization Test
SPF	:	Specific Pathogen Free
TCID50	:	Tissue Culture Infective Dose fifty
Vero	:	African green monkey kidney cell
VNT	:	Virus Neutralization Test
VP	:	Viral Protein
vvIBDV	:	very virulent IBDV

6- CONCLUSIONS.

From the results of this study, it was concluded that:

1. IBDV is still circulating in Egypt in many localities in Giza governorate.
2. Isolation of IBDV was best performed by passage on specific pathogen free embryonated chicken eggs (SPF-ECE).
3. Identification of IBDV was best performed using agar gel precipitation test (AGPT) and reverse transcription-polymerase chain reaction (RT–PCR) for amplification of the VP2 gene.
4. Sequence and phylogenetic analysis of the isolated IBDV VP2 gene revealed minimal substitution mutations and phylogeny revealed that the isolated strain emerging from a single node indicating the common ancestral gene with Egyptian vvIBDV.
5. On Studying pathogenicity of IBDV isolates from different localities in Giza governorate, the virulent IBD virus isolates caused mortality up to 40% in the inoculated chicks with signs of ruffled feathers, depression and diarrhea.
6. The inactivated oil adjuvanted vaccine prepared using local IBDV Giza-2014 isolate and Montanide ISA70VG adjuvant was immunogenic and potent compared with imported one due to the respectable humoral immune response measured using SNT and ELISA.
7. The prepared inactivated IBDV vaccine gave 14 weeks duration of immune response in vaccinated birds that started 3 weeks post vaccination and 100% protection percent.

7- SUMMARY

Infectious bursal disease virus (IBDV) is a member of the family Birnaviridae. IBD is an acute, highly contagious viral disease of young chickens and characterized by an enlargement of the bursa of Fabricius and severs renal damages. IBD was first reported in Egyptian flocks the early seventies**;** however, interest in IBDV antigenic characterization was triggered by the appearance of the very virulent IBDV in vaccinated flocks.

This study was carried out with an aim to isolate and identify IBD virus from different localities in Giza governorate as well as molecular analysis in order to show identity and diversity of the isolated IBDv Giza-2014 and other Egyptian and representative reference strains then it was subjected for studying pathogenicity and antigenicity by being used for preparation and evaluation of an inactivated IBDV vaccine.

From the applied experiments, it was revealed the following results:

1. Regarding on isolation and identification, forty two field samples from suspected chick flocks showing signs of IBD virus infection from different localities in Giza governorate (11 from Abo-Rawash, 8 from El-Ayat, 13 from El-Fayoum Road, 5 from El- Badrashin and 5 from El- Saff) were inoculated in specific pathogen free embryonated chicken eggs. It was found that 26 out of 42 samples (61.9%) induced signs on inoculated SPF-ECE by 4^{th} passage.
2. Field samples from suspected chick flocks positive for isolation on SPF-ECE were subjected for titration after each passage starting from the second passage**.** Titers of the isolates ranged from 5 $\log_{10}$ EID_{50}/ml to 8.5 $\log_{10}$ EID_{50}/ml after the 4^{th} passage.
3. It was found that 26 out of 26 isolated samples (100%) were positive using AGPT and RT-PCR.

4. After one step RT–PCR for amplification of the VP2 gene of IBD virus, the genomic DNA products of both reference strain and local isolates were subjected to gel electrophoresis which revealed that the reference IBD virus strain and the local isolates had the same size of VP2 gene fragment 640 bp, without significant differences between the strains.

5. Sequence of the hyper variable region of VP2 gene on the IBDV isolate and its alignment that of different IBDV strains (virulent, variant and vaccinal strains) revealed that minimal substitution mutations were observed in certain positions while other positions were nearly conserved.
6. Studying homology and phylogeny of the isolated virus with the other reference classical, very virulent, variant and vaccinal strains of IBDV revealed that IBDV Giza 2014 was in a separate branch and it was clustered more close to the Egyptian very virulent IBDV, Giza 2000 and Giza 2008 and the European and German IBDV but it was clustered at a far distance from Hel 2008 IM, Hel 2008, Bursine Plus and other strains which denote the continuous evolution and mutation of IBDV in Egypt which may affect the virus antigenicity and virulence.
7. On Studying pathogenicity of IBD virus isolates from different localities in Giza governorate, the virulent IBD virus isolates caused mortality up to 40% in the inoculated chicks with signs of ruffled feathers, depression and diarrhea.
8. The local variant strain was used for preparation of inactivated oil adjuvanted vaccine using Montanide ISA70VG and compared with the imported one which revealed sterility, safety, potency of the prepared vaccine.
9. The vaccinated birds were subjected to challenge test 3 weeks after vaccination both groups of vaccinated birds were of protection rate 100% and non-vaccinated challenged group was not protected deaths were 0%.