Animal Health Research Journal Vol. 8, No. 3, September 2020 pp. 121-132

ISSN: 2356-7767

Isolation and molecular detection of IBD virus and alteration of clinicopathological and immunological status in some chicken farms

Neven, M. Ramzy*; Ebtissam, N. Alshamy; Hala, N. Ibrahim*** and Farida, H. Mohamed****

Department of Virology, Ismailia Branch*, Department of Immunologym, Doki**, Department of Clinical Pathology, Ismailia Branch*** (AHRI) (ARC)

Received in 5/8/2020
Accepted in 3/9/2020

Abstract

In the current study, the infection bursal disease virus (IBDV) isolation and characterization from some chicken farms during the period of 2018-2020, were carried out and studied its impact on the hematological, biochemical and immunological parameters. A total of two hundred bursa and blood samples were collected from twenty farms of broiler chickens (3-6 weeks old, Belqas; Dakahlia), suffering from fever, dullness, anorexia, depression, head trembling, lameness and watery yellowish diarrhea with sudden onset of high mortalities. The obtained data of the postmortem findings were characteristic to IBDV infection where the affected chickens showed congested bursa with caseous exudate and haemorrhage on proventriculus and gizzard, nephrosis with kidney urates ,enlarged mottled liver, swollen necrotic spleen and sever echomotic hemorrhages on breast and thigh muscles. Isolates from bursal samples of commercial broiler farms exhibiting some clinical and postmortem signs with poor performance were used for Reverse transcription (RT) - polymerase chain reaction (PCR) assay. RT- PCR was used to amplify a 620-bp product within the hypervariable region of the IBDV VP2 gene. The RT- PCR results indicated that, 2 farms were positive to IBDV and showed specific bands at 620 bp on agrose gel. The hematological investigation revealed significant increase in (PCV), leukopenia with significant decrease in absolute lymphocyte count but increase in absolute count of heterophils and eosinophils in IBDV diseased chickens compared to apparently healthy one. On the other hand, marked elevation of hepatic enzyme activity (AST and ALT), increased level of creatinine and uric acid as compromised kidney function were detected. Furthermore, the lysozyme activity of diseased chickens was significantly higher than that of apparently healthy chickens. The serum total protein, albumin, total globulin and the A:G ratio were significantly decreased in IBDV diseased chickens compared to apparently healthy one. The antibody titer determined by ELISA had been shown significant difference in diseased chickens compared to apparently healthy chickens. Inhibition of the humoral immunity is attributed to the destruction of immunoglobulin-producing cells by the virus. It was concluded that the hematological, biochemical and immunological profile with IBDV naturally infected chickens showed slight, insignificant alterations rather than previously studied which may be due to isolation and molecular detection of the same strain.

Keywords: *IBD virus, PCR, hemato-biochemical alterations, immunosuppression.*

Introduction

Infectious bursal disease (IBD or Gumboro disease) is one of the most economically important diseases that affects commercially produced chickens **(van den Berg, 2000).** This immunosuppressive disease is caused by the IBD-virus (IBDV), a double-stranded RNA Avibirna virus **(Wang *et al.,* 2011).** IBDV was first recognized as a disease entity in "Gumboro" district of Delaware State in USA at 1957 by Gosgrove while it was first recorded in Egypt by **El-Sergany *et al.,* 1974 (Cosgrove, 1962; El-Sergany *et al.,* 1974).** The IBDV genome is divided into segments A

and B: segments A (3.4 kb) and B (2.8 kb). The large segment A encodes 4 viral proteins, the two capsid proteins VP2 (48 kDa) and VP3 (32-35 kDa), the viral protease VP4 (24 k Da) and a nonstructural protein VP5 (17-21 kDa), while the smaller segment B encodes VP1 (90 kDa), an RNA-dependent RNA polymerase. Expression studies have shown VP2 aa positions 206 to 350 to represent a major conformational and neutralizing antigenic domain called hyper variable region (HVR) **(Xu *et al.,* 2011)** which includes the most variable region important for cell antigenic and pathogenic variation. Most exchange of amino acid residues in VP2 occur in the four hydrophilic loops of the viral capsid **(Coulibaly *et al.,* 2010).** IBDV infection may induce a temporary or permanent destruction of the bursa of Fabricius and other lymphoid tissues **(Eterradossi & Saif, 2008** and **2013).** Destruction of B cells and macrophages, and their functions contribute to IBDV-induced immuno- suppression **(Sharma *et al.,* 2000** and **Rautenschlein *et al.,* 2003).** Serotype 1 IBDV causes economically significant immunosuppressive disease in young chickens. Within serotype 1, two subtypes are identified, classic and variant **(Wu *et al.,* 2007).** Some studies suggests that a virulent IBDV strain could maintain its virulence for a long period in the same chicken farm and the strain is highly stable under normal environmental conditions **(Li *et al.,* 2009).** Rapid and sensitive investigation for this virus in recent years is based on molecular diagnosis methods by reverse transcription-polymerase chain reaction (RT-PCR) for amplification of the IBDV VP2 gene. Conventional RT-PCR has been useful in detecting IBDV serotypes and, to a lesser extent, differentiating IBDV subtypes. Conventional RT-PCR, amplifying the VP2 hyper variable region, in combination with DNA sequencing of the PCR product, can differentiate classic, variant, and vvIBDV strains because variant and vvIBDV have characteristic nucleotide and amino acid substitutions, these methods potentially allow for more rapid, sensitive, and specific detection and differentiation of IBDV classic, very virulent and variant subtypes. This approach is a valuable tool for molecular epidemiological studies on IBDV **(Islam *et al.,* 2012).** Since IBDV is very resistant in the environment under farm conditions, the sanitary measures commonly applied on poultry farms are not sufficient to prevent this infection. Therefore, vaccination is an essential tool for the prevention of IBDV. Different modified live vaccines (MLVs) have been developed and classified as "mild", "intermediate", "intermediate plus" IBDV vaccines, depending upon their ability to break through maternally derived antibodies (MDAs) that can neutralize the vaccine virus **(Eterradossi &Saif, 2013).** IBDV infection in chickens is always associated with damage to the bursa of Fabricius and immunosuppression **(Sharma *et al.,* 2000; Silveira *et al.,* 2019 and Lupini *et al.,* 2020).** The acute phase of the disease lasts for 6-10 days and is characterized by atrophy of bursa along with depletion of B-cells in bursal follicles **(Uddin *et al.,* 2012),** the other lymphoid organs such as spleen and cecal tonsils are also affected. Immunosuppression occurs in clinical and subclinical form where both humoral and cellular immune responses are compromised and thus making birds more vulnerable to other secondary infections and reduced response to vaccination **(Musa *et al.,* 2010)**. Several pathological changes as part of the pathogenesis of the disease explained the biochemical changes in relation to the effect of the virus in several organs liver and kidney included **(Abidin *et al.,* 2014).**

Aim of the study:

In this study, 20 broiler farms over the period 2018-2020 were investigated to detect IBDV using RT-PCR. Evaluation of immune response as well as hematological and some biochemical parameters alterations of IBDV infected chickens were carried out.

Materials and Methods

1. Broiler Flocks:

Two hundred bursa samples were collected from twenty 3-6 weeks broiler chicken farms (10 samples from each farm which was located in Belqas, Dakahlia governorate, nearby different villages), chickens were vaccinated by the intermediate attenuated vaccine (D78) at 6^{th} and17^{th} day old chickens during the period of 2018-2020. Clinical observation was dullness, anorexia, depression, head trembling, lameness

and watery yellowish diarrhea with sudden onset of high mortalities. The obtained data of the postmortem findings were characteristic to IBDV infection where the affected chickens showed congested bursa with caseous exudate and hemorrhage in proventriculus and gizzard, nephrosis with kidney urates, enlarged mottled liver, swollen necrotic spleen and sever echomotic hemorrhages on breast and thigh muscles.

2. Sampling

A total of 200 blood samples (10 samples from each of the 20 farms) were collected from apparently healthy and diseased chickens from the wing vein. Blood samples were divided into two portions; the first portion was collected in vial containing dipotassium EDTA as an anticoagulant for hematological study. Then, the second part of blood kept in incubator at 37^{o}C for one hour then refrigerated at 4^{o}C for 1 h and centrifuged at 4,000 rpm for 15 min. The supernatant was collected in a 1.5ml Ependorf tube and immediately stored at -20^{o}C for evaluation of the immunological status of chickens (antibody titer by ELISA technique, lysozyme activity, electrophoretic pattern) and some biochemical analysis (serum creatinine, uric acid, ALT and AST).

Samples were obtained from the apparently healthy and diseased chickens which were vaccinated by the intermediate attenuated vaccine (D78) at 6th and17 th day old chickens respectively as the farm's vaccination schedule protocol.

3. Clinical samples:

Bursa samples were collected from different poultry farms. Bursa samples (pooled) were homogenized in sterile phosphate buffered saline to obtained 10% tissue suspension. The suspensions were clarified by centrifugation. All aliquots of the tissue suspension were used for virus isolation and RNA extraction.

4. Isolation of IBD virus on SPF ECE:

IBDV was isolated by inoculation of centrifuged supernatant fluid on SPF ECE in chorioallantoic membrane at 13th day. Then, the inoculated eggs were incubated at 37^{o}C for 5 days. Daily observation of the inoculated eggs and the dead one were chilled at 4^{o}C overnight and after that examined for lesion (thickening of CAM while embryos appeared congested with head edema and greenish liver) and confirmed by RT-PCR **(OIE, 2018)**.

5. RNA extraction. RNA extraction from samples was done using the QIAamp viral Mini kit (Cat. No. / ID: 57704, Qiagen, Germany, GmbH). According to **Manufacturer's instructions**, 140 µl of the sample suspension was incubated with 560 µl of AVL lysis buffer and 5.6 µl of carrier RNA at room temp. for 10 min. After incubation, 560 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 60 µl of AE elution buffer provided in the kit.

Oligonucleotide Primers and probe. Primers used were supplied from **Metabion (Germany)** is listed in table (1).

PCR amplification. Primers were utilized in a 25- µl reaction containing 12.5 µl of Quantitect probe Rt-PCR Kit (Cat. No. / ID: 204443, Qiagen, Germany, GmbH) according to **Metwally *et al.,* (2009)**,1 µl of each primer of 20 pmol concentration, 0.25 µl of rt-enzyme, 5.25 µl of water and 5 µl of RNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler.

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products was loaded in each gel slot. A gene ruler 100 bp DNA Ladder (Fermentas, Germany) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Table (1). Primers and probe sequences, target genes, amplicon sizes and cycling conditions.

Target gene	Primers sequences	Amplified segment (bp)	Reverse transcription	Primary denaturation	Amplification (35 cycles)			Reference
					Secondary denaturation	Annealing	extension	
VP2	TCAC-CGTCCTCAGCTTAC CCACATC GGATTTGG-GATCAGCTCGAAGT TGC	620	50C 30 min.	94˚C 15 min.	94˚C 30 sec.	59˚C 40 sec.	72˚C 1 min.	**Metwally *et al.*, (2009)**

6. Hematological studies:
Red blood corpuscles (RBCs 10^6/µl), hemoglobin (Hb gm/dl), packed cell volume (PCV%), total leukocytic count (WBCs 10^3/ µl), and differential leukocytic count were determined according to a routine hematological examination and standard blood smear **(Jain, 2000)**.

7. Serum biochemical parameters:
Liver and kidney functions:
Aspartate and alanine amino transferases (AST and ALT) were quantitatively estimated according to the method described by **Reitman and Frankel, (1957); Breuer, (1996)**; and **Thefeld (1974)** respectively. Serum creatinine was determined according to **Henry (1979)** and uric acid was to **(Caraway, 1963).** All these parameters were determined by using fully automated random-access clinical chemistry analyzer Erba Mannheim XL- 180 from ErbaLachema S.R.O. Company, using Syspacks reagents for Erba XL Analyzers from ErbaLachema S.R.O. Company.

8. Immunological assays:
a. Lysozyme activity assay:
Serum lysozyme activity was assessed according to **Peeters and Vantrapen (1977)**. Addition of 25 µl of each serum sample to wells cut in agarose gel (1% in PBS, pH 6.3) in which *micrococcus lysodeikticus* bacteria (50 mg /100 ml agarose) were dispersed. The diameter of clear zone formed around the cells after 24 hours was measured. The concentration of lysozyme was obtained from logarithmic curve using standard lysozyme solution.

b. Electrophoretic pattern of serum protein:
The estimation was conducted according to **Sonnen Wirth and Jaret (1980)** and calculated according **Syn Gene S.** No. 17292* 14518 sme*mpcs. Immunoglobulin determination was by zinc sulphate turbidity according to **(McEwan, *et al.,* 1970)** and calculated according **Syn Gene S.** No. 17292* 14518 sme*mpcs.

c. Enzyme-linked immunosorbent assay (ELISA):
IBD antibodies were assayed using ELISA kit **(ID. Vet laboratory, France).** The manufacturer's instructions were adhered to strictly. Cat No. IBDS- 5P/ Batch No. G56.

Statistical analysis:
Data of hemogram, serum biochemistry, lysozyme activity, electrophoretic pattern and antibody titer by ELISA of chickens were analyzed firstly for the means and standard errors of diseased chickens compared to apparently healthy one according to **Snedecor and Cochran (1980)**. Then, significance of the results was evaluated using analysis of variance **(ANOVA)** using **SPSS** software version 14.

Results

1. Post mortem findings:
Affected chickens from 20 flocks showed congested bursa, severe echomotic hemorrhages on breast and thigh muscles, nephrosis of kidney with mottled appearance and bones were bended and easily broken with history of high mortalities (11-15%) in 3, 6 day. The rest of the survived flocks showed dehydration with slight muscular congestion and slightly enlarged bursa.

2. Virus Propagation and Titration:
Harvested chorioallantoic membrane 5 days post inoculation showed congestion and thick-

ening while embryos appeared congested, and head edema with greenish liver. Virus titer was 10^6 EID_{50} per 1 ml of CAM homogenate.

3. Detection of IBDV by RT-PCR: Out of 20 broiler chicken farms bursal samples (pooled) tested with RT- PCR, 2 farms were found positive. All RT-PCR positive samples showed specific bands at 620 bp on agrose gel .

4. Haematological studies:
The results of haematological studies are presented in table (2). It is evident that there was significant increase ($p< 0.05$) in PCV (31.09± 0.31) while RBCs count (2.38 ± 0.023) and Hb concentration (11.79 ± 0.11) showed non-significant changes in diseased chickens compared to apparently healthy group. Meanwhile, total leucocytic count (TLC) declared significant decrease ($p< 0.05$) (17.59 ± 0.7) which accompanied by a significant decrease ($p\leq0.01$) in lymphocytic count (5.02± 0.17), however, hetrophils (10.84± 0.4) ($p\leq0.05$) and eosinophils (0.60±0.02) ($p\leq0.01$) showed significant increase in diseased chickens in comparison to apparently healthy group.

5. Biochemical analysis:
The results of biochemical analysis in apparently healthy and IBDV diseased chicken are presented in table (3)**.** It is evident from serum biochemical analysis that high significant ($p< 0.05$) increase in activities of AST (96.50±1.81), ALT (49.60±2.14), creatinine (1.03±0.01) and uric acid (7.09 ±0.51) in comparison to with value of apparently healthy one.

Table (2). Mean value (±SE) of hematological parameters in IBDV diseased chickens compared to apparently healthy one. (n = 20).

Groups / Parameters	Apparently Healthy chicken (n=20)	IBDV Diseased chicken (n=20)
RBCs ($\times10^6$ /µl)	2.42 ± 0.03	2.38 ± 0.023
Hb (g/dl)	11.77 ± 0.07	11.79 ± 0.11
PCV (%)	27.55 ± 0. 34^a	31.09 ± 0.31^b
WBCs ($\times10^3$/µl)	24.71 ± 1.1^a	17.59 ± 0.7^b
Heterophils ($\times10^3$/µl)	8.33 ± 0.22^a	10.84 ± 0.4^b
Lymphocytes ($\times10^3$/µl)	14.93 ± 0.29^a	5.02 ± 0.17^b
Monocytes ($\times10^3$/µl)	0.93 ± 0.2	0.96 ± 0.3
Eosinophils ($\times10^3$/µl)	0.36 ± 0.017^a	0.60 ± 0.02^b
Basophils ($\times10^3$/µl)	0.16 ± 0.1	0.17 ± 0.3

Table (3). Mean value (±SE) of some serum biochemical parameters in IBDV diseased chickens compared to apparently healthy one . (n = 20)

Group / Parameters	Apparently healthy chickens (n=20)	IBDV diseased chickens (n=20)
AST (U/L)	66.75 ±0.62^a	96.50±1.81^b
ALT (U/L)	18. 90 ±0.66^a	49.60 ±2.14^b
Creatinine (mg/dl)	0.44±0.01^a	1.03 ±0.01^b
Uric acid (ml/dl)	3.8±0.15^a	7.09 ±0.51^b

Values represent means ± SE Significance at $p\leq0.05$
Means with different superscript indicate significance at the same raw

6. Immunological assay:
Effect of IBDV disease on serum lysozyme activity, serum electrophoretic pattern and antibody titer of apparently healthy and diseased chickens was illustrated in table (4 & 5).

A. Lysozyme activity:
Result of serum lysozyme activity of diseased chickens was significantly higher than that of apparently healthy chickens ($p< 0.05$).

B. Electrophoretic pattern of protein:
Concentration of serum total protein revealed significant reduction ($p< 0.05$) as severe hypoproteinemia occurred in IBDV diseased chickens compared to apparently healthy group. In addition, highly significant hypoalbuminemia was detected as well in the same group of chickens. Furthermore, immunoglobulin fractions α, β and γ showed drastic reduction significantly in infected chickens. Consequently, hypoproteinemia reflected on A/G ratio results which showed significant decrease in diseased chickens in comparison to apparently healthy group.

Table (4). Mean value (±SE) of Lysozyme activity and serum electrophoretic pattern in IBDV diseased chickens compared to apparently healthy group. (n = 20)

Groups / Parameters	Apparently Healthy group (n=20)	IBDV infected group (n=20)
Lysozyme µg/ml	60.08±5.86[a]	165.81±15.48[b]
Total protein (gm/dl)	19.20±1.43[a]	4.35±0.18[b]
Pre albumin	0.69± 0.065[a]	0.35±0.018[b]
Albumin	6.97±0.33[a]	0.75±0.05[b]
T. albumin	7.66±0.30[a]	1.09±0.06[b]
α globulin	5.23±0.48[a]	1.19±0.11[b]
β globulin	2.78±81[a]	0.95±0.08[b]
γ globulin	3.36±0.38[a]	1.16±0.05[b]
T. globulins	11.54±1.13[a]	3.31±012[b]
A/G ratio	0.68±.04[a]	0.33±0.015[b]

Values represent means ± SE Significance at $p \leq 0.05$
Means with different superscript indicate significance at the same raw

C. ELISA assay:
On the other hand, the antibody titer determined by ELISA had been shown significant difference of diseased chickens compared to apparently healthy chickens. Meanwhile, positive antibody titer declared significant increment in comparison to negative antibody titer of IBD virus infected chickens.

Table (5). Mean value (±SE) of serum antibody titer in IBDV diseased chickens compared to apparently healthy group. (n = 20)

Groups / Parameters	Apparently healthy chickens	Diseased chickens	
		-ve Ab titer	+ve Ab titer
Antibody titer	1185±79.54[a]	129.15±23.36[b]	4631.73±920.45[c]

Values represent means ± SE Significance at $p \leq 0.05$
Means with different superscript indicate significance at the same raw

Discussion

Because of huge economic losses caused by this virus to poultry industry in Egypt, vaccination has therefore been applied intensively to control the infection. **Kusk *et al.,* (2005)** found that effective vaccination depends on rapid and accurate diagnosis of the subtype present in a flock. Post-mortem findings reported in this study were characteristic to IBDV infection. Affected chickens showed congested bursa and sever peticheal hemorrhages on breast and thigh muscles, liver was congested, enlarged and mottled. The severity of these lesions depends upon virulence of the virus, age of birds and the presence or absence of passive immunity **(Hassan, 2004; Rauw *et al.,* 2007).** It was suggested that pathogenesis and viral multiplication is more pronounced in very virulent strains comparing to the milder strains **(Van den Berg *et al.,* 2000).**

In this study, a method for IBDV detection was tested by the amplification of a 620-bp PCR product, which includes the hypervariable region of the VP2 gene **(Ikuta *et al.,* 2001).** Therefore, revising the vaccination programs in Egypt is recommended. Conventional RT-PCR has been useful and sensitive test in detecting IBDV serotypes. Hematological studies have helped in the diagnosis of infectious and other diseases for many years. Hemograms determined during the course of disease conditions coupled with history and clinical signs, often assist to suspect the disease. Their use as a diagnostic aid in avian species, especially pet birds, is becoming increasingly popular. Quantitative changes in a particular leukocyte type indirectly reflect the nature of the disease process and the body's response **(Jain, 1986).**

In IBDV infected chicken increased hematocrit values which have previously been observed by **(Chineme and Cho 1984).** It is believed that dehydration, which is one of the clinical and postmortem finding in IBD is a major cause of the increase in the hematocrit value this result is agreed with our results that indicated significant increase of hematocrit value. Our results leukopenia with lymphopenia is in accordance with those obtained by **(Campbell and Coles, 1986)** who showed that lymphopenia occurred in acute viral disease due to glucocorticoid eccesses. Specifically, IBDV causes destruction of B- lymphocytes within bursa of fabricius **(Silveria *et al.,* 2019 and Lupini *et al.,* 2020)** the organ of maturation and differentiation of B- lymphocytes before they migrate into blood stream**. Weiss and Kaufer-Weiss, (1994),** detected IBDV antigen in the bursa of fabricious 11 hours post infection thus 24 hours provide an adequate period for the effect of B-lymphocyte destruction to be evident in the blood in cases where an IBDV infection has been established. Heterophilia observed in infected chicks is evidence of massive tissue destruction **(Coles, 1986).** Eosinophilia is associated with tissue destruction, these tissues have high concentrations of mast cells, and during tissue destruction these cells degranulate resulting in histamine release, which is chemotactic to eosinophils **(Jain, 1986).** The ALT, AST values were significantly increased in IBDV infected birds. IBDV causing hepatocytes necrosis and rupturing of hepatocytes that elevated ALT values **(Abidin *et al.,* 2013).** In IBDV infection liver and kidney were adversely affected that leads to increase values of serum enzymes. Similar findings were reported by **(Abidin *et al.,* 2014** and **Roosevien, 2006)**, that come in agreement with our results which revealed increasing activities of AST, ALT creatinine and uric acid indicating pathological changes as part of the pathogenesis of the disease.

In the current study, the immunological status was evaluated of apparently healthy and diseased chickens which ranged from moderate to severely affected birds. As lysozyme is a primary humoral factor of innate immunity in both animals and humans. It is known to be effective against some viruses **(Angelo, 1965; LeeHuang *et al.,* 1999)** and Gram-positive bacteria **(Aliev, 1973; Buharin & Vasilev, 1974; Blotskyi, 1976)** .

Meanwhile , there was a significant increase in lysozyme activity in disease chickens than that of apparently healthy chickens which supported the involvement of innate immune response during IBDV infection **(Palmquist *et al.,* 2006)**. This agreed with **Farhana *et al.,* (2018)** who found upregulation of cytokines, chemokines including the lysozyme and NOS2 which indicate the occurrence of the inflammatory

response in the infected bursa, **(Khatri *et al.*, 2005)** and the involvement of macrophages, an important effector of innate immune response during IBDV infection **(Palmquist *et al.*, 2006)**. However, lysozymes was known to be one of lysosomal enzymes attacks mucopeptide in cell walls of various bacteria **(Moore *et al.*, 2006)** and have important role as bactericidal activity during phagocytosis **(Tizard, 1996).** The increase in lysozyme may be due to its leakage from lysosomal granules. Since the toxicant binds to metallthionin and the complex formed is stored in lysosomal granules when the storage exceeding their storage capacity may be bursting of lysosomes and release of lysosomal granules **(Bussolaro *et al.*, 2008).** The antiviral activity of lysozyme is ascribed to its direct interaction with the virus (the blockade of viral fusion protein gp41) or with the cell (the blockade of CD4 receptor on lymphocytes) (**Bahbahani *et al.*, 2018). Zhang *et al.*, (2008)** investigated that lysozyme produced by the marine strain of bacteria from the genus *Bacillus* inhibited the replication of pseudo rabies virus in porcine kidney cells PK-15, not via direct interactions with the virus or the cell but during/after the infection of a cell, which is synchronize with that of **Małaczewska *et al.*, (2019).**

Screening of serum antibody titer of chickens using ELISA assay revealed significant difference of diseased chickens compared to apparently healthy chickens. Meanwhile, positive antibody titer declared significant increment in comparison to negative antibody titer of IBD virus infected chickens. Infection with IBDV at an early age significantly compromises the humoral and local immune responses of chickens. The cell mediated immune response (CMI) is also compromised, but to a lesser extent and for a shorter period. The immunosuppression could be as a result of lysis of B cells directly or their precursors. Other mechanisms of immunosuppression may be, notably referred to the development of suppressor cells **(Saif, 1991).** Moreover**,** inhibition of the humoral immunity is attributed to the destruction of immunoglobulin-producing cells by the virus. Meanwhile, altered antigen presenting and helper T cell functions may also be involved **(Sharma *et al.*, 2000).** The IBD virus replicates extensively in IgM+cells of the bursa and chickens may die during the acute phase of the disease, although IBD virus-induced mortality is highly variable and depends, among other factors, upon the virulence of the virus strain. The sub-clinical form is more common than clinical IBD because of regular vaccination on breeding farms. Infection at an early age significantly compromises the humoral and local immune responses of chickens because of the direct effect of B cells or their precursor **(Balamurugan and Kataria, 2006).**

Blood proteins are considered an important factor in the evaluation of health status of chickens, as well as of production features **(Filipović *et al.*, 2007)**. In addition, blood proteins have many important physiological roles in the body including the maintenance of homeostasis **(Piotrowska *et al.*, 2011**).

The present study revealed hypoproteinemia and hypoalbuminemia and significant decrease in A/G ratio in diseased chickens **.**The induced hypoproteinemia and hypoalbuminemia in IBD infected chicken may attributed to reduced production by the affected liver or loss of proteins through impaired kidney in IBD infection **(Ley *et al.*, 1983 and Nunoya *et al.*, 1992**). Since liver is the central organ for the synthesis of protein, impaired liver function in IBDV infection could cause hypoprotenemia, whereas impaired kidney would result in loss of small molecular size and osmotic sensitivity protein such as albumin resulting in hypoalbuminaemia **(Bain, 2003)** .Moreover, The anorexia and the diarrhea in the IBD infected bird in the present study could also contribute for the hypoprotenemia. Furthermore, the consuming of the proteins in the process of white blood cell and antibody production, tissue repair, maintenance of plasma osmolality and enzyme production **(Hochleithner, 1999)** may also cause hypoproteinemia in IBDV infected birds.

Also, in IBD chickens the level of immunoglobulin fractions α, β and γ showed drastic reduction in infected chickens than the normal group, which reflected as significantly decreased gamma globulins and total protein. This hypoglobulinemia may attributed to the inhibitory effect of IBD infection on IgM production from B cells within bursa of fabricius **(Silveria *et al.*, 2019 and Lupini *et al.*, 2020).**

This finding was in contrary to previous stud-

ies which reported increased in gamma-globulin **(Ley *et al.,* 1983)** and total globulin **(Panigraphy *et al.,* 1986)** following IBDV infection. These discrepancies could be related to the age, strain of the virus and immune status in the affected chicks. Moreover, the true serum gamma-globulin concentration might have been masked by neutralization of the antibody **(Okoya and Uzoukwu, 1990).** The A:G ratio has been used extensively in analysis of sera as an indicator of infection and antibody (**Griminger, 1986**). In the present study, there was significantly decrease in the A: G ratio in infected chickens than the normal chickens. This finding was agreed with **(Panigraphy *et al.,* 1986)**. The reduction in A:G ratio in IBDV infection has also been attributed to hypo-albuminaemia and hyper-gamma-globulinaemia **(Evans, 2003).** However, the decreased A: G ratio in the IBD infected chicken in the current study was not associated with an increase in globulin in this group because both globulin and albumin were significantly decreased in IBD group than the normal group but the significant decreased A:G ratio was due to the relatively increased globulin fractions in relation to albumen fraction within the IBD group only.

Conclusion

The infectious bursal disease virus continuous to be a serious problem in Egypt, concerning our study, 20 broiler farms over the period 2018-2020 were investigated in an attempt to molecular characterization IBDV using RT-PCR, isolation to provide information about the existence of vvIBDV. The evaluation of the electrophoretic protein and immunoglobulin fractions revealed significant changes in infected growing broiler chickens ,hence, affect its productivity. Screening of serum antibody titer declared significant difference of infected chickens with respect of the positive antibody titer that showed significant increment in comparison to negative antibody titer infected chickens. In these cases the immunosupression may be transient and convalescent chickens may recover most humoral immune functions which might be concomitant with secondary infection during the transient immunosuppression which can lead to drastic economic losses. Consequently, immunosuppression as well as alterations in blood indices and some serum biochemical parameters were declared obviously that were consistent with the pathogenicity of IBDV infection which may be as related to the damage affected bursa of fabricius, liver and kidney. So the need, immunostimulants' supplementation preferred to be used to enhance herd immune status as well as pinpoint on the high level of maternal antibody that help to minimize early infection and consequently immunosuppression.

References

Abidin, Z.; Khatoon, A.; Butt, T.M.; Hussain, S.; Kanwal, A.; Ali, S., and Aziz, A. (2014). Isolation and molecular identification of infectious bursal disease (IBD) virus from commercial poultry: Effects of field isolate on cell mediated immune response and serum biochemical parameters in broilers. Int. J. Innov. appl. Res., 2: 8-20.

Abidin, Z.; Khan, M.Z.; Khatoon, A.; Saleemi, M.K.; Khan, A. and Javed, I. (2013). Ameliorative effects of L-carnitine and vitamin E upon OTA induced haematological and serum biochemical alterations in white leghorn cockerels. Br. Poult. Sci., 54: 471-477.

Aliev, E.A.; A.M. Tevosov and F.G. Saltanova (1973). Brucellosis resistance in sheep of different breeds." Reports of the Soviet Academy of Agricultural Science, No 6, 3940.

Angelo, P. (1965). "Ricerche sull'azione del lisozima neiconfronti del virus del difterovaiolo dei polli". Acta Medica Veterinaria (Napoli), 11, 135-140.

Bain, P.J. (2003). Liver. In Duncan and Prasse's Veterinary Laboratory Medicine and Clinical Pathology, Eds, K.S. Latimer, E.A. Mahaffey and K.W. Prasse, 4th ed., Blackwell Limited, Ames, Iowa,USA, pp: 193-214.

Balamurugan, V. and Kataria, J.M. (2006). Economically important non-oncogenic immunosuppressive viral dis-eases of chicken—current status.Veterinary Research Communications,30(5), 541–566.

Behbahani, M.; Nosrati, M. and Mohabatkar, H. (2018). "Inhibition of human immu-

nodeficiency type 1 virus (HIV-1) life cycle by different egg white lysozymes."Appl Biochem Biotechnol.;185:786–798.

Blotskyi, I.A.; A.I. Kundryukov; N.Y. Terescenko and V.K. Shilyakin (1976). "Lysozyme titre in pigs with leptospirpsis.: Veterinariya, 4, 55-56.

Breuer, J. (1996). Report on symposium (drug effect in clinical chemistry methods). Eur J Clin Chem Clin Biochem 34(4), 385- 386.

Buharin, O.V. and N.V. Vasilev (1974). Lysozyme and its Role in Biology and Medicine". University of Tomsk's Publishing House, 138-153.

Bussolaro, D.; Filippo, K.F.; Ganrgioni, R.; Fernandes, L. and Ribeiro, C.A. (2008). "The immune response of peritoneal macrophage due to exposure to inorganic lead in house mouse Musmusculus. "Toxicology in Vitro, 22: 254-260.

Campbell, T.W. and Coles, E.H. (1986). Avian clinical pathology. In: Coles E.H. Ed., Veterinary clinical pathology. Philadelphia, PA, USA, WB Saunders, p. 289-290.

Chineme, C.N. and Cho, Y. (1984). Clinicopathological and morphological changes in chickens experimentally infected with infectious bursal (Gumboro) disease virus. Trop. Vet., 2: 218-224.

Caraway, W.T. (1963). Uric acid. Stand Methods Clin. Chem. 4: 239-247.

Coles, E.H. (1986). "Veterinary Clinical Pathology" 4th ed. WB Saunders Company, Philadelphia.

Cosgrove, A.S. (1962). An apparently new disease of chickens - avian nephrosis. *Avian* Dis., 6: 385-389.

Coulibaly, F.; Chevalier, C.; Delmas, B. and Rey, F.A. (2010). Crystal structure of an Aquabirnavirus particle: insights into antigenic diversity and virulence determinism. J. Virol 84, 1792-1799.

El-Sergany, H.A.; Ann Moursi; Saber, M.S. and Mohamed, M.A. (1974). A Preliminary investigation on the occurrence of gumboro disease in Egypt. J. Vet. Sci. 11, 185-208.

Eterradossi, N. and Saif, Y.M. (2008). Infectious bursal disease. In Y.M. Saif., A.M. Fadly, J.R. Glission, L.R. McDougald, L.K. Nolan, &D.E. Swayne (Eds.), Diseases of Poultry12th edn (pp. 185–208).Ames, IA: Blackwell.

Eterradossi, N. and Saif, Y.M. (2013). Infectious bursal disease. In D. Swayne, J.R. Glisson, L.R. McDougald, L.K. Nolan, D.L. Suarez, &V. Nair (Eds.), Diseases of Poultry13th edn (pp. 219–246). Ames, IA: Wiley-Blackwell

Evans, E.W. (2003). Proteins, lipids andcarbohydrates. In Duncan and Prasse's Veterinary Laboratory Medicine and Clinical Pathology, EdsLatimer, K.S., E.A. Mahaffey and K.W. Prasse,4th ed., Blackwell Limited, Ames, Iowa, USA, pp: 162-192.

Farhanah, M.I.; Yasmin, R.; Isa, N.M.; Hair-Bejo, M.; Ideris, A.; Powers, C.; Oladapo, O.; Nair, V.; Khoo, J.; Ghazali, A.; Yee, W. and Omar, A. (2018). Bursal transcriptome profiling of different inbred chicken lines reveals key differentially expressed genes at 3 days post infection with very virulent infectious bursal disease virus. Journal of General Virology, 99: 21–35 .

Filipović, N.; Stojević, Z.; Milinković-Tur, S.; Ljubić, B.B. and Zdelar-Tuk, M. (2007). Changes in concentration and fractions of blood serum proteins of chickens during fatten-ing. Vet. Arhiv., 77(4): 319-326.

Griminger, P. (1986). Lipid metabolism. In Avian Physiology, Ed., Sturkie, P.D., 4th ed., edSpringer-Verlag, New York, pp: 345-358.

Hassan, M.K. (2004). Very virulent infectious bursalsisease virus in Egypt; epidemiology, isolation and immunogenicity of classic vaccine. Vet. Res. Commun 28, 347-356.

Henry, R.J. (1979) Colorimetric methods for determination of serum creatinine. Clinical chemistry, principals and techniques, 2nd ed. Harper and Row ; 525.

Hochleithner, M. (1999). Biochemistries. In Avian medicine: principles and application, Eds.,B.W. Ritchie, G.J. Harrison and L.R. Harrison.Brentwood International, incorporated, pp: 223-245. **ID.Vet laboratory, France**: info@innovative-diagnostics.com

Ikuta, N.; El-Attrache, J.; Villegas, P.; García, M.; Lunge, V.R.; Fonseca, A.S.K.; Oliveira, C. and Marques, E.K. (2001). Molecular characterization of Brazilian infectious bursal disease virus.*Avian Dis*., 45: 297-306.

Islam, M.R.; Rahman, S.; Noor, M., Chowdhury, E.H. and Muller, H. (2012). Differ-

entiation of infectious bursal disease virus (IBDV) genome segment B of very virulent and classical lineage by RT-PCR amplification and restriction enzyme analysis. Archives of virology 157, 333-336.

Jain, N.C. (2000). " Schalm's veterinary haematology"8th Ed. Lea and Febiger, Philadelphia, USA.

Jain, N.C. (1986). Schalm's Veterinary hematology, 4th Edn. Philadelphia, PA, USA, Lea and Febiger, p. 747-748.

Khatri, M.; Palmquist, J.M.; Cha, R.M. and Sharma, J.M. (2005). Infection and activation of bursal macrophages by virulent infectious bursal disease virus. Virus Res., 113:44 –50.

Kusk, M.; Kabell, S.; Jorgensen, P.H. and Handberg, K.J. (2005). Differentiation of five strains of infectious bursal disease virus: development of a strain-specific multiplex PCR Vet. Microbiol. 109, 159-167.

LeeHuang, S.; P.L. Huang; Y. Sun; Hf. Kung; D.L. Blithe and H.C. Chen (1999). "Lysozyme and RNases as anti-HIV components in beta-core preparations of human chorionic gonadotropin." Proceedings of National Academy of Science (USA), 96, No 6, 2678-2681.

Ley, D.H.; R. Yamamoto and A.A. Bickford (1983). The pathogenesis of infectious disease: serologic, histopathologic and clinical chemical observations. Avian Disease, 27: 1060-1085.

Li, Y.; Wu, T.; Cheng, X. and Zhang, C. (2009). Molecular characteristic of VP2 gene of infectious bursal disease viruses isolated from a farm in two decades. Virus Genes 38, 408-413.

Lupini, C.; Felice, V.; Silveira, F.; Mescolini, G.; Berto, G.; Listorti, V.; Cecchinato, M. and Catelli, E. (2020). Comparative in vivo pathogenicity study of an ITA genotype isolate (G6) of infectious bursal disease virus. Transbound. Emerg. Dis. 67(2):1025-1031.

Małaczewska, J.; Kaczorek-Łukowska, E.; Wójcik, R. and Siwicki, A.K.; (2019). Antiviral effects of nisin, lysozyme, lactoferrin and their mixtures against bovine viral diarrhoea virus. BMC Vet Res. 2019; 15: 318.

McEwan, A.D.; E.W. Fisher; I.E. Salman and W.J Penhale (1970). "A turbidity test Chem.Acta27,155-163.

Metwally, A.M.; Yousif, A.A.; Shaheed, I.B.; Mohammed, W.A.; Samy, A.M. and Reda, I.M. (2009). Re-emergence of Very Verulent IBVD in Egypt International Journal of Virology. 5, (1):1-17.

Moore, M.M.; Allan, J.I. and Mcveigh, A. (2006). "Enviromental prognostic: an integrated model supporting lysosomal stress as predictive biomarkers of animal health status." Marine Enviro. Res., 61: 278-304.

Musa, I.W.; Saidu, L.; Adamu, J.; Mbuko, I.J.; Kaltungo, B.Y. and Abdu, P.A. (2010). Outbreaks of gumboro in growers in Zaria, Nigeria. Niger. Vet. J., 31: 306–310.

Reitman, S. and Frankel, S. (1957). A colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Amer. J. Clin. pathol., 28 (1) : 56-63.

Nunoya, T.Y.O.; M. Hiraga and T. Saito (1992). Occurrence of acute infectious bursal disease withhigh mortality in Japan and pathogenicity offield isolates in specific-pathogen-free chicks. Avian Disease, 36: 597-609.

Okoye, J.O.A. and M. Uzoukwu (1990). Pathogenesis of infectious bursal disease in embryonallybursectomised chicks. Avian Pathology, 19: 555-569.

Palmquist, J.M.; Khatri, M.; Cha, R.M.; Goddeeris, B.M. and Walcheck, B. (2006). In vivo activation of chicken macrophages by infectious bursal disease virus. Viral Immunol., 19: 305–315.

Panigraphy, B.; L.D. Rowel and D.E. Corrier (1986). Hematological values and changes in blood chemistry in chicks with infectious bursal disease. Research in Veterinary Science, 40: 86-88.

Peeters, T.L. and Vantrapen, G.R. (1977). Factors influencing lysozyme determination by lysoplate..Clin. Chim.Acta,74: 217-225.

Piotrowska, A.; Burlikowska, K. and Szymeczko, R. (2011). Changes in blood chemistry in broiler chickens during the fattening period. Folia Biol. Krakow, 59(3-4): 183-187.

Rautenschlein, S.; Yeh, H.Y. and Sharma, J.M. (2003). Comparative immunopathogenesis of mild, intermediate, and virulent strains of classic infectious bursal disease virus. Avian Dis. 47: 66– 78.

Rauw, F.; Lambrecht, B. and Van den Berg, T. (2007). Pivotal role of ChIFNgamma in the pathogenesis and immunosuppression of infectious bursal disease. Avian pathology: journal of the W.V.P.A.36, 367-374.

Reitman, S. and Frankel, S. (1957). Determination of serum glutamic oxaloacetic and glutamic pyruvic. J. Clin. Path. 28: 56.

Roosevien, F.N.R. (2006). Molecular characteristics and pathogenecity of infectious bursal disease virus isolated in Malaysia. MVSc thesis. Faculty of Veterinary medicine, Uni Putra Malaysia, pp. 99-132.

Saif, Y.M. (1991). Immunosuppression induced by infectious bursal disease virus. Veterinary Immunology and Immunopathology,30, 45–50.

Sharma, J.M.; Kim, I.J.; Rautenschlein, S. and Yeh, H.Y. (2000). Infectious bursal disease virus of chickens: pathogenesis and immunosuppression. Developmental and Comparative Immunology, 24, 223–235.

Snedecor, G.W. and Cochran, W.G. (1980). Statistical Methods. Ames Lowa State Univ. Press,7th Ed, 593, USA.

Silveira, F.; Felice, V.; Franzo, G.; Mescolini, G.; Catelli, E.; Cecchinato, M.; Berto, G.; Listorti, V. and Lupini, C. (2019). Inoculation of specific pathogen-free chickens with an infectious bursal disease virus of the ITA genotype (G6) leads to a high and persistent viral load in lymphoid tissues and to a delayed antiviral response. Vet. Microbiol. 235: 136–142.

Sonnenwirth, A.C. and Jarett, C.V. (1980). "Gradwohl's Clinical Laboratory Methods and Diagnosis."Vol. 9, 8th Ed., Mosby. Pp. 2339.

SPSS, 14 (2006). Statistical Package for Social Sience, SPSS for Windows Release 14.0.0,2006.Standard Version ,Copyright SPSS Inc.,1989-2006,All Rights Reserved,
Copyright® SPSS Inc.

Tizard, I.R. (1996). "Veterinary Immunology." An introduction by W.B. Saunders Comp. A division of Harcourt Brace and Company,5th Edition, Philadelphia, London, Montreal, Sydney, Tokyo. .

Thefeld, W. (1974). Annuals' of Clinical Biochemistry 12:142. Cited by Wooton and Freemon Pp: 79.

Uddin, M.M.; Islam, M.S.; Basu, J. and Khan, M.Z.I. (2012). Distribution and quantification of lymphocytes in the major lymphoid organs of naturally gumboro infected broilers. Int. J. Morphol., 30: 1585–1589.

Van den Berg, T.P. (2000). Acute infectious bursal disease in poultry: a review. Avian Pathology, 29, 175–194.

Weiss, E. and Kaufer-Weiss, I. (1994). Pathology and pathogenesis of infectious bursal disease. In: Proc. International symposium on infectious bursal disease and chicken infectious anaemia, Rauischholzhausen, Germany, 21-24 June, 1994.

Wang, Y.; Kang, Z,; Gao, H.; Gao, Y.; Qin, L.; Lin, H.; Yu, F.; Qi, X. and Wang, X. (2011). A one-step reverse transcription loop -mediated isothermal amplification for detection and discrimination of infectious bursal disease virus. Virology journal 8, 108.

Wu, C.C.; Rubinelli, P. and Lin, T.L. (2007). Molecular detection and differentiation of infectious bursal disease virus. Avian diseases 51, 515-526.

Xu, X.G.; Tong, D.W.; Wang, Z.S.; Zhang, Q.; Li, Z.C.; Zhang, K.; Li, W. and Liu, H.J. (2011). Baculovirus virions displaying infectious bursal disease virus VP2 protein protect chickens against infectious bursal disease virus infection. Avian diseases 55, 223-229.

Zhang, X.; Wu, F.X.; Sun, M.; Wang, Q.Y.; Wang, Y.J. and Yang, X.K. (2008). Study on antimicrobial and antiviral activities of lysozyme from marine strain S-12-86 in vitro."Agric Sci China. 7:112–116.