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Molecular and pathological investigations of hemorrhagic syndrome associated viral infection in broilers and sasso breeds in Dakahlia Governorate

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ABSTRACT

Chicken infectious anemia (CIA) is a worldwide, highly contagious immunosuppressive viral disease of young chickens 4-6 week of age caused by chicken anemia virus (CAV). It is implicated in several field problems of economic significance after maternal antibodies have waned. CAV threaten poultry industry alone or either coinfection with infectious bursal disease virus (IBDV) or inclusion body hepatitis (IBH). This paper aimed to understand the pathogenesis of this existing condition. Three hundred chickens from fifteen selected commercial broiler flocks (7 Cubb and 8 Native breeds) were collected from different areas of Dakahlia Governorate Egypt during the period from October 2019 to September 2020. Necropsy was carried out on freshly dead or diseased birds beside blood samples for hematological changes. Tissue samples were subjected to PCR assay for detection of CAV DNA, IBDV RNA and IBH DNA. Conventional PCR test indicated that all tissue samples collected form Cubb and Native breed farms were either PCR positive for CAV and IBDV coinfected with CAV while all samples were negative for IBH virus. Hematological findings showed a significant decrease ($p<0.05$) in RBCs count, Hb concentration and PCV in CIA and coinfected chickens. Grossly, emaciation was characteristic in carcasses moreover, lymphoid organs were congested and atrophied beside several multifocal hemorrhagic areas in skeletal muscles accompanied with pale bone marrow. Microscopically, lesions noticed were more intense and more severe in CIA coinfection with IBDV. Generalized lymphoid cell depletion in lymphoid organs and clusters of bacterial aggregations were common. Hemopoietic elements of bone marrow showed necrosis beside extensive depletion. It could be concluded that disseminated hemorrhages mainly in skeletal muscles and viscera beside lymphoid depletion of immune organs were responsible for high mortalities in CIA infected flocks which became higher in intensity in coinfection with IBDV. Presence of bacterial colonies in various examined organs following immunosuppression. Great economic losses from concomitant and subclinical infection by CIA beside carcass condemnation so, CIA vaccination of broiler chickens is recommended.

INTRODUCTION

High production pressure in poultry industry directly affected chickens which became more susceptible for many diseases, secondary infections and immunosuppression. This recent huge industry may be resulted in improper vaccination programs. Immunosuppressive viruses cause many clinical or subclinical conditions in

poultry **(Lütticken, 1997)** The mechanism of this state of immunosuppression, is due to immune failure that recognize numerous signals **(Babiuk et al. 2003).**

From these viruses Infectious Bursal Disease (IBDV**),** Chicken Infectious Anemia **(**CIAV**)** and Inclusion Body Hepatitis (IBHV) viruses cause severe economic losses **(Islam et al. 2002*).*** These three viruses cause destruction of lymphoid tissues, poor response and depletion of immune cells which responsible for this state of immunosuppression beside mortalities and areas of congestion and hemorrhage in muscles and visceral organs **(Dhama et al. 2002*).***

Chicken Anemia virus (CAV) is considered one of the smallest, non- enveloped, single stranded, circular negative sense DNA virus (Li et al., 2017a; Rosario et al. 2017). The virus DNA encodes viral proteins of VP1, VP2 and VP3 **(Ducatez et al. 2008)** belonging to the genus Gyrovirus of the family Cicroviridae, and has 23-25 nm in size. It causes Chicken Infectious Anemia (CIA) which is an acute highly contagious disease of young chickens, after an incubation period of 1 to 14 days with clinical symptoms in young chickens while it is a sub-clinical disease in older ones, which is the only natural host of the virus **(Cardona et al., 2000b).** CIA characterized by severe anemia, weakness, anorexia, ruffled feathers, stunted growth, generalized lymphoid atrophy and increased mortalities (5%-10%) up to 60% **(Todd 2004 Dhama et al. 2008 McNeilly et al. 1991).**

CIA is mainly noticed in broiler chicks of up to 3–4 weeks of age, which usually acquire the infection vertically **(Pope 1991 Todd 2000 Dhama et al. 2008)** but after 3 weeks of age the susceptibility to clinical disease decline. CIA infected birds developing an immunosuppression in the presence of other viruses such as Fowl adenovirus (FAV) (**Toro et al. 2001) and NDV (De Boer et al. 1994).** Beside attenuated immune response against several viral vaccines, resulting in vaccination failures **(Todd 2000 Schat 2003 Toro et al. 2006 Dhama et al. 2008).** Maternal antibodies prevent the clinical signs of disease but do not prevent infection and transmission of the virus or its immunosuppression effect **(Sommer and Cardona, 2003).** Among the histopathological changes, severe depletion of cortical thymocytes and erythroblastoid cells in the bone marrow which leads to immunodeficiency and anemia **(Noteborn and Koch 1995**). Diagnosis of CAV infection depends on history, clinical signs, hematological, pathological findings: gross and histopathological lesions which were confirmed by PCR as a developed tool of diagnosis (**Gowthaman et al. 2014 Al-Ajeeli et al. 2020).**

Infectious bursal disease (IBD) or Gumboro disease is an acute highly contagious disease of young chickens, characterized by severe immunosuppression in young chicks of 3-6 weeks of age **(Sachan et al. 2019).** Gumboro disease is caused by IBD virus (IBDV), is a nonenveloped double stranded RNA virus transmitted by oral fecal route **(Smith et al. 2015)**. IBDV is belonging to family Birnaviridae, genus Avibirnavirus, which is responsible for major economic losses in poultry industry worldwide.

The virus has two serotypes: serotype1 (pathogenic strain) which is classified into classical (intermediate and very virulent strains) **(Jayasundara et al. 2017)** and serotype 2(non- pathogenic one). IBDV causes cytolysis of dividing cells in the bursa of Fabricius in the infected chicks resulting in severe immunosuppression **(Ciccone et al. 2017**). This is severe state of immunosuppression due to apoptosis of B cell of the bursa and cytokine storm which considered the main factors of the severity of Gumboro that, may responsible for secondary infections beside failure of vaccination **(Cubas-Gaona, et al. 2018).** Gumboro disease is controlled either by live attenuated or inactivated (killed) IBDV vaccines which is preferred than live attenuated one. High levels of humeral immunity occurred by using killed vaccine for chickens at pre laying stage for inducing antibodies for a period of two weeks. Infectious Bursal Disease (IBD) causes a state of immunosuppression for infected chickens **(Hon et al. 2008**). Outbreaks occurred among the vaccinated flocks resulted in a chick that more susceptible to secondary infections by either

viral or bacterial agents (**Sachan et al. 2019).** Hemorrhages in skeletal muscles, high mortalities and poor weight gain that considered as fatal complications of the disease **(Zachar et al. 2016**). Clinical diagnosis of Gumboro (acute form) is based on disease evolution in the form of mortality peak followed by recovery within 5-7 days **(Van den Berg et al. 2000).** Also, noticed symptoms, characteristic pathognomonic lesions in bursa of Fabricius beside histopathological investigations together with demonstration of viral antigen by immunohistochemistry **(Balamurugan and Kataria, 2006).** RT-PCR allows rapid identification of IBDV virus **(Toroghi et al. 2003).**

Inclusion Body Hepatitis (IBH) is one of immunosuppressive disease of poultry, caused by Fowl adeno virus (FAV). IBHV is a nonenveloped virus belonging to the adenoviridae family **(Balamurugan et al. 2001**) which mainly affects hepatic, endothelial and lymphoid cells. This disease affects poultry of 3-6 weeks old chicks, characterized by sudden onset, enlarged and mottled friable liver, that showing intranuclear inclusion bodies (IBHs) in the hepatocytes **(Gowda and Satyanarayana, 1994)** with high mortalities. FAV and CAV are transmitted vertically **(Toro et al. 2001)**. There is a relationship between occurrence of IBH and presence of IBD and CIA viruses **(Shane and Jaffery, 1997).** FAV needs impairment of immune system of the bird to produce its pathogenic effect. So, IBD and CIA viruses predispose to FAV by their immunosuppression effect on chickens. Recently, IBH virus has reported as a virulent virus that is able to cause IBH as a primary disease without previous predisposing infection by IBDV and CIAV. **(Susantha et al. 2006).** Microscopically, it causes severe depletion of lymphocytes in medullae of bursal follicles. Molecular technique as PCR is a specific diagnostic method for demonstration of adenovirus **(Balamurugan and Kataria, 2006).**

The aim of the present study to focus light on etiology and pathologic findings of widespread hemorrhagic syndrome of broiler chickens among some flocks (foreign and native) breeds reared in Dakahlia, Egypt in the period between October 2019 to September 2020.

MATERIAL AND METHODS

Chickens

Three hundred chickens were examined from fifteen commercial broiler flocks; (7 Cubb and 8 Native sasso) breeds were used for detection of CAV and coinfection with IBDV and IBHV during October 2019 to September 2020 in this study. Broiler Cubb farms aged 3-6 and 4-8 weeks old for sasso farms in different localities of Dakahlia Governorate, Egypt. All chicken flocks had been vaccinated against IBD, Newcastle, Avian Influenza and Infectious Bronchitis diseases. All birds with a history of mild respiratory manifestations, off food, depression, diarrhea with morbidity rate (20-70) % and low mortalities were subjected to study. The selected flocks were reared under cage system with standard managemental conditions.

Hematological changes

Blood samples were collected from wing vein of diseased and control healthy birds in tubes containing anticoagulant (dipotassium salt of EDTA 1mg/ 1ml blood) for RBCs count and determination of hemoglobin contents and Packed cell volume (PCV) using the automatic cell counter system XT (2000 IX) according to **(Feldman et al. 2000).**

Detection of CAV DNA, IBDV RNA, and IBHV DNA by Conventional PCR

Twenty pooled tissue specimens represented five selected examined flocks (4 birds selected from each flock), including bursa of Fabricious (for detection of IBDV RNA), liver, thymus and spleen (for detection of CAV DNA and IBHV DNA). Specimens were homogenized and subjected for conventional PCR. Tissue samples transported on ice from field to Animal Health Research (Provinicial-Lab Mansoura) and were labelled and stored at -20 C until used for PCR testing for detection of presence of CAV, IBDV and IBHV genome in the Biotechnology unit Reference lab for veterinary quality control on poultry production (Animal health research institute, Dokki, Giza, Egypt).

Nucleic acid extraction. Whole nucleic acid extraction from samples was performed using the QIAamp minielute virus spin kit (Qiagen, Germany, GmbH). Briefly, 200 µl of the sample suspension was incubated with 25 µl of Qiagen protease and 200 µl of AL lysis

buffer at 56^{O}C for 15 min. After incubation, 250 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 100 µl of elution buffer.

Oligonucleotide Primers.

Supplied from (**Metabion Germany)** are listed in table (1).

Table (1). Primers sequences, target genes, amplicon sizes of different examined viruses

Target agent	Target gene	Primer sequence (5'-3')	Length of amplified product (bp)	Reference
Adeno	Hexon protein	CAA RTT CAG RCA GAC GGT TAG TGA TGM CGS GAC ATC AT	897 bp	**Gaba et al., 2010**
IBD	VP2	TCACCGTCCTCAGCTTACCCACATC GGATTTGGGATCAGCTCGAAGTTGC	620 bp	**Metwally et al., 2009**
CAV	VP1	CTAAGATCTGCAACTGCGGA CCTTGGAAG CGGATAGTCAT	418 bp	**Hussein et al., 2002**

PCR amplification.

IBD PCR: Primers were utilized in a 25- µl reaction containing 12.5 µl of Quantitect probe rt-PCR buffer (QIAgen, Gmbh), 1 µl of each primer of 20 pmol concentration, 0.25 µl of rt-enzyme 5.25 µl of water, and 5 µl of template. The reaction was performed in in an Applied biosystem 2720 thermal cycler. Reverse transcription was applied at 50 ^{O}C for 30 min, a primary denaturation step was done at 95 ^{O}C for 5 min, followed by 35 cycles of 94^{O}C for 30 sec., 59^{O}C for 40 sec. and 72^{O}C for 45 sec. min. A final extension step was done at 72^{O}C for 10 min.

CAV and Adeno PCR.

Primers were utilized in a 25- µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 5.5 µl of water, and 5 µl of DNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler. Primary denaturation step was done at 95 ^{O}C for 5 min, followed by 35 cycles of 94^{O}C for 30 sec., (Adeno: 57^{O}C for 40 sec; CAV: 50^{O}C for 40 sec) and 72^{O}C for 45 sec. min. A final extension step was done at 72^{O}C for 10 min.

Analysis of the PCR Products.

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 generuler 100 bp DNA ladder (Fermentas, Thermo) 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.

Macroscopic Examination.

Both freshly dead and diseased birds suffering from the clinical signs were subjected for necropsy and record gross lesions.

Microscopic Examination.

Specimens from bursa of Fabricus, thymus, spleen, liver, proventriculus, kidneys and skeletal muscles (breast and thigh) were collected from PCR positive cases then fixed in 10% Neutral Buffered Formalin solution, dehydrated in a gradual ethanol 70-100%, cleared in Xylene then embedded in Paraffin. Five microns sections were prepared then prepared and routinely stained with Hematoxylin and Eosin stain (H&E) according to **(Suvarna et al. 2013)** then examined microscopically.

Bone marrow is collected from the mid-shaft femur region of bird and the plug of marrow is submitted to microscopic examination by previously mentioned routine technique.

Statistical analysis

The data was statistically analyzed by using computerized SPSS program version 16 using one way ANOVA. The comparison of means

was carried out with Duncan's multiple range test (Tamhane and Dunlop 2000).

RESLTUS

Clinical signs

Generally, all commercial Cubb and Sasso chickens either infected with CIA and/or coinfection with IBDV had some mild signs or severe particularly in coinfected and complicated flocks. Clinically, birds suffered dullness, off food, ruffled feathers, diarrhea, sometimes accompanied with some respiratory manifestations and a high morbidity rate reached 70% with low mortality rate (5-10 %).

Detection of CAV, IBDV and IBHV viruses by Conventional PCR

CAV DNA was detected in the liver, thymus and spleen of all pooled tissues from the five selected flocks (5/5 flocks). IBDV RNA and CAV DNA coinfection were detected in pooled tissues from two selected flocks (2/5 flocks), on the other hand, chickens were found negative for IBHV DNA in all pooled liver, thymus and spleen tissue samples of the five tested flocks using PCR that resulted in PCR amplifications L [Gelpilot100 bp ladder (Qiagen, 100- 1000 bp]. (Fig. 1 a & b).

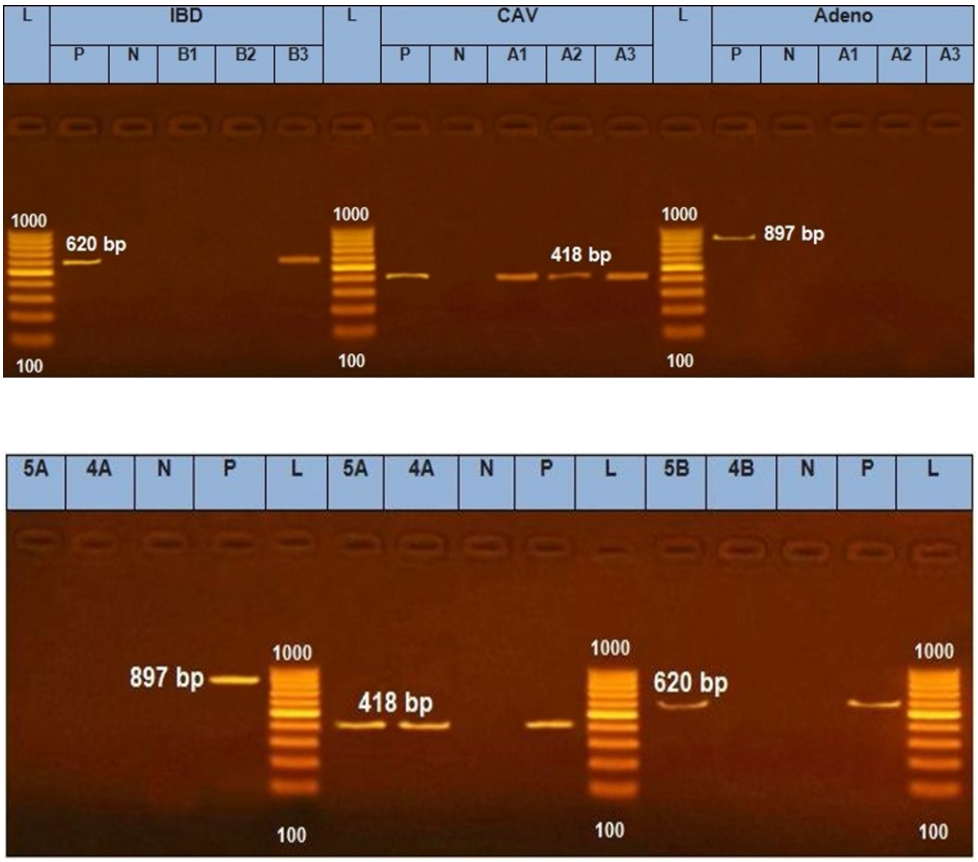

Fig. 1: (a & b): PCR results for CAV, IBDV and IBHV virulence gene showing positive amplifications of CAV gene in all tested samples, positive amplifications of IBDV gene in 2 pooled tested samples of 2 flocks and negative amplifications of IBHV gene in all tested samples. Positive amplifications and negative amplifications of different genes were reported. 1) CAV (418 bp), 2) IBDV (620 bp) and 3) IBHV (897 bp). L [Gelpilot100 bp ladder (Fermentas, Thermo, 100- 1000 bp].

A1, 2, 3, 4, 5 (liver, thymus and spleen samples)

B1, 2, 3, 4, 5 (bursa samples)

P (positive control) N (negative control)

Macroscopic Findings

Gross changes: Necropsied chickens infected with CAV showed emaciation, multifocal scattered muscular hemorrhages either on thigh (Fig. 2) or pectoral muscles (Fig.3) or both and some viscera and under skin. Atrophied and congested lymphoid organs (thymus, spleen and bursa of Fabricius) beside hemorrhagic areas (Fig. 4). Pale and enlarged liver contained greyish dark red areas on its surfaces. On the other side CAV coinfected with IBDV revealed more intense and severe hemorrhagic areas on breast and thigh muscles (Fig. 5). Kidneys showed diffuse hemorrhage, pale in color with distended ureters containing white materials. And the lymphoid organs appeared more congested with inflamed bursa of Fabricius (Fig. 6).

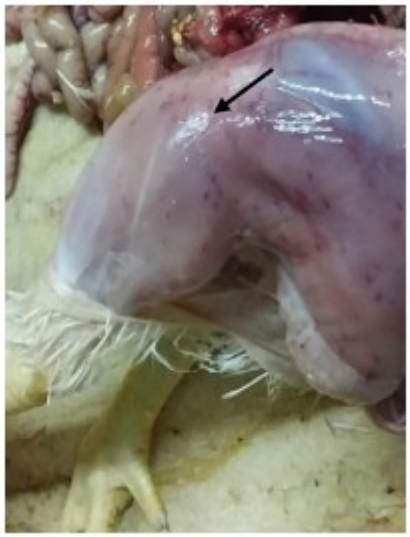

Fig. 2. Cubb chicken of 28 days old infected with CIA showing multifocal hemorrhage on thigh muscle

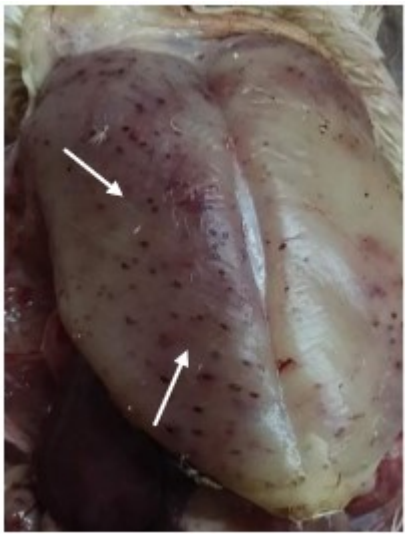

Fig. 3 Cubb chicken of 30 days old infected with CIA showing multifocal hemorrhage on pectoral muscles.

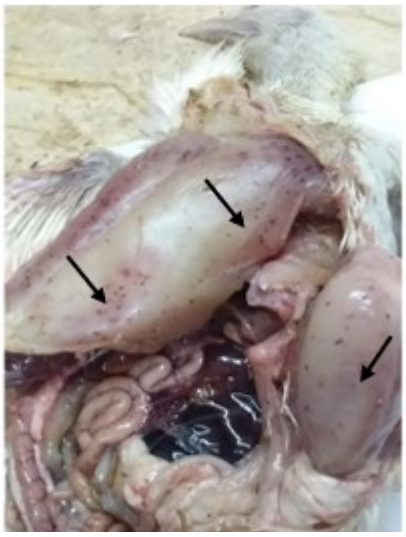

Fig. 4 Cubb chicken 22days old infected with CIA showing minute hemorrhage and atrophy of thymic lobules

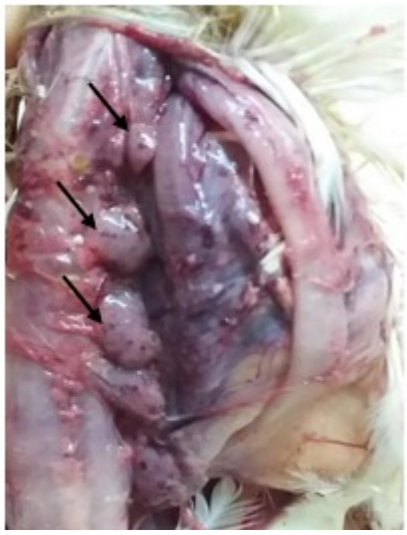

Fig.5 Cubb chicken of 32 days old coinfected with CIA and IBDV showing petechial hemorrhagic areas disseminated within the thigh and breast muscle.

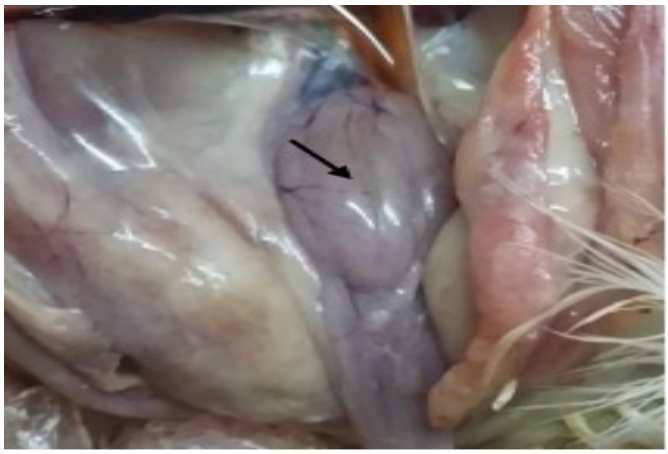

Fig.6 Cubb chicken of 35 days old coinfected with CIA and IBDV showing congested and inflamed bursa of Fabricius.

Hematological picture

Table (2) Hematological findings in blood of chickens either infected with CIA or coinfected with IBDV n=5

	RBCs count 10^6 /ul	Hb gm/dl	PCV %
Coinfection CIA +IBDV	2.00 ± 0.06	7.10* ± 0.01	29.35 ± 0.76
CIA	2.11* ± 0.04	7.70* ± 0.04	30.35 ± 0.22
Control gp (non infected)	2.50 ± 0.05	8.70 ± 0.05	35.01 ± 0.33

A significant decrease ($p< 0.05$) in RBCs count, Hb concentration and PCV in CIA and coinfected chickens which indicates anemia in infected chickens which follow bone marrow lesions and depression of hematopoietic centers by the virus.
* represents significant decrease than normal

Histopathological Findings:

Chicken Virus Anemia (CVA) Infection:

Liver: Disseminated hemorrhagic areas scattered in the hepatic parenchyma with congested hepatic blood vessels and sinusoids were seen (Fig.7). Some portal blood vessels had hyalinized wall and others contained bacterial emboli beside heterophilic infiltrations and necrotic or degenerative changes in the hepatic cells were common.

Skeletal Muscles: Recent or organized hemorrhagic areas with or without basophilic bacterial aggregations which usually encircled by fibrous tissue infiltrated by mononuclear cells were common in old hemorrhages were seen (Fig. 8). Focal myomalecia replaced by extravasated erythrocytes, degenerated heterophils, fibrin and necrotic debris were encountered. Intermuscular fat suffered from necrotic changes.

Lymphoid tissues: Spleen, Thymus, Bursa of Fabrecius exhibited intense lymphoid depletion, necrosis, atrophy together with edema, congestion and hemorrhages (Figs. 9&10). Proliferations of reticuloendothelial cells were common where sometimes appeared laden with hemosiderin could be seen. Clusters of basophilic bacterial aggregates and bacterial emboli were encountered in lymphoid organs (Fig. 11).

Bone marrow: Moderate depletion and atro-

phy of some hemopoietic centers with extensive replacement by fat cells were common (Fig.12). Sometimes regenerative attempts could be seen in some cases.

Coinfection of Chicken Infectious Anemia agent (CVA) and Infectious Bursal Disease Virus (IBDV):

The lesions in examined organs appeared more intense and wide disseminated in the examined organs and characterized by the following:

Liver: The hepatic parenchyma showed intense lesions characterized by portal edema, hemorrhages, congestions and leukocytic aggregates mainly lymphocytes (Fig.13). The latter may be extended to interlobular tissue. The adjacent hepatocytes suffered from various degenerative or necrotic changes.

Skeletal Muscles: The majority of extravasated blood became organized with hyaline degeneration or necrosis in the adjacent muscle fibers (Fig.14). Sometimes bacterial aggregations were detected in spaces between muscle fibers and intravascular with or without inflammatory cells mainly heterophils and lymphocytes.

Lymphoid Tissues: Bursal lymphoid follicles showed intense depletion and necrosis with presence of necrotic debris in the center (Fig.15). Hyperplastic epithelial covering with vesicle formation could be seen. Thymus and Spleen had necrotic lymphoid elements beside focal congestion and hemorrhages within the thymic lobules.

Bone Marrow: The hemopoietic elements suffered from necrosis and extensive depletion which appeared shrunken and replaced by fat cells (Fig.16).

Proventriculus: Partial loss of epithelial with intense hemorrhage in mucosa and submucosa (Fig.17). some compound glands showed necrosis beside lymphoid infiltrations.

Kidney: Pronounced hemorrhagic areas scattered in the renal parenchyma with necrotic renal tubules were common (Fig.18). Cellular and hyaline casts were seen inside the lumina of some tubules. Contracted glomerular tuft and proliferative mesangial cells were seen.

Photomicrographs of (H&E) stained sections from different organs of cubb and sasso chickens suffering from hemorrhagic syndrome:

Plate (1)

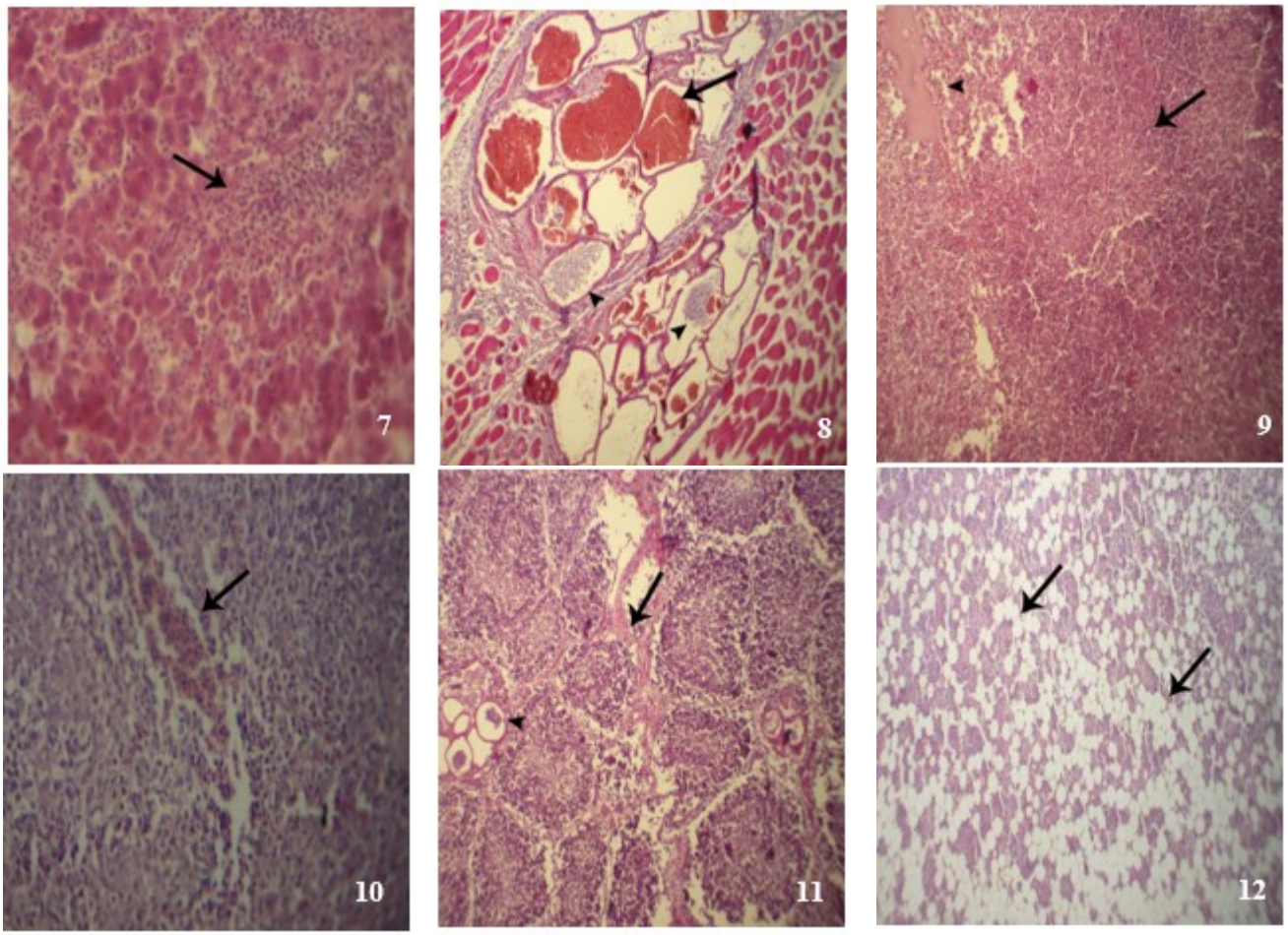

Fig.7: Liver of broiler chicken (CIA) showing disseminated hemorrhages (arrow) and hyperemic sinusoids within the hepatic parenchyma beside some inflammatory cell aggregations. H&E, X 400

Fig.8: Skeletal Muscles of chicken (CIA) showing hemorrhagic areas (arrow) and bacterial colonies aggregates (arrow head) encircled by fibrous tissue containing inflammatory cells. H&E, X400

Fig.9: Spleen of chicken (CIA) showing intense lymphoid depletion (arrow) and edema (arrow head) in the splenic tissue. H&E, X 200

Fig.10: Thymus of chicken (CIA) showing congestion and hemorrhages (arrow) with depletion and necrosis mainly in medulla of thymic lobules. H&E X400

Fig.11: Bursa of Fabricious of chicken (CIA) showing atrophied follicles with depleted medulla (arrow) beside bacterial aggregates (arrow head). H&E X200

Fig.12: Bone marrow of chicken (CIA) showing depleted and atrophied hemopoietic centers with partial replacement by fat cells. H&E, X 200

Plate (2)

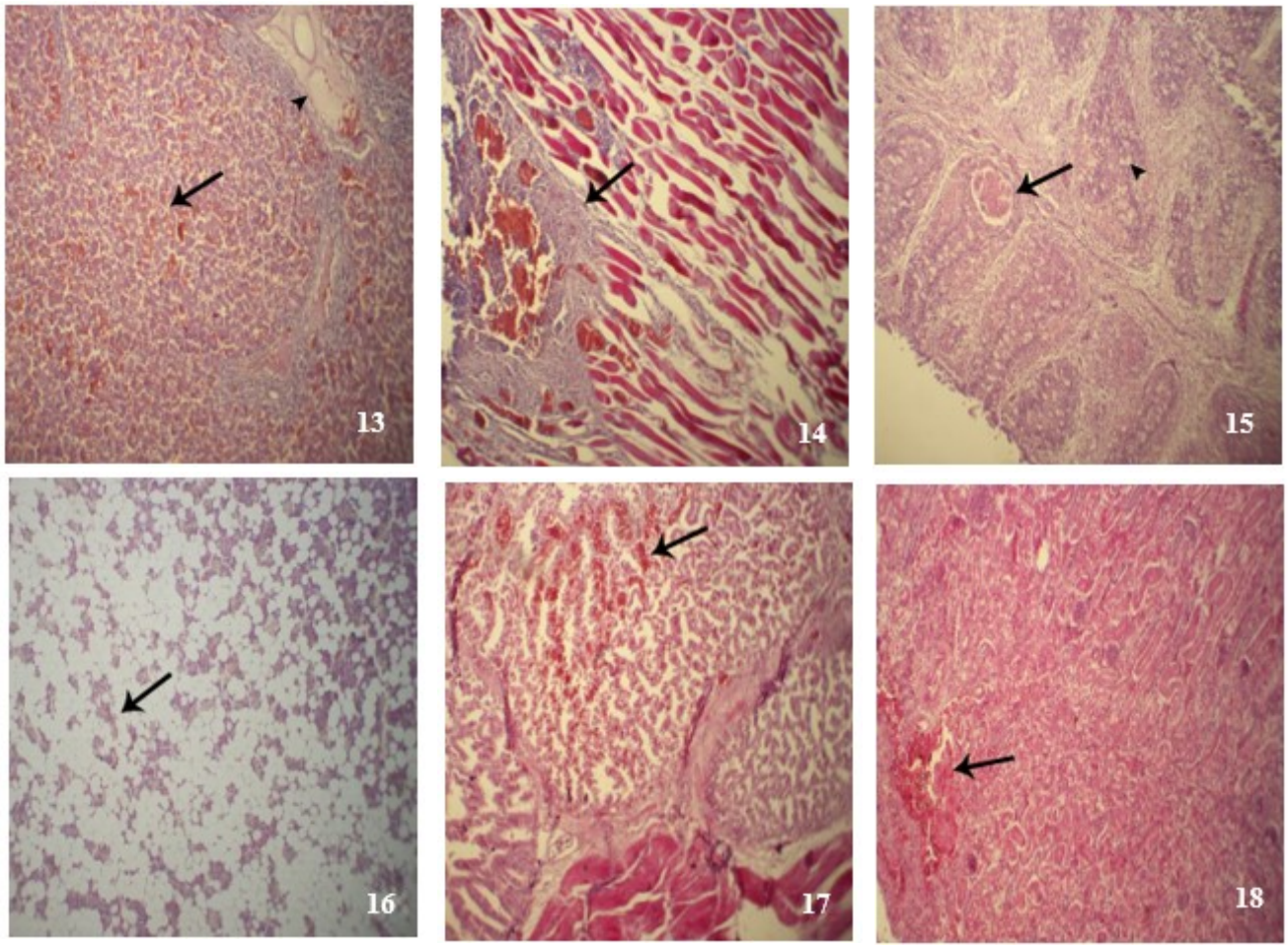

Fig.13: Liver of chicken (Coinfection CIA& IBD) showing severely congested sinusoids (arrow) with edema, hemorrhages (arrow head) and leukocytic aggregates in portal areas and interstatium. H&E, X 100

Fig.14: Skeletal Muscles of chicken (Coinfection CIA& IBD) showing organized hemorrhages (arrow). H&E, X 200

Fig.15: Bursa of Fabrecius of chicken (Coinfection CIA& IBD) showing necrotic debris in the center of some follicles (arrow) and minute cysts in the depleted follicles (arrow head). H&E, X 100

Fig.16: Bone Marrow of chicken (Coinfection CIA & IBD) showing extensive depletion and shrinkage of hemo-poietic centers (arrow) and replaced by numerous fat cells. H&E, X200

Fig.17: Proventriculus of chicken (Coinfection CIA& IBD) showing mucosal and submucosal hemorrhages (arrow). H&E, X200

Fig.18: Kidney of chicken (Coinfection CIA& IBD) showing hemorrhages and hemosiderosis (arrow) in the renal parenchyma. H&E, X200

Discussion

The present study revealed that all examined samples by conventional PCR of liver, thymus and spleen of broiler chickens were positive amplification for CAV gene while all of them were negative amplification for IBHV gene, while IBDV gene was detected in the bursa tissue samples from two tested flocks as coinfection of CAV. Gross lesions include emaciated carcasses beside congestion and atrophy of lymphoid organs accompanied with scuttered multifocal hemorrhagic areas in skeletal muscles and viscera. Moreover, pale bone marrow was seen.

Our results declared that Chicken infectious anemia (CIA) could negatively influence the immune system of chickens alone or when combined with infectious bursal disease (IBD) **(Balamurugan and Kataria, 2006)** with or without causing clinical disease that badly affects poultry production and industry because chickens need a healthy and responsive immune system **(Toro et al. 2009).**

PCR was used as an important diagnostic tool for CAV detection from chickens suspected to be infected by the virus **(AboElkhair et al. 2014 Al-Kateb et al. 2017).**

In the present study, CVA DNA was detected in all tested samples which revealed hemorrhagic syndrome in Cubb and Sasso breeds that indicates the high prevalence findings in samples screened by PCR **(Bhatt et al. 2011 Gowthaman et al. 2014).**

The clinical signs in investigated flocks which were characterized by depression, decrease food and water intake, diarrhea and 70% morbidity rate accompanied with low mortalities 5%-10%. Some flocks suffered some respiratory manifestations that is on line with that described by **(McNeilly et al. 1991; Bhatt et al. 2011).** It is worthy to mention that all recorded clinical signs appeared on foreign breed at 20-32 days while sasso (Native) breed showed manifestations during 25-45 days then decreased in severity later on. Our results agree with **(Hu et al. 1993a)** who attributed reduction of intensity to humeral immunity and the ability of birds to produce antibodies to the virus.

The results of erythrogram indicate significant reduction in RBCs count, Hb and PCV which reflects bone marrow picture which usually follow hemopoietic centers destruction and lymphoid organs depression by CIA virus. These changes became pronounced in chicks coinfected with CIA and IBDV virus and indicates greater effect of the virus upon erythropoiesis and immune organs. Our results are in agreement with **(Shahira and Dalia 2019).** The hematological pictures were in accordance with microscopic picture of bone marrow lesions in CIA infected chickens and other coinfected birds.

Macroscopically, the general gross lesions represented by intense atrophy of lymphoid organs (thymus, spleen and bursa of Fabricius) accompanied with pale bone marrow seen mainly in emaciated carcasses. Loss of body weight may have a potential contribution of lymphoid organs to immunosuppression pathogenesis by decreasing proliferative activities of T and B lymphocytes in immune organs which led to a significant decrease in immunoglobulin (IgG, IgM and IgA) levels in all body fluids as described by (**Dhama 2002).** Severe multifocal muscular hemorrhages in skeletal muscles which presented also, under skin and allover internal organs beside hemorrhagic proventriculitis. Hemorrhages, was resulted from primary destruction of thrombocytes which led to thrombocytopenia (impairment of clotting process) as described by **(Pope 1991).** Also, apoptosis of thrombocytes causes a decrease in thrombocytes as mentioned by **(Toro et al. 2009).**

Regarding to microscopical lesions of CIA, our results conclusively showed lesions mainly in bone marrow, thymus, bursa of Fabricius, spleen, liver and skeletal muscles. Thymus showed lymphoid cell depletion specially in thymic cortex beside necrosis and hemorrhages, these findings were in agreement with that described by (Dhama 2002). On the other hand, lymphoid depletion due to apoptosis of lymphocytes in thymic cortex by VP3. However, CAV has 3 viral proteins (VP). Vp1 is the only protein in the virus particles. VP2 needed for the proper folding of VP1. VP3 causes apoptosis in the infected cell. CAV can only replicate in the dividing cells because it does not cod the enzymes needed for DNA replication. Target cells of CAV are hemocytoblasts, in bone marrow, T lymphocyte precursor and

mature cells. Infection of these target cells by the virus resulted in apoptosis causing cell depletion of lymphoid organs which results in impairment of innate and acquired immune response as mentioned by **(Toro et al. 2009).**

Our histopathological findings in flocks coinfected by CIA and IBDV were severe. In our view, this severity of lesions was due to secondary bacterial infections as mentioned before. However, it could be argued that apoptosis of heterophils and thrombocytes cause a decrease in both two cells which are important phagocytic cells and cytokines production which lead to increase bacterial infections. Thrombocytes plays a key role in thymus atrophy resulting in lack of T helper (Th) cells and cytotoxic T lymphocytes (CTL). Generally, any immune suppressive agent can influence the acquired humeral immunity development against CAV which prolong viral replication and induce massive and severe damage to the immune system as mentioned by **(Toro et al. 2009).**

Balamurugan and Kataria (2006) and Toro et al. (2009) considered that the infection by IBDV or vaccination with hot vaccines increase the cell death of lymphoid cells in bursa of Fabricius (IgM cells which serve as targets for the IBDV). The latter usually led to lymphoid depletion then final destruction of the bursal follicles which considered the pathognomonic lesion of IBDV. The later usually led to infection. This bursal atrophy that increasing the susceptibility to other secondary bacterial infections as mentioned by (**Arafat et al. 2017).** However, this is confirming the potential contribution of IBDV as an important risk factor that negatively affecting the immune responses to other pathogens including CAV and increasing clinical signs (anemia) and mortalities.

Conclusion

The current results explained that infection of CAV either alone or in combination with IBDV (infection or hot vaccines) cause massive economical losses in poultry industry as a result of immunosuppression and severe hemorrhages. Also, our results suggest that the protection of chickens either Foreign or Native breeds in Egypt, achieved by vaccination against CIA which is considered an important and necessary tool to overcome this economical problem.

Furthermore, most broiler chickens have maternal immunity (virus- neutralizing antibodies) against CAV either from natural infection or vaccination of parent flock protecting them against infection until 2-3 weeks of age that explains subclinical infections occurring after maternal antibodies had weaned.

Therefore, CAV vaccine might help in disease control and interfering with this problem in short-lived chickens (broilers and sasso breed) in the field.

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