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Avian Disease

Efficacy of The Turkey Herpes virus-IBDV Vector Vaccine Against Recent Egyptian Very Virulent IBDV in Commercial Layers

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ABSTRACT

The infectious bursal disease virus (IBDv) is widespread in poultry flocks all around the world. During the period between 2016- 2017, 22 IBD outbreaks were investigated in El-Minoufiya Governorate in different chicken sectors (11 broilers, 7 commercial layer pullets and 4 native baladi varieties). The disease occurred at 23-42 days of age. The mortality ranged between 3-9 % in commercial broilers farms, 5.8-12% in native breed varieties and 5-10 % in commercial layer replacement pullets. Two hundred one-day-old commercial male layer chicks was used for assessment of protection obtained after vaccination with HVT-IBDV vector vaccine "Vaxxitek" against challenged with vvIBDV local field isolate "Lay./Men.Egypt/17" at 46th-day of age. Maternal derived antibodies (MDA) was followed up by ELISA to determine MA waning and the suitable age of chicks for IBDV vaccination and/or challenge. Chicks divided into 4 groups of (50 birds/ of each), G1 (vaccinated with HVT-IBD vector vaccine at 1 day old of age and non-challeneged) and G2 (vaccinated with HVT-IBDV at one-day of age and challenged with vvIBDV local field isolate "Lay./Men.Egypt/017" at 46-day of age), G3 (non- vaccinated and non-challenged) and G4 (non-vaccinated and challenged chickens). Our results revealed that the mortality percentage of commercial male layer chickens challenged with recent vvIBDV local field isolate "lay./Men.Egypt/017" was (0%) in G2, while in G4 was (90%), at 7-days post challenge. The highest bursa body ratio observed was (5.32 and 5.31) in non- vaccinated and non-challenged birds (G3) and birds vaccinated with the VAXXITEK HVT-IBDv vaccine (G1). Conclusion, the recombinant VAXXITEK HVT-IBDv vaccine has provided protection for commercial male layer chicks against challenge with recent vvIBDv isolate. Vaccination with vector vaccine in endemic areas with vvIBDV, protect against mortality, partially against bursal atrophy and decreases immunosuppressive effect of vvIBDV.

Keywords: *HVT-IBD vector vaccine, vvIBDV and Infectious bursal disease (IBD).*

INTRODUCTION

Infectious bursal disease (IBD) is one of the most economically important contagious diseases of young chickens (3-6 weeks-old) caused by infectious bursal disease virus (IBDV) and characterized mainly by hemorrhagic syndrome, severe damage in bursa of fabricius, immunosuppression and high mortalities (Eterradossi and Saif, 2013). The economic importance of this disease is manifested in 2 ways. First losses due

to morbidity and mortality as some virus strains may cause up to 60% mortality in chickens with 3 weeks of age and older. The second, and more important, manifestation is a severe, prolonged immunosuppression of chickens infected at an early age (Tippenhauer et al., 2012; Cubas-Gaona et al., 2018 and Gimeno and Schat, 2018).Variant and vvIBD viruses are the most important antigenic mutants of IBDV that threaten chickens, causing high economic losses and vaccination failure because their irreversible immunosuppressive effect on the young chicks (Withers et al., 2005). The IBDV is a single-shelled nonenveloped with a diameter of 65–70 nm, double-stranded bisegmented linear RNA virus that belongs to Birnaviridae family, genus Avibirnavirus (Fauquet et al., 2005). Its genomic RNA consists of segments A that codes to polypeptides cleaved into two structural proteins, VP2 and VP3, a serine protease, VP4 and a non-structural VP5 while the smaller segment B encodes VP1 (Durairaj et al., 2011). The VP2 is responsible for serotype specificity; conversely, VP3 is a group-specific antigen that is recognized by non-neutralizing antibodies, which may cross-react with both serotypes (Oppling et al., 1991).Two different serotypes of Infectious bursal disease virus (1, 2) (McFerran et al., 1980). The only pathogenic to chickens was serptype-1 which differed obviously in their virulence and pathogenicity (Winterfield and Thacker, 1978). Different Modified Live Vaccines (MLVs) containing classical viruses are commercially available and are classified according to their degree of attenuation as "mild", "intermediate", "intermediate plus" and "hot" IBD vaccines (Saif, 1998; van den Berg, 2000 and Muller et al., 2003). Determination of the proper time of vaccination of young maternally immunized chicks with live attenuated IBDV vaccine consider major problem , whereas monitoring antibody levels in a breeder flock or its progeny (flock profiling) can aid in determining the proper time to vaccinate (De Herdt et al., 2005; Block et al., 2007 and Eterradossi and Saif, 2013). With the advancement of technology, next-generation vaccines have been developed with the advantage of Maternally derived antibodies (MDAbs) and are commercially available in the market such as the IBD vector vaccine using turkey herpes virus (HVT) as a vector for the IBDV viral protein 2 (VP2) gene (Bublot et al., 2007;Le Gros et al., 2009; Rojs et al., 2011and Dacic et al., 2018), and the Immune-complex vaccine that is a mixture of of a certain amount of IBDV-specific antibodies obtained from the sera of hyperimmunized chickens and infectious IBD vaccine virus (Whitfill et al., 1995 and Zahid et al., 2017).

In Egypt, IBD was diagnosed for the first time on the basis of its characteristic pathological lesions at 1974 (El-Sergany et al., 1974). The interest with antigenic characterization of IBDV was triggered by the appearance of very virulent infectious bursal disease virus (vvIBDV) strains in vaccinated Egyptian flocks (Khafagy et al., 1991). The vvIBDV strains are antigenetically very similar to the classical ones with a marked increase in virulence and break through high levels of maternal antibodies leading to great economic losses in chicken farms (Xiumiao et al., 2012). Presently, the presence of both very virulent and variant IBDV strains in Egypt were reported in several studies and become serious problems circulating in chickens vaccinated with the classical strain vaccines (Metwally et al., 2009; Mohamed et al., 2014; Mawgod et al., 2014). Thus, in present study we investigated recent IBDV outbreaks in El-Minoufiya Governorate, by agar gel precipitation test (AGPT) for detection of IBDV antigen in the cloacal bursa of the affected chickens then RT-PCR assay was applied. Also, evaluation of the efficacy of HVT-IBDv vector vaccine "Vaxxitek" against challenge with vvIBDV local field isolate "Lay./Men.Egypt/17"at 46-day of age was done.

MATERIALS AND METHODS

1- Samples collected for IBDV detection:

Postmortem examination was performed on a variable number of freshly dead birds, which succumbed to Gumboro after onset of mortalities on the examined farms. Bursa of fabricius were collected and kept at -20oC till be used.

2- IBD virus propagation and titration in embryonated chicken eggs:

Specific pathogen free (SPF) embryonated chicken egg (ECE):

A total of 60 SPF-ECE were obtained from (KoomOshiem, Fayoum, Agriculture Research Center- Ministry of Agriculture, Egypt). The fertile chicken eggs were kept in incubator at 37C° till the age of 9-11day old and inoculated Via Chorioallantoic membrane (CAM) route for propagation and titration of the very virulent local field

IBDV isolate "Lay./Men.Egypt/17" Kindly supplied from Dr.Hesham Sultan prof. of poultry disease department of birds and rabbits medicine faculty of veterinary medicine Sadat city university to be used for challenge in vaccination experiments.

3- Chickens:

Two hundred one-day-old commercial male layer chicks (Hy-line) were obtained from a commercial hatchery. Chicks possess MDA against IBDV, acquired from their parents that were vaccinated with live and inactivated oil emulsion IBDV vaccines according to a specific vaccination program. Serological fol-low up of MDA using ELISA to determine MA waning and the suitable age of chicks for IBDV vaccina-tion and/or challenge also assessment of their protection after vaccination with HVT-IBD vector vaccine "Vaxxitek" against challenge with vvIBDV local field isolate "Lay./Men.Egypt/17".

4- Reference antigens, antisera, vaccines, and viruses:

-The reference positive and negative precipitating antigensbesidepositive and negative precipitating reference antisera against IBDV were obtained from Intervet, Inter. B. V. Boxmeer, Holland for the AGPT.

- HVT-IBDV vaccine "Vaxxitek®" (Merial Limited, Duluth, GA), in Egyptian market was obtained from local agencies (IFT) and was used in the vaccination program studies.

- A local field isolate of vvIBDV designated as "Lay./Men.Egypt/17", in the form of bursal extract was diluted 1:10 in phosphate buffer saline, Kindly supplied from Dr.Hesham Sultan prof. of poultry disease department of birds and rabbits medicine faculty of veterinary medicine Sadat city university, to be used for challenge in vaccination experiments.

5- Laboratory vaccination experiments:

Two hundred of one-day-old commercial male layer chicks from one hatch was used for determination of serological response and degree of protection of commercial layer chicken vaccinated with Vaxxitek vaccine and challenged at 46-day of age with vvIBDV local field isolate "Lay./Men.Egypt/17". The MDAbs' waning in these chicks were followed from one-day-old until 53-day of age by ELISA. The chickens were divided into 4 groups which were treated as shown in **table (1).**

6- Blood samples:

Chicken blood were collected from wing vein or by slaughtering and kept in slop position at 37C for one hour then at 4C overnight. Sera then separated by centrifugation at 3000 rpm/10 minutes and stores at -20 C till tested. Sera were inactivated at 56 C for 30 minutes before testing.

7-Agar gel precipitation test:

IBDV antigen for AGPT was prepared according to **Hirari and Shimakura (1974).** Agar gel medium (2% DIFICO Laboratories Detroit, Michigan, USA) was prepared as described by **Wood et al. (1979).** The test was used to demonstrate the presence of antibodies to 1BDV in examined chicken sera and for detection of 1BDV antigen (s) in the bursa of affected chickens as described by **Wood et al. (1979).**

8- RNA extraction from bursal homogenate of the selected samples:

Extraction of genomic RNA: The viral RNA was extracted from bursal homogenates by Viral Gene-Spin ™ Viral DNA/RNA Extraction Kit (iNtRON Biotechnology) according to the instructions of the manufacturer. Viral Gene-Spin ™ Viral DNA/RNA Extraction Kit contains; Lysis buffer, Binding Buff-er, Washing Buffer A, Washing Buffer B, Elution Buffer and Spin Columns.

Amplification of Vp2 gene of IBDV: The one-step RT-PCR was conducted with Verso™ 1-step RT-PCR Kit (Thermo scientific) according to the manufactures instruction with RT-PCR thermal cycling program according to **Dolz et al. (2005).** The RT-PCR product was analyzed with a garose gel electrophoresis using 2 % agarose gel stained with ethidium bromide.

IBDV specific primers

Two primers were used to amplify 480 bp specific fragment in the VP2 gene of IBDV **Dolz et al. (2005)** . The primers sequences were as following:

Forward primer

5` ACAGGCCCAGAGTCTACA 3` was located at nucleotide sequence 733-750

Reverse primer

5`AYCCTGTTGCCACTCTTTC 3` was located at nucleotide sequence 1194-1212

9- Enzyme Linked Immunosorbant Assay (ELISA):

ELISA kits (ProFlock® IBD plus and classic IBD supplied by Symbiotic Corporation, 11011 via Frontex, San Diego. CA 92127) were employed for detection of IBD antibodies in chicken sera of

experimental purpose as well as measuring MAbs'.

10-Histopathological examination:

Bursae of Fabricius of experimentally infected and control birds, were fixed in neutral buffered 10% formalin solution**. Bancroft et al. (1996)**

11- Statistical analysis:

Whenever necessary, data were analyzed by students T-test or by analysis of variance ANOVA test followed by application of Dunean's new multiple range test to determine the significance of differences between individual treatment and corresponding control.

RESULTS

Results of serological response of commercial male layer chickens vaccinated with HVT-IBDV at one-day of age:

The waning of maternal antibody in commercial male layer chickens used for vaccination experiment judged by (IBD plus and classic IBD) ELISA titers, which were highest at one-day of age (20090, 9093) and decrease till (400, 150) at 53-day of age and also judged by AGPT, which were 6/10(60%) at 11-day of age and decreased till 0/5(0%) at 25-day of age, (Table 2). Assessment of antibody response of commercial male layer chickens vaccinated with HVT-IBDV vaccine at one-day of age, (G1) and non-vaccinated chickens, (G3) judged by (IBD plus and classic IBD) ELISA titers, were (17872 and 5150) and (17032 and 6227), respectively, at 11-day of age, while at 18-day of age were (14845 and 3362) and (13225 and 3332), respectively, and also judged by AGPT, which were 4/5(80%) and 3/5(60%), respectively, at 11-day of age, while at 18-day of age were 2/5(20%) and 1/10(10%) in G1 and G3, respectively, (Table 3). Serological response weekly examined from age 18-day until age 42-day of commercial male layer chickens in (G1) and (G3), judged by (IBD plus and classic IBDV) ELISA titers, were (10957 and 2526) and (10638 and 2900) at 25-day of age, respectively, while at 32-day of age were (7733 and 2351) and (7196 and 2096), respectively, also at 42-day of age were (10542 and 2505) and (2500 and 1000), respectively, and at 46- day of age were (12636 and 3934) and (950 and 370) in G1 and G3, respectively. Serological response also judged by AGPT, which were 2/5(40%) and 0/5(0%), respectively, at 25-day of age, while at 32-day of age were 3/5(60%) and 0/5(0%), respectively, also at 42-day of age were 9/10(90%) and 0/10(0%), respectively, and at 46-day of age were 5/5(100%) in G1 and 0/5(0%) in G3, (Table 4 and figure 1). The Bursal body weight ratio (B/BR), Bursal index (BI) and Mean severity index (MSI) of G1 and G3 at 42-day of age (3-days before challenge) were (4.75 and 6.25), (0.76 and 1) and (0.4 and 0) respectively, (Table 5 and figure 2). IBDV by RT/PCR detected in bursae of commercial male layer chickens at 42-day of age were 5/5(weak positive) and 5/5 (negative) in G1 and G3, respectively, and by AGPT were 0/5(0%) in G1and G3.

Results of serological response and degree of protection of commercial male layer chickens vaccinated with HVT-IBDV challenged at 46-day of age during 3- and 7-days post challenge:

The detection of IBDV by RT/PCR at 49-day of age (3-day post challenge) in bursae of commercial male layer chickens groups were 5/5 (positive) in G1, chickens group vaccinated with HVT-IBDV at one-day of age and challenged with vvIBDV local field isolate "Lay./Men.Egypt/017" at 46-day of age (G2) and non-vaccinated challenged chickens (G4) while RT/PCR was 5/5 (negative) in G3. The detection of IBDV by AGPT were 0/5 (0%) in all groups except (G4) was 5/5 (100%), (Table 6).

Thebursal body weight ratio (B/BR) of commercial male layer chickens in G1 and G3 were (4.52 and 5.3) respectively versus (4.66 and 6.1) in G2, and G4, respectively, at 3-dpc, The Bursal index (BI) in G1 and G3 were (0.85 and 1), respectively, versus (0.87 and 1.15) in G2 and G4, respectively, at 3dpc,The Mean severity index (MSI) in G1 and G3 were (0.2 and 0), respectively, versus (1.2 and 2.75) in G2 and G4, respectively, at 3dpc, (Table 7 and Figures 3-5). The mortality percentage of commercial male layer chickens challenged with recent vvIBDV local field isolate "lay./Men.Egypt/017" was (0%) in G2, while G4 was (90%), at 7-days post challenge, (Table 8 and Figures 6-8).

Serological response of commercial male layer chickens at 7-dpc judged by (IBD plus and classic IBDV) ELISA titers, were (10191 and 3963) and (400 and 150) in non-challenged G1 and G3 respectively versus (10989 and 10224) and (5490 and 17414) in challenged G2 and G4, respectively. While detection of IBD antigen in serum samples by AGPT were 5/5(100%) in all groups except G3 was 0/5 (0%), as shown in table (9).

IBDV detected by RT/PCR in bursae of commercial male layer chickens in all groups were5/5 (positive) except G3 was 0/5 (negative).The detection of IBDV by AGPT in all groups was 0/5 (0%), as shown in table (10). The bursal body weight ratio (B/BR) of commercial male layer chickens were (5.31 and 5.32) in non-challenged groups G1 and G3, respectively versus (4.89 and 1.36) in challenged groups G2 and G4, respectively, at 7-days post challenge with vvIBDV local field isolate "lay./Men.Egypt/017", The Bursal index (BI) were 0.99 and 1 in non-challenged groups G1 and G3, respectively, versus 0.91 and 0.25 in challenged groups G2 and G4, respectively at 7dpc, The Mean Severity index (MSI) was 0 in non-challenged groups G1 and G3 versus 0.5 and 3.5in challenged groups G2 and G4, respectively, at 7dpc, (Table 11; figures,. 9-12). The histopathological Bursal changes at 7^{TH} days post challenge of G2 showed hyperplasia and metaplasia of lining epithelium but G4 showed characteristic starry sky appearance due to depletion of lymphocytes in both cortex and medulla, all results compared to apparently normal architecture at non- vaccinated non-challenged (G3).

DISCUSSION

Our study was directed to evaluate the efficacy of HVT-IBDV vector vaccine in commercial layers against challenge with recent vvIBDV isolate, based on clinical signs, mortality percentage, postmortem gross lesions, detection of IBDVantigen (s) in the cloacal bursa of dead birds, Bursa body weight ratio, bursal index and histopathological examination. Very virulent infectious bursal disease (vvIBDV) charac-terized by immunosuppression or mortality in immature chickens at age of 3-6 weeks of age, the main target of the virus is Bursa of Fabricius causing severe depletion of immature B lymphocyte (Eterradossi et al., 2004). Acute IBD cause up to 30 and 90 % mortality in broiler and pullet flocks, respectively (Chettle and Wyeth, 1989 and Van den Berg, 2000), VvIBDV strains succeeded in surviving in the Egyptian farms despite of the application of intensive vaccination programs (Sultan, 1995; El-Khayat, 2003 and Sultan et al.,2011). IBDV was detected in 11 commercial broilers, 4native baladi variety and 7 commercial layers farms distributed in El-Minoufiya governorate. The incidence of the disease ranged between 23-42 days of age and was characterized by typical clinical signs and gross lesions, similar to those described by (Box, 1989; Sultan, 1995; Hassan et al., 2002 and Abd El-Razik, 2008). The course of the disease was acute lasting 7-10 days with most of the mortalities occurred within 3-5 days after the onset of the disease. Most affected birds showed profuse watery yellowish diarrhea with soiled vent feathers, vent picking, anorexia, depression, trembling, prostration and finally death. The gross lesions were mainly dehydration, extensive hemorrhage on the muscles of the thigh and breast, swollen pale or congested kidneys with prominent tubules and distension of urates. The BF was constantly involved and was either enlarged, edematous yellowish pink or hemorrhagic and contained blood in the lumen or atrophied bursae and hemorrhages at the junction between proventriculus and gizzard, these data are in agreement with these reported by (El-Khayat, 2003; Abd El-Razik, 2004; Jeon et al., 2008 and Shaimaa, 2008). The same observations were reported by (Giambrone et al., 1982; Solano et al., 1985 and Khafagy et al., 1990) and Sultan (1995) who reported that all investigated flocks had been vaccinated several times between 9-34 days by various classes (intermediate and/or intermediate plus) of standard serotype1 live IBDV vaccines.Although all the chickens had been vaccinated against IBDV, high mortalities were recorded, ranged between 3-9% (average 4.7%) in commercial broilers, 5.8-12% (average 8.5%) in native breed varieties, and 5-10% (average 6.87 %) in commercial layer replacement pullets farms. These data agreed with previous studies done by (El-Khayat, 2003; Aly et al., 2004; Rautenschlein et al., 2005; Abd El-Razik, 2008; Sultan et al., 2009 and Jackwood, 2011). The possible causes of vaccination failure could be due to the high field virus exposure, timing of IBDV vaccination, error in application of the vaccines and IBDV challenge strain. All investigated flocks were progenies of different parent flocks vaccinated with live and inactivated oil-emulsion IBD vaccines, so these progenies are expected to carry variable levels of maternal antibodies which may interfere to a great or less extent with vaccination particularly at an early age (Ismail and Saif, 1990; Khafagy et al., 1990 and Jackwood, 2011).In addition, results of the current study go in parallelism with the report of (Anon, 1990; Khafagy et al., 1990 and Juneja et al., 2008) who reported that IBDV was detected by

AGPT which is the most practicable and economic method could be used for detection of IBDV specific antigen in extracts from bursal tissues or group specific antibodies. AGPT was applied on bursal homogenates collected from both acute and sub clinical affected birds which appeared as a precipitation lines the successful use of the AGPT as a diagnostic means for IBDV antigen in the cloacal bursa of acutely affected birds. The development of precipitation lines was suggested by Hirai and Shimakura (1974) may be attributed to the difference in the diffusion rates of IBDV antigens. Reverse transcriptase polymerase chain reaction (RT/PCR) considered the most rapid, sensitive and accurate method for detecting IBDV these assays detected nucleotide segments specific to viral genome. By using RT/PCR assay it became possible to detect and classify IBDV to use such information in studying the epidemiology of the viruse (Abdel-Alim and Saif, 2001; Corley et al., 2002; Pages et al., 2004 and Sreedevi and Jackwood, 2007). In the vaccination-challenge experiment we evaluated the efficacy of HVT-IBDV vector vaccine in commercial layers against challenge with recent vvIBDV isolate. The serological response has been tested using two ELISA kits, the classic IBD kit and an "improved" kit, the BD-plus kit. Classic IBD kits uses an antigen derived from a classical strain grown in tissue culture while IBD plus kits uses a native bursal derived classical strain antigen IBD plus test allows a more accurate detection of IBDV protective VP2 antibodies. Waning of MDA antibodies were more detected at 25-32 day of age using the IBD plus kit that confirm the sensitivity of this kit (table 2) agreed with (Prandini et al., 2008 and Le Gros et al., 2009). These results suggested that the serological examination of optimum vaccination for each flock is required to effectively control IBDV in the field (Tsukamoto et al., 1995). There were detectable positive antibody response measured by (IBD plus and classic IBD) ELISA mean titers on day 42 day of old in commercial male layer chickens vaccinated on day one with HVT-vector (table, 4) concerning HVT-IBDV vector vaccine,VP2 protein, containing most of the neutralization sites, is the primary host-protective immunogen of IBDV and has been the target protein for recombinant vaccine studies using a variety of different expression systems (Darteil et al., 1995; Tsukamoto et al., 1999; Butter et al., 2003; Prandini et al., 2008 ; Pradhan et al., 2012; and Huang et al., 2004). Our results agreed with Darteil et al. (1995) Who reported that expression of vp2 protein by an HVT recombinant virus was able to induce production of anti-vp2 neutralizing antibodies in chickens, in the same context, Bublot et al. (2007) who compared an IBD vectored vaccine (vHVT13), in which turkey herpesvirus (HVT) is used as the vector, demonstrated that this vaccine is able to induce an immune response in birds with a high level of MDA. Moreover, Rojs et al. (2011) studied the efficacy of three commercially available vaccines against infectious bursal disease (IBD) in commercial broilers raised in a high (IBDV) risk area. Three broiler flocks were vaccinated subcutaneously with a turkey herpesvirus (HVT)-IBD vector vaccine at one day old and conducted a significant increase in antibody titers detected in flocks vaccinated with the vector vaccine indicated its ability to induce an immune response in birds with a high level of maternally derived antibodies. There was complete protection against mortality in vaccinated challenged group G2 versus 90% of mortality in non-vaccinated challenged chicken G4 (Table 8 and figure 6), these results are agreed with Darteil et al. (1995) who was the first reported (100%) protection against IBDV challenge after vaccination of 1-day- old chickens with a single injection of HVT recombinant vaccine. In the same context, Bublot et al. (2007) who used an IBD vectored vaccine (vHVT13), in which turkey herpesvirus (HVT) is used as the vectordemonstrated that this vaccine is able to protect chickens against various IBD virus (IBDV) challenge strains including very virulent, classical, and USA variant IBDV, also similar results obtained in a recent study (Dačić et al., 2018). IBDV causes acute lytic infections and high titers of anti-IBDV antibodies, it replicates in actively dividing IgM^+ B cells in the bursa of Fabricius. results in lymphoid depletion and severe atrophy of the bursa as the predominant feature of the pathogenesis of this disease (Van den Berg, 2000 and Ratuenschaleine et al., 2005) and so good vaccine should be evaluated on the basis of protection of bursa from depletion and atrophy these parameter were included in our experiments. The results of the bursal body weight ratio (B/BR), Bursal index (BI) and the mean severity Index (MSI) noticed on 42-day of age (3-days before challenge), at 49-day of age (3-day post

challenge with vvIBDV local field isolate "lay./Men.Egypt/017") and at 53-day of age (at 7-days post challenge), results indicating that vaccination with HVT-vector vaccine protect chicks against bursal atrophy and damage (Tables, 5, 7, 11 and figures 9-10). It agreed with Perozo et al. (2009) that reported the recombinant HVT-IBDV confers protection against bursal damage as indicated by significantly lower bursal lesion scores in the vaccinated birds. These results were expected because the viral vector of the vaccine, by loading only the gene of immunogenic protein of IBDV, is not able to synthesize virulent viral particles, and therefore it does not cause infection and lysis of B-lymphocytes (Bublot et al., 2007) .In the same context Roh et al. (2016) demonstrated that the HVT-IBD vector vaccine is recommended as the vaccine of choice to avoid the safety problems associated with persistent bursal lesion and atrophy. Moreover, the result of our study agreed with Dačić et al. (2018) that reported the recombinant vaccine provided protection against bursal atrophy compared to liveintermediate vaccines.

CONCLUSION

The protective efficacy of the recombinant VAXXITEK HVT-IBDv vaccine against the challenge with local field isolate "lay./Men.Egypt/017" (vvIBDv) was evaluated in this study. The recombinant VAXXITEK HVT-IBDv vaccine has provided protection for commercial male layer chicks against mortality and against bursal atrophy after challenge with the vvIBDv strain. Studies showed that vaccination with vector vaccine in endemic areas with vvIBDV, decreases immunosuppressive effect of vvIBDV.

Table (1): Experimental design for assessment of protection of commercial male layer chickens vaccinated on one-day-old with HVT-IBDV against challenge with vvIBDV local field isolate "Lay./Men.Egypt/17".

Group No.	Chicken No.	Vaccination regime		IBDV challenge at 46- day of age	Assessment
		Vaccine	Age/days		
G1	50	HVT-IBDV	1	-	**1-** Clinical signs. **2-** Mortality %. **3-** Gross lesions. **4-** B/BR and BBI. **5-** Seroconversion. (ELISA and AGPT) **6-** Histopathology. **7-** RT-PCR 3-day pre and 3, 7-day post challenge.
G2	50	HVT-IBDV	1	+	
G3	50	Non	-	-	
G4	50	Non	-	+	

IBD challenge virus= Occulonasal challenge on day 46 with 100µl/bird (contain 3.5 log-10 EID50) of IBDV local field isolate "Lay./Men.Egypt./17 ." HVT-IBDV= IBD vectored vaccine, in which turkey herpes virus (HVT) is used as the vector. B/BR= Bursal body weight ratio. BBI= Bursa body weight ELISA= Enzyme linked immunosorbent assay
AGPT= Agar gel precipitation testRT-PCR= Reverse Transcriptase polymerase chain reaction

Table (2): Waning of maternal derived antibodies (±Sd) (MDA) in commercial male layer chickens. acquired from their parents that were vaccinated with live and inactivated oil emulsion IBDV vaccines, Chicks divided into 4 groups (50 birds/each).

Age/days	**ELISA titer (Mean ± S. E)**		**AGPT**
	IBD plus	**classic IBD**	
1	20090±20.90Aa	9093±23.23Ab	Nd.
11	17032±32.75Ba	6227±22.24Bb	(6/10) 60%
18	13225±22.23Cb	3332±33.34Ca	(1/10) 10%
25	10638±33.58Da	2900±29.30Db	(0/5) 0%
32	7196±17.88Ca	2096±11.20Eb	(0/5) 0%
53	400±14.20Ea	150±10.55Fb	(0/5) 0%

ELISA= Enzyme linked immunosorbant assay. AGPT= Agar gel precipitation test. N.D.=Not done.
Capital litter: Means within the same column of different litter are significantly differ at ($P < 0.05$). Small litter: Means within the same row of different litter are significantly differ at ($P < 0.05$).

Table (3): Serological response of commercial male layer chickens vaccinated with HVT-IBDV vac-cine at one-day of age and non-vaccinated chickens, at 11-18 day of age, the progeny used in this experiment regarding MDA from profile 2 of which as there is no difference between at one day of age.

Group no.	Chicken no.	Age/ days	Vaccination regime		ELISA titer (Mean ± S. E)		AGPT
			Vaccine	Age/day	IBD plus	classic IBD	
G1	50	11	HVT-IBDV	1	17872±70.72Aa	5150±50.53Bb	(4/5) 80%
G3	50	11	--	--	17032±32.50Ba	6227±22.15Ab	(3/5) 60%
G1	50	18	HVT-IBDV	1	14845±44.50Ca	3362±26.15Cb	20% (2/5)
G3	50	18	--	--	13225±62.22Da	3332±23.22Db	10% (1/10)

no.=Number. ELISA= Enzyme linked immunosorbant assay. AGPT= Agar gel precipitation test
HVT-IBDV= IBD vectored vaccine, in which turkey herpesvirus (HVT) is used as the vector. Capital litter: Means within the same column of different litter are significantly differ at ($P < 0.05$). Small litter: Means within the same row of different litter are significantly differ at ($P < 0.05$).

Table (4): Serological response of commercial male layer chickens vaccinated with HVT-IBDV by ELISA and AGPT.

Group no.	Chicen no	Age/ days	Vaccination regime		ELISA titer (Mean ± S. E)		AGPT
			Vaccine	Age/ days	BD plus	IBD classic	
1	50	25	HVT-IBD	1	10957	2526	(2/5) 40%
3	50		--	--	10638	2900	(0/5) 0%
1	50	32	HVT-IBDV	1	7733	2351	(3/5) 60%
3	50		--	--	7196	2096	(0/5) 0%
1	50	42	HVT-IBDV	1	10524	2505	(9/10) 90%
3	50		--	--	2500	1000	(0/10) 0%
1	50	46	HVT-IBDV	1	12636	3934	(5/5) 100%
3	50		--	--	950	370	(0/5) 0%

ELISA= Enzyme linked immunosorbent assay. AGPT= Agar gel precipitation test.
HVT-IBDV=IBD vectored vaccine, in which turkey herpes virus (HVT) is used as the vector.

Table (5): Bursal body weight ratio, Bursal index and Mean severity index of commercial male layer chickens vaccinated with HVT-IBDV at 3-days pre-challenge.

Group no.	Chicken no	Age/ Days	Vaccination regime		B/BR Mean	B/BI mean	Bursal lymphocytic tissue lesion (SI)		MSI
			Vaccine	Age/days			Lymphocytic necrosis	Lymphocytic depletion	
1	50	42	HVT-IBDV	1	4.75	0.76	0.5	0.3	0.4
3	50		--	--	6.25	1	0	0	0

BR=Bursal body weight ratio.Lucio and Hitchner (1979) B:BI=Bursal body weight index .Lucio and Hitchner (1979). SI= Severity index of bursal lymphoid tissue lesion.Sharma et al. (1989) MSI= mean severity index. Sharma et al. (1989) HVT-IBDV= IBD vectored vaccine, in which turkey herpesvirus (HVT) is used as the vector.

Table (6): Detection of IBDV by AGPT and RT/PCR in bursae of commercial male layer chickens vaccinated with HVT-IBDV at 3-dpc with recent vvIBDV local field isolate "lay./Men.Egypt/017":

Group no.	Chicken no	Vaccination regime		Chall- at 46-day of age	RT/PCR for IBDv 3-dpc.	AGPT at 3-dpc.
		Vaccine	Age/days			
1	10	HVT-IBDV	1	-	(5/5) positive	(0/5) 0%
2	40	HVT-IBDV	1	+	(5/5) positive	(0/5) 0%
3	10	--	--	-	(5/5) negative	(0/5) 0%
4	40	--	--	+	(5/5) positive	(5/5) 100%

Dpc = day post challenge. Chall= challenge. RT/PCR=Reverse Transcriptase polymerase chain reaction. AGPT= Agar gel precipitation test. HVT-IBDV=IBD vectored vaccine, in which turkey herpesvirus (HVT) is used as the vector.

Table (7): Bursal body weight ratio, Bursal index and Mean severity index of commercial male layer chickens vaccinated with HVT-IBDV at 3-dpc with vvIBDV local field isolate "lay./Men.Egypt /017":

Group no.	Chicken no.	Vaccination regim		Chall.- at 46-days of age	Assessment of protection 3 – days post challenge				
		Vaccine	Age/ days		B/BR Mean	B/BI mean	Bursal lymphocytic tissue lesion (SI)		MSI
							Lymphocytic deplation	Lymphocytic necrosis	
1	10	HVT-BDV	1	-	4.52	0.85	0.5	0	0.2
2	40	HVT-BDV	1	+	4.66	0.87	1.5	1	1.2
3	10	-	-	-	5.3	1	0	0	0
4	40	-	-	+	6.1	1.15	2.5	3	2.75

IBD challenge virus= Oculnasal challenge at 46th day of age with 100µl/bird contain $10^{3.5}$ EIDS-50 of vvIBDV local field .isolate"lay./Men.Egypt./017". Chall. = challenge. Dpc = day post challenge
B:BR= Bursal body weight ratio.Lucio and Hitchner (1979) B:BI=Bursal body weight index.Lucio and Hitchner, (1979)
SI= severity index of bursal lymphoid tissue lesion. Sharma et al. (1989) MSI= Mean severity index. Sharma et al. (1989)
HVT-IBDV=IBD vectored vaccine, in which turkey herpesvirus (HVT) is used as the vector.

Table (8): mortality% of commercial male layer chickens vaccinated with HVT-IBDV and challenged with vvIBDV recent field isolate "lay./Men Egypt/017"on 7 dpc.

Group no.	Chicken no.	Vaccination regime		Challenge at 46-days of age	Mortality at 7-days post challenge	
		Vaccine	Age/days		rate	%
2	40	HVT-IBDV	1	+	0	0%
4	40	--	--	+	36	90%

dpc = day post challenge. HVT-IBDV=IBD vectored vaccine, in which turkey herpesvirus (HVT) is used as the vector.

Table (9): Serological response and AGPT of commercial male layer chickens vaccinated with HVT-IBDV at 7-day post challenged with recent vvIBDV local field isolate "lay./Men.Egypt/017".

Group no.	Chicken no.	Vaccination regime		Chall at 46-days of age	ELISA titer at 7- days post challenge		AGPT
		Vaccine	Age/ Days		BD+	IBD classic	
1	10	HVT-IBDV	1	--	10191	3963	(5/5) 100%
2	40	HVT-IBDV	1	+	10989	10224	(5/5) 100%
3	10	--	--	--	400	150	(0/5) 0%
4	40	--	--	+	5490	17414	(5/5) 100%

chall. = challenged.ELISA= Enzyme linked immunosorbent assay. AGPT= Agar gel precipitation test. HVT-IBDV= IBD vectored vaccine, in which turkey herpesvirus (HVT) is used as the vector.

Table (10): Detection of IBDV virus by AGPT and RT/PCR in bursae of commercial male layer chickens vaccinated with HVT-IBDV at 7-dpc with vvIBDV recent local field isolate "lay./Men.Egypt/ 017".

Group no.	Chicken no	Vaccination regime		Chall- at 46-days of age	RT/PCR for IBDV at 7-dpc.	AGPT at 7-dpc.
		Vaccine	Age/days			
1	10	HVT-IBDV	1	--	5/5 Positive	(0/5) 0%
2	40	HVT-IBDV	1	+	5/5 Positive	(0/5) 0%
3	10	--	--	--	0/5 Negative	(0/5) 0%
4	40	--	--	+	5/5 Positive	(0/5) 0%

dpc = day post challenge. chall.=challenged. RT/PCR=Reverse Transcriptase polymerase chain reaction. AGPT= Agar gel precipitation test.HVT-IBDV=IBD vectored vaccine, in turkey herpesvirus (HVT) is used as the vector.

Table (11): Results of degree of protection commercial male layer chickens vaccinated with HVT-IBDV vector vaccine at 7-dpc with vvIBDV recent field isolate "lay/Men.Egypt/017".

Group no.	Chicken no	Vaccination regime		Challenge at 46-days of age	Assessment of protection 7 – days post challenge				
		Vaccine	Age/ days		BBR mean	BBI mean	Lymphocytic Deplation	Lympho-cytic necrosis	MSI
1	10	HVT-IBDV	1	-	5.31	0.99	0	0	0
2	40	HVT-IBDV	1	+	4.89	0.91	1	0	0.5
3	10	--	--	-	5.32	1	0	0	0
4	40	--	--	+	1.36	0.25	4	3	3.5

B:BR= Bursal body weight ratio. Lucio and Hitchner (1979) BBI=Bursal body weight index. Lucio and Hitchner, (1979) MSI= Mean severity index. Sharma et al. (1989) HVT-IBDV=IBD vectored vaccine, in which turkey herpesvirus (HVT) is used as the vector.

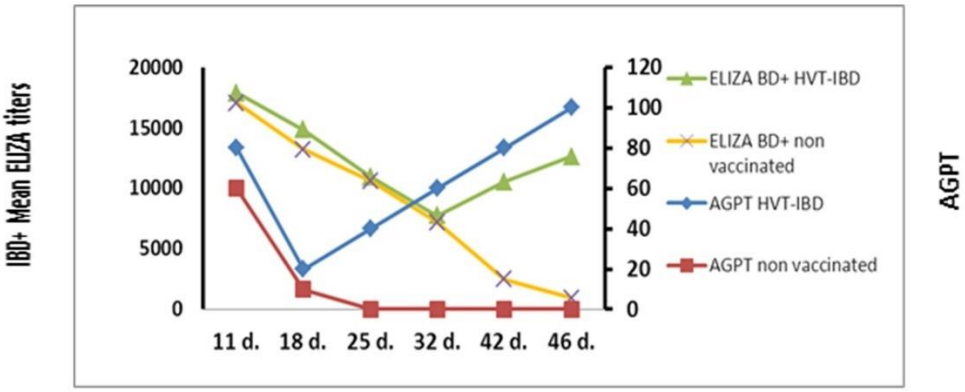

Fig (1): Serological response of commercial male layer chickens vaccinated with HVT-IBDV vaccine (G1) and non-vaccinated group (G3) by IBD plus ELISA and AGPT.

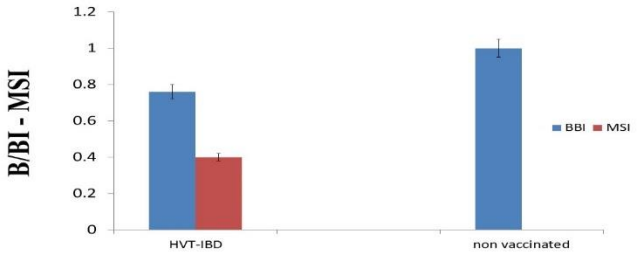

Fig. (2): B/BI and MSI of commercial male layer chickens vaccinated with HVT-IBDV and non-vaccinated group at 42-day of age (3-day pre-challenge**)**

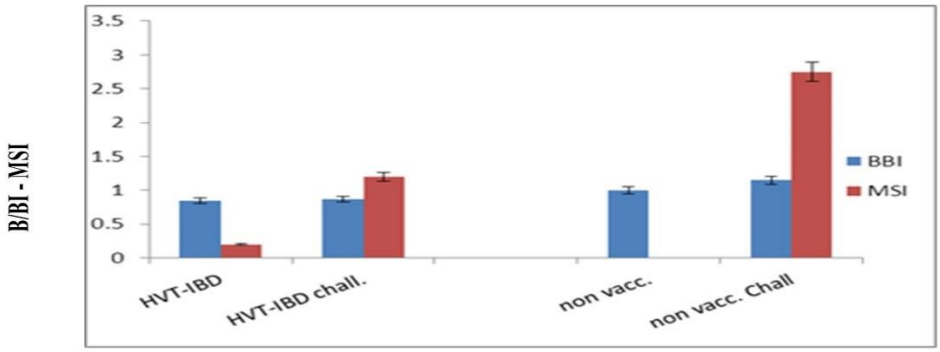

Fig. (3): B/BI and MSI of commercial male layer chickens vaccinated with HVT-IBDV and non-vaccinated groups at 3dpc with vvIBDV local field isolate "lay./Men.Egypt/017" at 46-day of age.

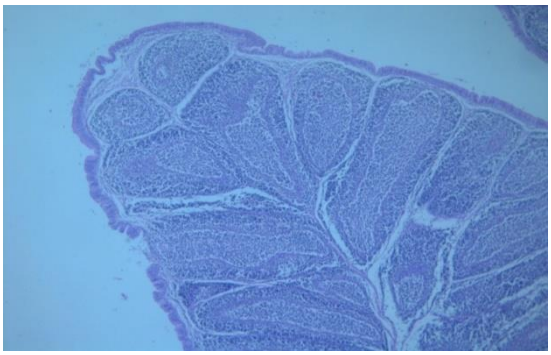

Fig (4): Bursa of 49-day old of commercial layer chickens vaccinated with HVT-IBDV at one-day of age and challengedwithv-vIBDV local field isolate "Lay./Men.Egypt/017" at 46-dayof age (G2), showed apparently normalbursa (HandE X100).

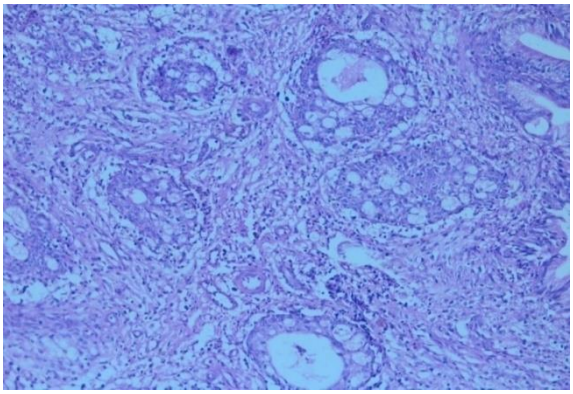

Fig (5): Bursa of 49-day of old commercial male layerchicken non-vaccinated challenged with vvIBDV local field isolate "Lay./Men.Egypt/017" at 46-day of age (G4) showed severe interfollicular connective tissue formationwith atrophy, severe depletion and necrosis of bursalfolliclesnwith cysts formation (HandE X100).

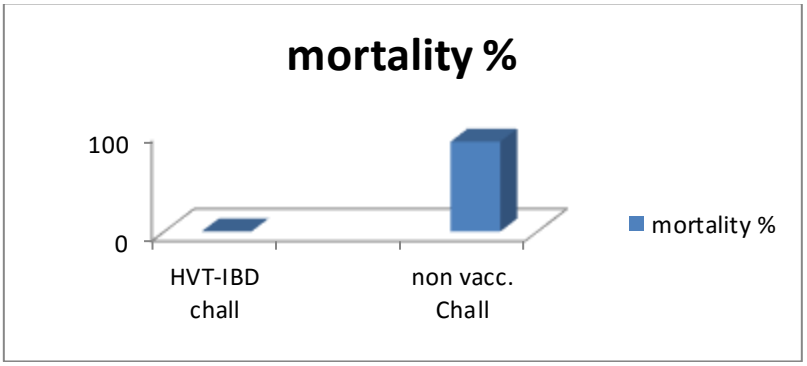

Fig (6): mortality % of IBDV vectored vaccinated challenged group and non-vaccinated challenged group at 7dpc.

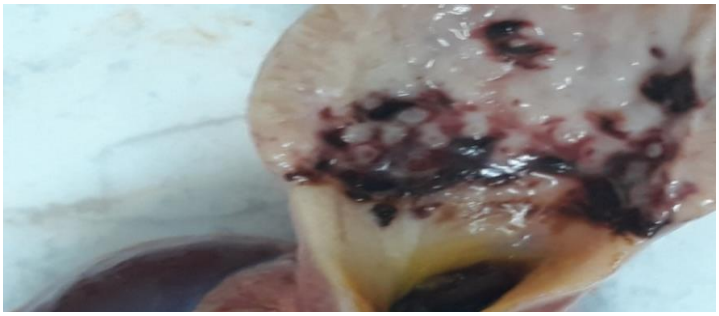

Fig. (7) Proventriculus of non-vaccinated challenged group with vvIBDV local field isolate "lay./Men. Egypt/017" (G4) showed ecchymotichaemorrhages in the mucosa at 7 day post challenge.

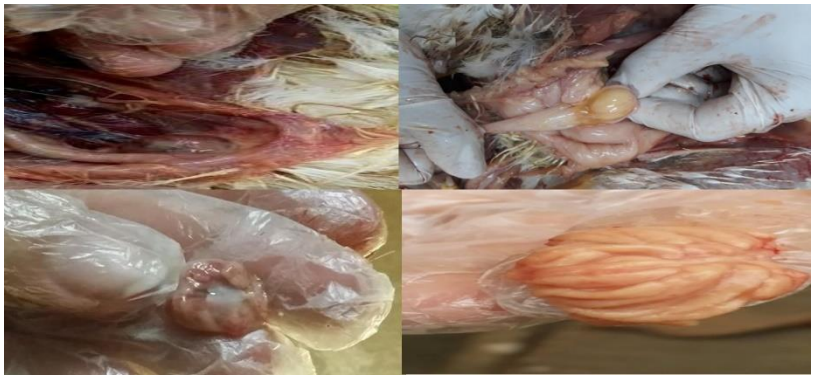

Fig. (8): Bursae of non-vaccinated challenged group with vvIBDV local field isolate "lay./Men. Egypt/017" (G4) enlarged showed edema, agelatinous yellowish transudate and longitudinal striation become prominent.

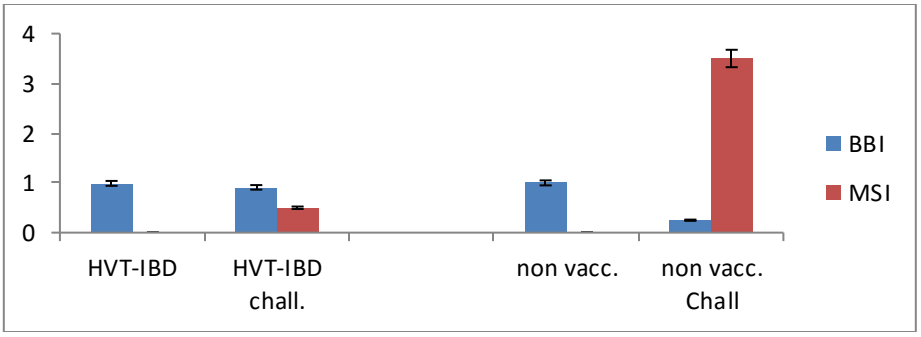

Fig. (9) B/BI and MSI of commercial male layer chickens vaccinated with HVT-IBDV and non-vaccinated groups at 7dpc challenged with vvIBDV local field isolate "lay./Men.Egypt/017" at 46-day of age.

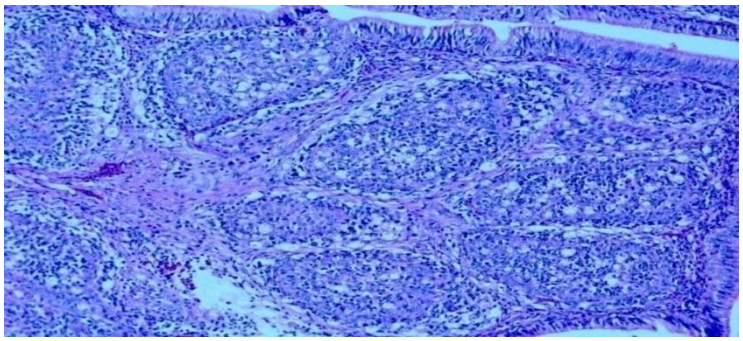

Fig (10): Bursa of 53-day old of commercial layer chickens vaccinated with HVT-IBDV at one-day of age and challenged with vvIBDV local field isolate "Lay./Men. Egypt/017"at 46-day of age (G2) showed hyperplasia and metaplasia of lining epithelium (HandE X100).

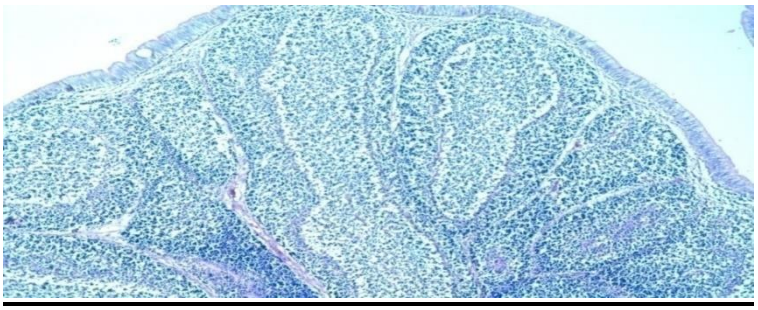

Fig (11): Bursa of 53-day of commercial layer chickens non-vaccinated challenged with vvIBDV local field isolate "Lay./Men.Egypt/017"at 46-day of age (G4) showed characteristic starry sky appearance due to depletion of lymphocytes in both cortex and medulla (HandE X100).

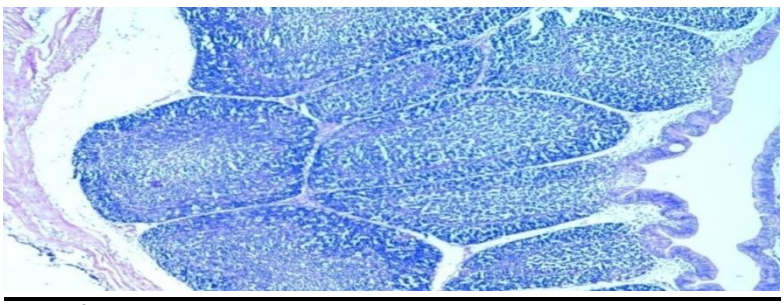

Fig (12): Bursa of 53-day old of commercial male layer chickens non- vaccinated non-challenged (G3) showed apparently normal architecture (HandE X100).

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