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Production and evaluation of rabbit IgG against IBDV in broiler chickens

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Abstract: Infectious bursal disease is an acute highly contagious disease affecting young chickens causing increased mortality and severe immunosuppression. Therefore, additional approaches together with vaccination are required to overcome its endemicity. Here, ten males Bosch rabbit of 3 months old (2-3 kg/ weight) were used for production of IgG against IBDV. The produced rabbit IgGs were titrated using Passive Hemagglutination (PHA) and evaluated experimentally for their protection against vvIBDV challenge in commercial broiler chickens. Daily observation of clinical signs, mortalities and postmortem changes were recorded till 10 days post-infection. The spleen and bursa were collected for histopathological examination and cloacal swabs were collected to evaluate viral shedding by real time-PCR. The results indicated the effectiveness of the anti-vvIBDV IgG in protection against vvIBDV and in the reduction of viral shedding. These results suggested that IgG produced in rabbits may help in IBDV control and decrease its commercial economic losses.

Key words: Infectious bursal disease virus; Rabbit IgG; PHA test, ELISA; q-RT-PCR

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1. Introduction

Infectious bursal disease (IBD), Gumboro, is an acute and highly contagious disease caused by the infectious bursal disease virus (IBDV) affecting mainly young chickens (**Eterradossi and Saif, 2020)**. The virus belongs to the Birnaviridae family, consisting of two segments of double stranded RNA bounded with a non-enveloped capsid (**Muller et al., 1979)**. In 1962, IBD was first detected in the USA subsequently causing extreme economic losses due to high mortality rate in susceptible broiler (30%) and layer (60%) chickens due to vvIBD. In addition, the IBDV impaired the growth rate, and leads to immunosuppression (**Phong et al., 2003; Van den Berg et al., 2004**) as it destroys the precursor antibody-producing cells in the bursa of Fabricius, particularly B-lymphocytes, inducing bursal atrophy (**Sharma et al., 2000).** In Egypt, the IBD was recorded in 1974 **(ElSergany et al., 1974),** while the virus was isolated and identified in 1976 **(Ayoub and Malek 1976).**

There are two antigenically distinct serotypes of the IBDV. Serotype I strains which are pathogenic and diverge in their virulence to chickens, and serotype II strains which infect turkeys only **(Enterradossi and Saif, 2020).** The IBD virus is resistant to different environmental conditions leads to their persistence in the poultry farms; therefore, effective vaccination together with strict hygiene management is crucial for the control of IBDV (**Al-Natour et al., 2004).** However, IBDV is evolving quickly in the field (**Jackwood and Sommer-Wagner, 2011)**, resulting in the emergence of IBDV antigenic variant strains in the early 1980s **(Rosenberger et al., 1985)** and very virulent IBDV (vvIBDV) strains in the late 1980s (**Chettle et al., 1989)**. In Egypt, several non-classical antigenically different IBDV strains of IBDV were recorded (**Abdel Mawgod et al., 2014; Alkhalefa et al., 2018)**, but the most prevalent IBDV strain in the field is vvIBDV (**Sedeik et al., 2018)** and thus, the situation with IBDV is becoming more complex (**Müller et al., 2012)**. The development of the IBD virus's antigenicity and virulence make the vaccination more challenging for the control of IBDV (**Eterradossi and Saif, 2020).**

It is well known that the chick is protected by maternal antibodies in their early life **(Hamal et al., 2006; Gharaibeh et al., 2008).** However, the maternal antibodies half-life in chicken is relatively short (**Patterson et al., 1962)**. It lasts for 10–14 days, then the protective antibodies are significantly reduced and chickens become susceptible for infection (**Gharaibeh et al., 2008)**.

Vaccination is one of essential tool for controlling the IBDV prevalence (**Hornyak et al., 2015)**. Conventional attenuated live vaccines (mild, intermediated, and intermediate plus vaccines), vector, immune-complex, as well as killed vaccines are commercially available and most used all over the world (**Muller et al., 2003; Liew et al., 2016, Eterradossi and Saif, 2020)**. The next-generation vaccines were developed to overcome the maternal –derived antibodies (MDAbs) by using turkey herpes virus (HVT) as a vector for the IBDV viral protein 2 gene (**Bublot et al., 2007)**. Moreover, the Immune-complex vaccine, which is a mixture of intermediate plus strain and antibodies, is taken up by macrophages till the MDAbs decreased **(Prandini et al., 2016)**.

Passive hyper immune therapy is an alternative way to control virus infection and has the advantage of immediate immunization once injected. The antibodies were broadly used to avoid and treat different infections like hepatitis A, measles, varicella, vaccinia, and tetanus (**Su et al., 2011; Mahgoub et al., 2012)**. Passive immunization overcome the use of partially virulent viruses in active immunization, overcoming the possibility of neutralization of maternal antibodies following active vaccination at young age and reducing the possible immunosuppressive effect of attenuated vaccine. However, the major complication of its application is its relatively high cost **(Aizenshtein et al., 2016)**. Hyper immune serum and egg yolk antibodies have respectable effects on the IBD virus especially at the early infection but are limited due to the high

cost and poor safety. Recently, the genetically engineered antibodies represented a viable alternative for the treatment and prevention of IBDV infection **(Xu et al., 2011; Li et al., 2015).**

The diagnosis of any disease is the first and foremost requirement for its effective control. Several serodiagnostic tests are available to diagnose the clinical cases including agar gel precipitation **(Castello et al., 1987**), enzyme linked immuno-sorbent assay (ELISA) (**Nicholas et al., 1985; Cao et al., 1995**), and passive hemagglutination (PHA) test which considered to be inexpensive, quick and easy to perform (**Aliev et al., 1990; Rahman et al., 1994**).

In this study, IgG hyperimmune sera were prepared in rabbits against vvIBDV and their effectiveness for protection against experimental infection with vvIBD in broiler chicks was evaluated.

2. Materials and Methods

2.1. Animals

Ten males Bosch rabbit 3 months old (2-3 kg/ weight) were housed in animal facility joined with the Animal Health Research Institute (AHRI) in Tanta as 1 rabbit per cage and given diet "*ad libitum*" with full access to water. The rabbits were left for 2 weeks for acclimatization. The live vvIBDV combined with adjuvant used for preparation of rabbit hyperimmune sera against IBDV **(Barnes et al., 1982).**

A total of 50 one-day-old commercial Arbor Acres broiler chicks obtained from a local hatchery (El-kanana, Tanta, Egypt) were reared in clean well-ventilated floor pens with 10 cm depth fresh wood shavings litter. The birds were housed in an animal facility joined with the animal health research institute in Tanta. These chicks were used for the protection study against vvIBDV.

All animal experiments were performed according to the ethics of the Institutional Animal Care and Use Committee of Damanhour University under approval No. DMU/VetMed-2023/017.

2.2. Virus

Very virulent local field Egyptian strain of IBDV; a kind gift by **Sultan et al., (2022)** (GenBank Accession No. KX646373) was used in rabbit immunization to produce specific IgG. It was also used as an antigen in passive hemagglutination (PHA) test and in challenge experiment as challenge virus at $10^{3.5}$ EID_{50}/ml titer.

Infected bursae samples were collected from diseased birds and homogenized as a 10% w/v suspension in phosphate buffered saline. The suspension was centrifuged at 3000 rpm for 10 minutes and the supernatant was collected. vvIBD virus was titrated by passive hemagglutination test **(Rajeswar and Dorairajan, 1999).**

Both viruses were propagated in SPF embryonated chicken eggs (ECEs) according to **Rodriguez-Chavez et al., (2002)**. Third egg passage samples from bursa of Fabricius that showed clear lesions in embryos and on CAMs were selected. Viral titration was carried out according to **Reed and Muench (1938)**. The titer was expressed as log10 EID_{50}/ml. The virus titer was $10^{3.5}$ EID_{50}/ml. The virus was then frozen at -70°C for further studies.

2.3. Vaccine

A live attenuated intermediate plus IBD vaccine ((ME/IBD-IMP/818®), MEVAC, Egypt) was used in the challenge experiment.

2.4. Rabbit immunization schedule

Ten male rabbits were divided into 2 groups; 2 animals were kept as control and 8 animals were injected with live vvIBDV (**Sultan et al., 2022)** combined with adjuvant as described by **Hussain et al., (2004)** with some modifications. The live vvIBDV ($10^{3.5}$ EID_{50}/ml) was emulsified with equal volume of adjuvant MONTANIDE™ ISA 71 VG (Seppic Inc.) for Water-in-Oil (w/o) emulsion.

The rabbits were injected S/C in the neck region with 1 ml of combination of live virus and adjuvant in 3 different sites and the control rabbits were injected with PBS only every injection time. The rabbits were injected 5 times at a 1-week interval.

Rabbits were bled periodically every week from the ear vein then blood was left to coagulate and centrifuged at 3000 rpm for 30 minutes for collection of serum. Then, the antibody titer was determined against vvIBDV using PHA test according to **Hussain et al., (2003); Aliev et al., (1990); Rahman et al., (1994).** The collected sera were stored at -20 °C till use for detection of specific antibodies by PHA. All rabbits were sacrificed on the 14th day after the last injection (5th injection) and whole blood was collected.

2.5. Extraction and purification of IgG

Serum was collected from whole blood and the IgG was purified using ammonium sulfate precipitation and dialysis according to **Steinbuch and Audran (1969)**.

2.6. Challenge experiment

Chicks were allocated into five groups (10 chicks in each group**).** Group 1 (G1) was the negative control. Group 2 (G2) was challenged orally with 100 µL of $10^{3.5}$ EID50/ml vvIBDV. Group 3 (G3) was vaccinated only one time with live attenuated intermediate plus IBD-vaccine at 13th days of age. Groups 4 (G4) and group 5 (G5) were passively immunized through I/M route with rabbit-IBDV IgG (PHA titer 2^8) 24 h before challenge and 24 h after challenge, respectively.

Each bird received 12 ml of the purified IgG that was calculated according to **Ezeibe et al., (2013)** to reach PHA titer 2^6 in chickens (protective titer for IBD in chicken by PHA). At the 12th day, G3 birds were placed in specific isolators to be vaccinated with live attenuated intermediate plus IBDV vaccine. At 35th day, G1 birds were placed in specific isolators (control negative group non-challenged) and then birds in G2 – G5 were challenged orally with 100 µl of challenge virus ($10^{3.5}$ EID_{50}/chick). Daily observation for recording clinical signs and mortalities was practiced for 10 days post challenge (dpc) **(Khan et al., 1988a).**

2.7. Passive Haemagglutination (PHA) test

In each group, PHA test was performed to detect the maternal derived antibody (MDAb) at the 12th day of age and at 1, 3, 5, 7, 10 dpc to titrate the antibodies. All the serum samples used in PHA were heat inactivated at 56ºC for 25-30 minutes in a water bath **(Rahman et al., 1994)** and then processed for PHA. The test was performed using sensitized human (O) RBCs according to **Aliev et al., (1990); Rahman et al., (1994) and Hussain et al., (2003).**

2.8. Enzyme linked immunosorbent assay (ELISA)

In each group, an ELISA test was performed to titrate the antibodies at 1 and 10 dpc. Serological titration of IBD-antibodies was performed using commercial indirect classical ELISA kits **(ID Vet, France).** Serum samples used in ELISA were not heat inactivated. According to the manufacturer's instructions, IBD immune status was considered negative if ELISA titer was less than 875. Samples with a sample to positive ratio (S/P) of 0.2 or greater were considered positive.

2.9. Real time RT-PCR

Cloacal swabs were collected from 3 chicks from each group at 4th, 7th and 10th dpc to evaluate the viral shedding by real time qRT-PCR. The nucleic acid was extracted according to the manufacture's instruction using QIAamp Viral RNA Mini Kit Catalogue No. 52904 **(Qiagen, CA, USA).** The cycling conditions for the real time PCR was performed according to **Moody et al., (2000)**. The sequence of primers and probe used were forward primer 5' GAG GTG GCC GAC CTC AAC T 3', reverse primer 5' AGC CCG GAT TAT GTC TTT GAA G 3' and the probe (FAM)-TCC CCT GAA GAT TGC AGG AGC ATT TG-(TAMRA)-3.

2.10.Histopathological examination

Postmortem lesions for dead chickens were recorded (**Khan et al., 1988b)**. In addition, tissue samples from spleen and bursa of Fabricius were collected at 4th and 7th dpc for histopathological examination **(Haddad et al., 1997*)*.**

Tissues were collected and preserved in neutral buffer formalin solution (10%) for histopathological examination. After proper fixation, the specimens were dehydrated in different ascending grades of ethyl alcohol, cleared in xylene, embedded in paraffin, sectioned at 5 µm, stained by hematoxylin and eosin stains for histopathological

examination according to **Haddad et al., (1997); Bancroft and Gamble (2008).**

3. Results

3.1. Evaluation of antibody titers of IBDV in rabbits' serum by PHA test

The results showed that the titer increased gradually from the day of injection till reaching peak at 2 weeks after the last vaccination from 2^0 to 2^{10}; respectively, while the titer in the control group remained 2^0 during the same period **(Table 1).**

3.2. Clinical signs of challenged chickens

The clinical signs were observed 10 dpc, the results showed that in G1 (negative control) no clinical signs were reported and all birds were normal for all parameters (feed and water intake) without any mortality and with good performance till the end of the experiment. G2 (positive control) showed severe clinical signs. It started from 2nd dpc in the form of depression, ruffled feathers, severe prostration, watery diarrhea, anorexia and trembling and the cloaca was reddened. The morbidity rate was 100% (10/10), mortality rate was 30% (3/10). The clinical disease completely subsided in surviving birds at day 9 dpc. G3 (IBD-vaccine) showed mild clinical signs, which started at 3rd dpc and increased to moderate signs by 4th dpc, then all signs completely subsided at 8th dpc and the mortality rate was 0%. G4 (IgG 24 hr before challenge) showed mild clinical signs that started at 3rd dpc and increased to moderate signs by 4th dpc, then all signs completely subsided at 8th dpc and the mortality rate was 0%. G5 (IgG 24 hr after challenge) showed mild clinical signs, which started at 2nd dpc and reached moderate degree at 5th dpc, then all signs completely subsided at 8th dpc and the mortality rate was 0% in surviving birds.

3.3. Antibody titer of challenged chickens by PHA

In each group, serum samples were examined for IBDV-maternal derived antibody (MDAb) titer by PHA test at the 12th day. The results showed that the titer was 2^1. Following that, serum samples were examined for IBDV-PHA titer on the 1st day of challenge, 3rd, 5th, 7th, and 10th dpc as shown in **Figure 1.** In the control negative group (G1), the PHA titer did not change. The PHA titer in the control positive group (G2), at day of challenge was 0, then at 3rd dpc increased to $2^{0.67}$, which increased to reach $2^{3.76}$ at 5th dpc, then continued to rise again to reach 2^6 at 7th dpc and 2^8 at 10th dpc. The PHA titer in G3 (immunized with live attenuated IBD-vaccine at 13th day) at day of challenge was $2^{8.3}$, at 3rd dpc declined to 2^6, then increased to reach $2^{6.67}$ at 5th dpc and continued to rise to reach $2^{7.3}$ at 7th dpc and continued to rise to $2^{8.6}$ at 10th dpc. The PHA titer in G4 (immunized with IgG anti- IBDV 24 hours before challenge) at day of challenge was $2^{6.67}$, at 3rd dpc declined to be 2^4 then increased to reach 2^5 at 5th dpc, then continued to rise to reach 2^6 at 7th dpc and till $2^{8.67}$ at 10th dpc. The PHA titer in G5 (immunized with IgG anti- IBDV 24 hours after challenge) at day of challenge was 0, at 3rd dpc was $2^{5.67}$, then increased to 2^6 at 5th dpc, then slightly increased again to reach $2^{6.33}$ at 7th dpc and continued to rise to reach $2^{8.33}$ at 10th dpc.

3.4. Antibody titer of challenged chickens by ELISA

In each group, serum samples were examined for antibody titer by ELISA. Serum samples were examined for IBDV ELISA titer on the 1st day of challenge and 10th dpc as shown in **Figure 2.** In the control negative group (G1), the ELISA titer did not change and remained negative. The ELISA titer in group G2 (control positive), at day of challenge was negative, then at 10th dpc increased to 6891. The ELISA titer in group G3 (immunized with live attenuated IBD-vaccine), at day of challenge was 2943 then at 10th dpc increased to 12347. The ELISA titer in group G4 (immunized with IgG anti- IBDV 24 hours before challenge) at day of challenge was 430 (negative), then increased to reach 10916 at 10th dpc. The ELISA titer in group G5 (immunized with IgG anti- IBDV 24 hours after challenge) at day of challenge was 538 (negative), then increased to reach 10396 at 10th dpc.

Table 1. Antibody titers against IBDV in rabbit serum samples along the experiment as measured by PHA

	Mean PHA titer in serum	
	Immunized rabbits (injected with IBDV + adjuvant)	**Control rabbits (Injected with PBS)**
0 day (1st dose)	2^0	2^0
1st week (2nd dose)	2^2	
2nd week (3rd dose)	2^4	
3rd week (4th dose)	$2^{5.5}$	
4th week (5th dose)	2^8	
5th week	2^{10}	

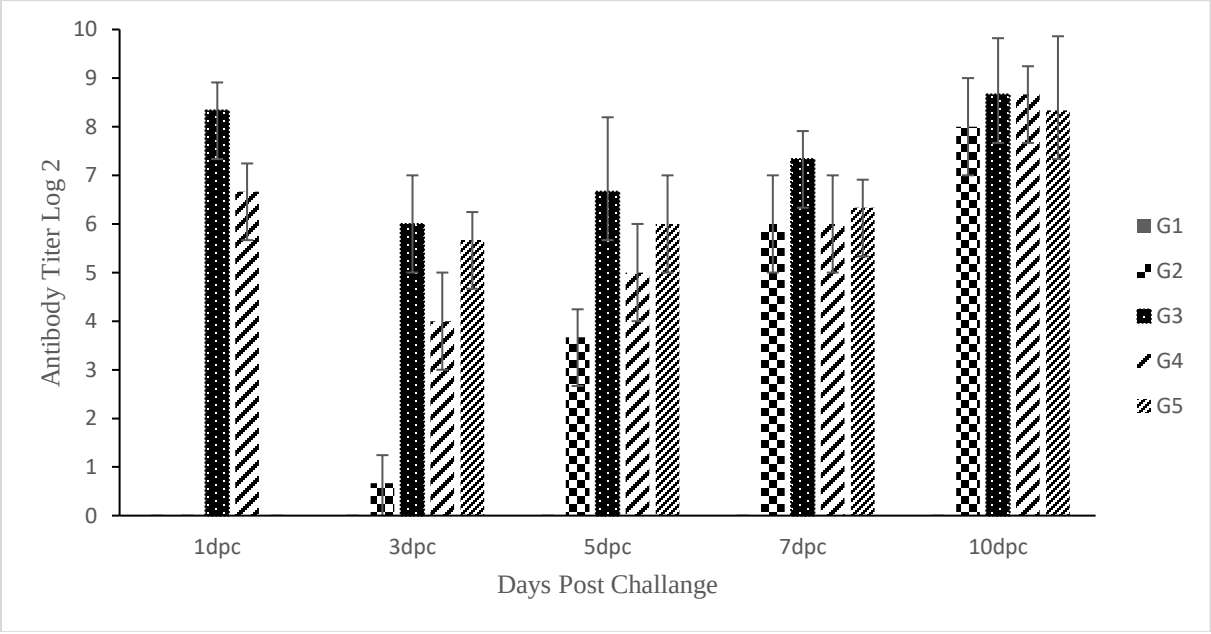


Figure 1. Serum titer against IBDV by PHA in the five experimental groups during 10-days post challenge. G1: negative control group, G2: challenged orally with 100 µl of challenge virus (control positive), G3: vaccinated with live attenuated intermediate plus IBD vaccine at 13th days of age then challenged with challenge virus, G4: passively immunized 24 h before challenge, G5: passively immunized 24 h after challenge.

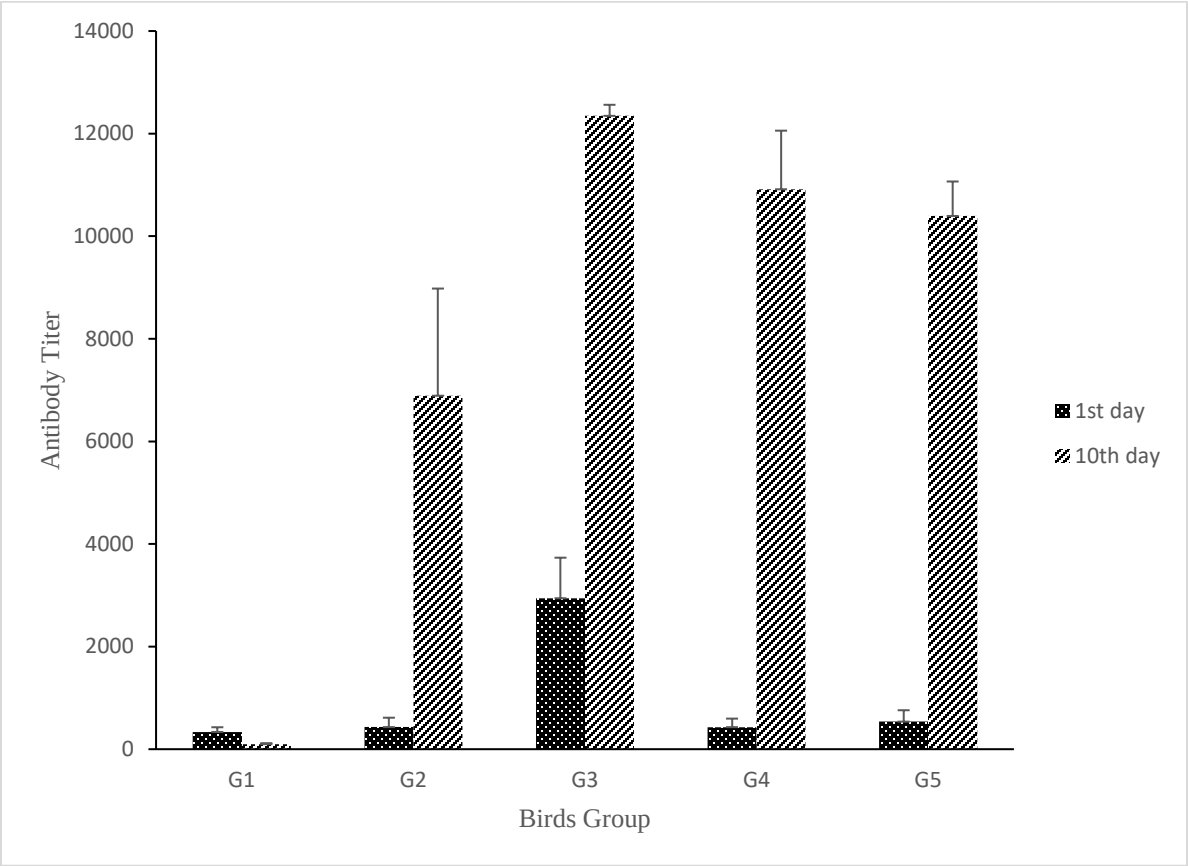


Figure 2. Serum titer against IBDV by ELISA in the five experimental groups during 10-days post challenge. G1: negative control group, G2: challenged orally with 100 µl of challenge virus (control positive), G3: vaccinated with live attenuated intermediate plus IBD vaccine at 13th days of age then challenged with challenge virus, G4: passively immunized 24 h before challenge, G5: passively immunized 24 h after challenge.

3.5. Virus shedding in cloacal swabs of challenged chickens

In each group, virus shedding was examined by qRT-PCR test. Cloacal swabs were examined for virus shedding on the 4th, 7th dpc and 10th dpc as shown in **Figure 3**. The virus shedding in group G2 (control positive) at 4th day of challenge was high, then began to decline till the 10th dpc. The virus shedding in group G3 (immunized with live attenuated IBD-vaccine) at 4th day of challenge was high as well, then began to decline till no shedding at 10th dpc. The virus shedding in group G4 (immunized with IgG anti- IBDV 24 hours before challenge) at 4th day of challenge was high, then began to decline till reaching nearly zero at 10th dpc. The virus shedding in group G5 (immunized with IgG anti- IBDV 24 hours after challenge) at 4th day of challenge was high, then began to decline till the 10th dpc.

The start of shedding in all challenged groups was on the 4th day then continued to the 7th day in all groups. In group G3, the shedding stopped on the 10th day, while it continued in group G2, G4, G5. At the 10th dpc, the shedding could still be detected in some samples of group G4 and G5.

3.6. Postmortem lesion and histopathological changes of challenged chickens

While the postmortem (PM) examination showed that the birds in G1 had normal appearance, G2 birds had severe hemorrhages in thigh, bursa of Fabricius, thymus and breast muscles and swollen kidney. G3, G4, and G5 birds showed mild to moderate lesion. PM examination showed hemorrhages in the thigh and breast muscles. The lesions in bursa ranged from flakes of pus to hemorrhages. Kidneys were also found to be swollen along with deposition of urates.

The histopathological examination showed some pathological changes in chickens of G3, G4, G5 but significantly lower than control positive group (G2) and no pathological lesions were found in examined organs of control negative group (G1) **(Figure 4)**. The bursa of Fabricus of control negative group (G1) birds showed normal bursal follicles filled lymphocytes and covered with mucosa. Bursa of Fabricius of G2 infected birds sacrificed at 4th dpc showed marked lymphoid necrosis of the germinal center of the bursal follicles that were replaced with endodermal cells and histocytes and remnant normal lymphocytes within the cortical area of the bursal follicles. Bursa of Fabricius of G2 infected birds sacrificed at 7th dpc showed marked atrophy of the bursal follicles associated with germinal histiocytic cells proliferation and severe interstitial fibrosis. The Bursa of Fabricius of G3 infected birds sacrificed at 7th dpc showed mild necrosis of the cortical area, marked necrosis of the germinal center, and pronounced interstitial fibrosis. Bursa of Fabricius of G4 infected birds sacrificed at 4th dpc showed mild lymphoid necrosis of the cortical area of the follicle with severe vacuolar degenerative changes of the germinal center. The Bursa of Fabricius of G4 infected birds sacrificed at 7th dpc showed decreased necrosis within the lymphoid follicle either within cortical or germinal or with marked interstitial fibrosis. The Bursa of Fabricius of G5 infected birds sacrificed at 4th dpc showed moderate degree of lymphoid necrosis of the cortical and the germinal center of the follicle. The Bursa of Fabricius of G5 infected birds sacrificed at 7th dpc showed decreased necrosis within the lymphoid follicle and marked decreased interstitial fibrosis.

The spleen of control negative group (G1) birds showed normal white and red pulps. Spleen of G2 infected birds sacrificed on the 4th day showed marked lymphoid necrosis of the white pulp. The spleen of G2 infected birds sacrificed at 7th dpc showed marked lymphoid cells depletion associated with marked histiocytic cells proliferation distributed all over the white pulp. The spleen of G3 birds sacrificed at 7th dpc showed congestion of the red pulp and mild degree of lymphoid depletion of white pulp with starry-sky appearance. The spleen of G4

birds sacrificed at 4th dpc showed mild degree of lymphoid depletion. Spleen of G4 birds sacrificed at 7th dpc showed marked lymphoid hyperplasia of the white pulp. The spleen of G5 birds sacrificed at 4th dpc showed normal white pulp cells. Spleen of G5 birds sacrificed at 7th dpc showed hyperplasia of the lymphoid cells within the white pulp.

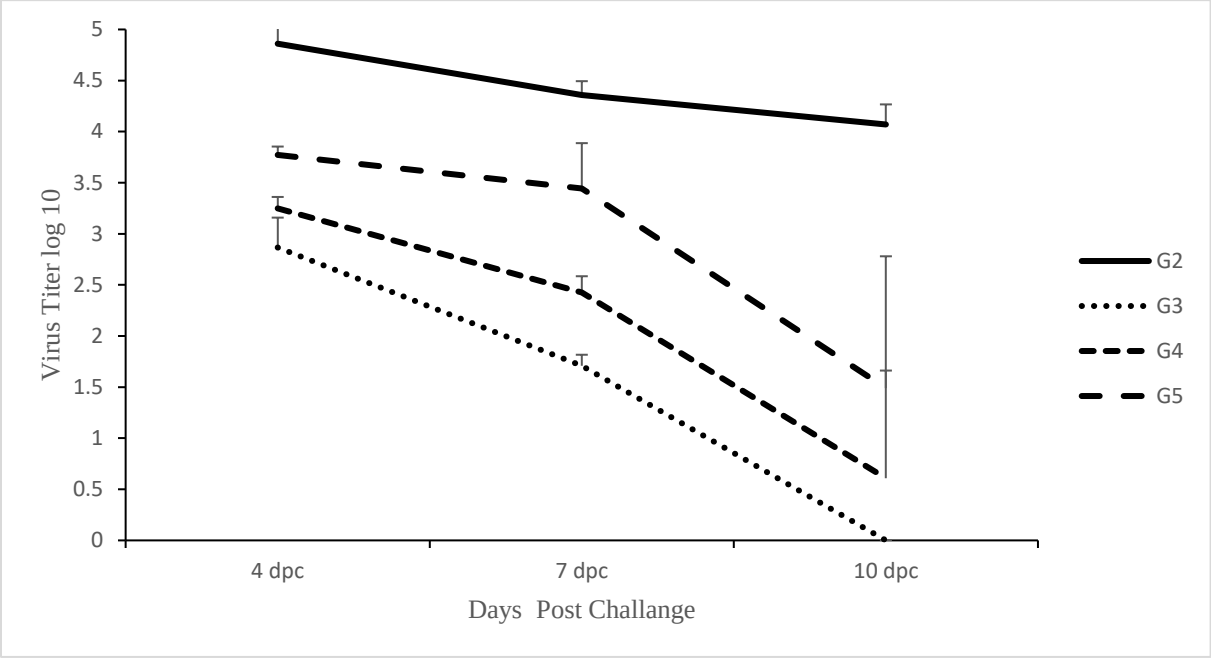
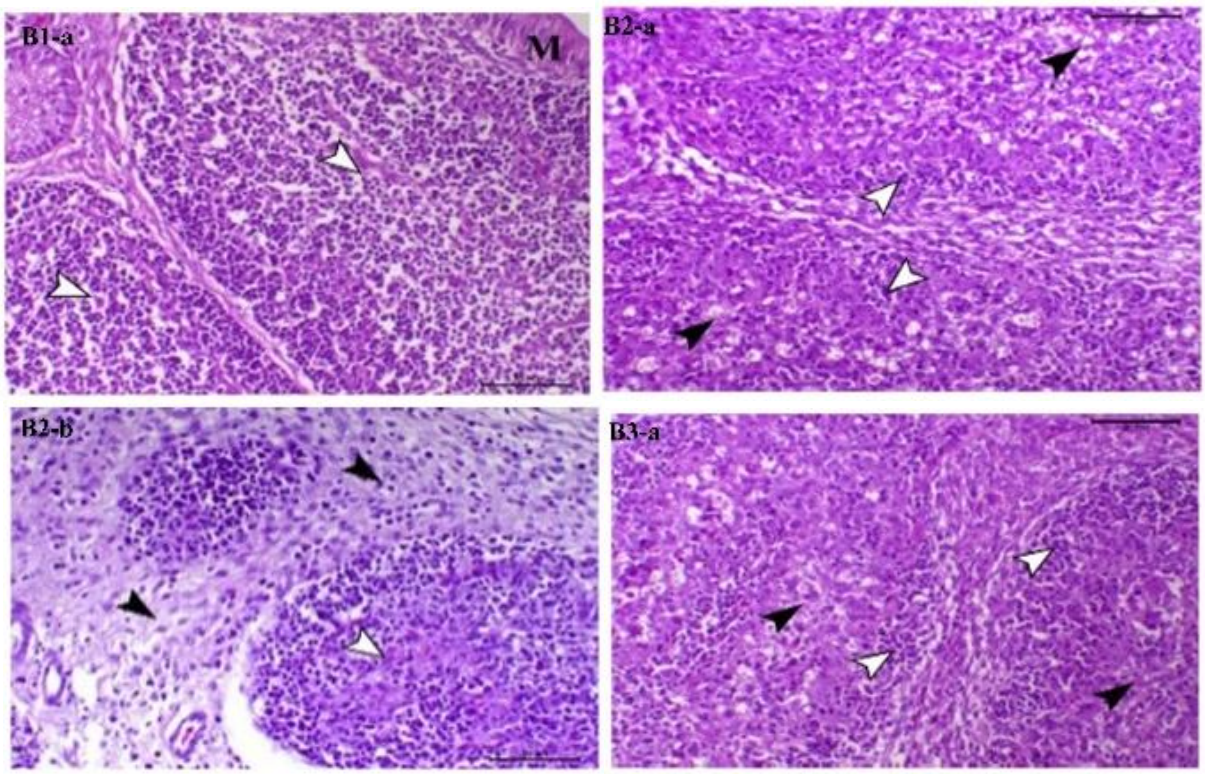
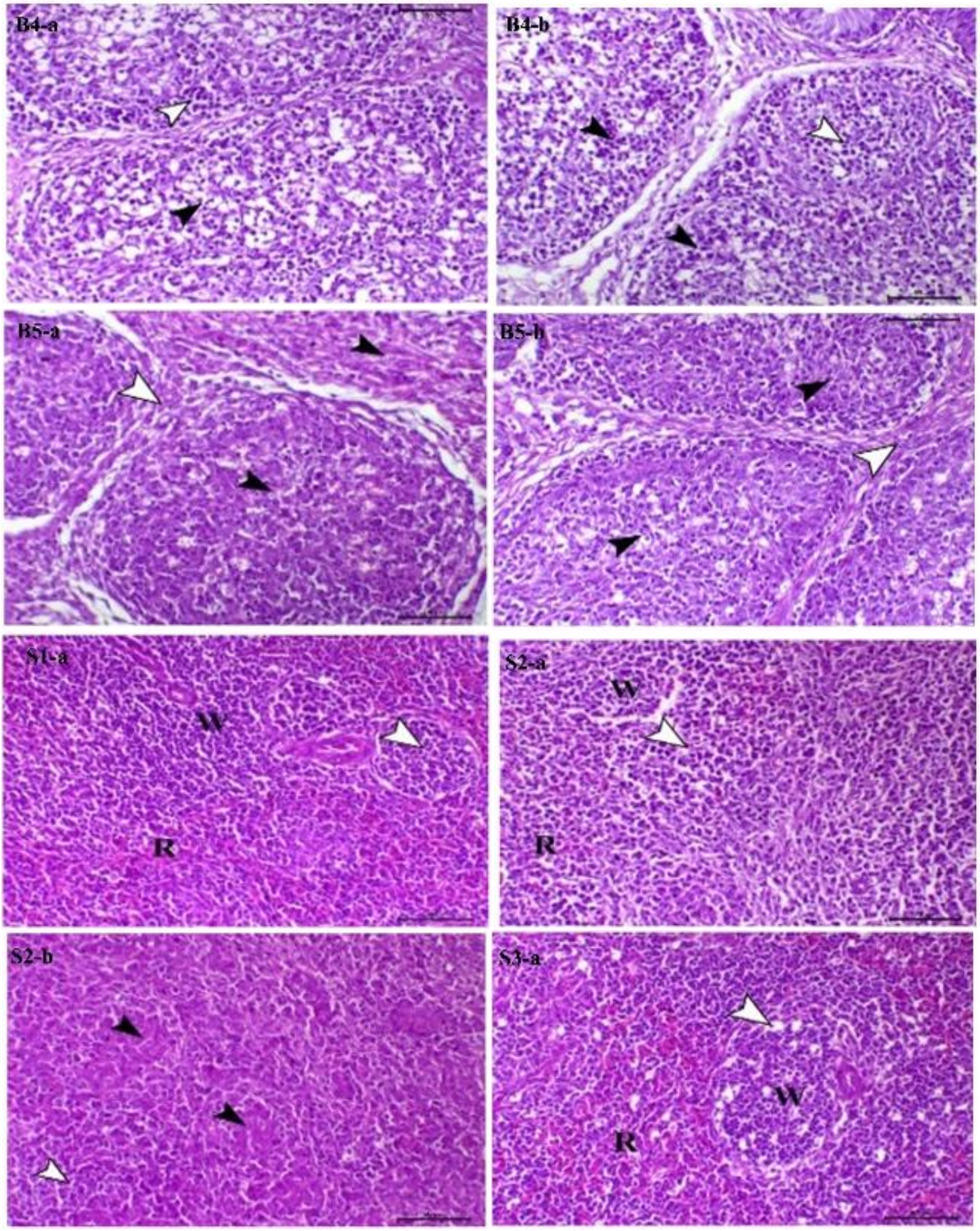


Figure 3. qRT-PCR analysis of virus shedding in cloacal swabs of challenged groups. G2: challenged orally with 100 µl of challenge virus (control positive), G3: vaccinated with live attenuated intermediate plus IBD vaccine at 13th days of age then challenged with challenge virus, G4: passively immunized 24 h before challenge, G5: passively immunized 24 h after challenge.




B4-a
B4-b
B5-a
B5-b
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R
S2-a
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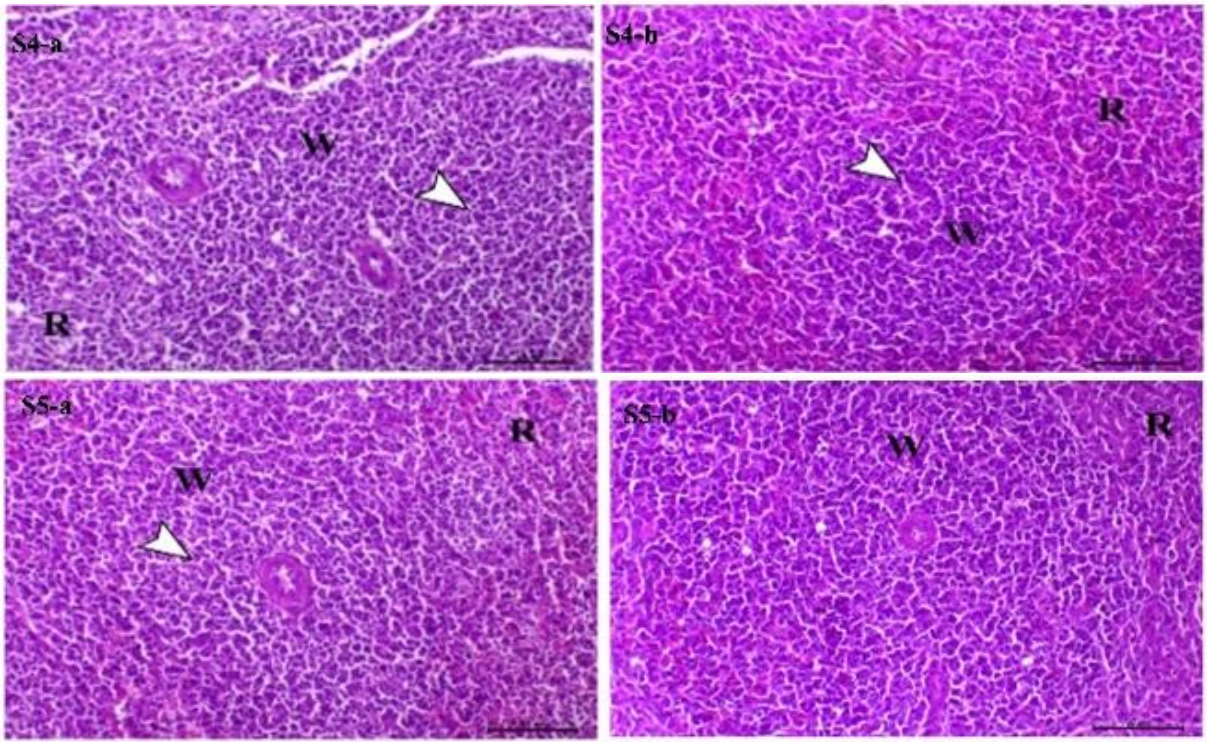

Figure 4. Histopathological changes in bursa of Fabricius and spleen of different challenged groups. G1: negative control group, G2: challenged orally with 100 µl of challenge virus (control positive), G3: vaccinated with live attenuated intermediate plus IBD vaccine at 13th days of age then challenged with challenge virus, G4: passively immunized 24 h before challenge, G5: passively immunized 24 h after challenge. Tissues were stained with H&E, bar= 50 µm. **B1-a:** Bursa of Fabricius of control bird (G1) showing normal bursal follicles filled lymphocytes in (arrowheads) and covered with mucosa (M). **B2-a:** Bursa of Fabricius of infected bird (G2) sacrificed at 4th dpc showing marked lymphoid necrosis of the germinal center of the bursal follicles (black arrowheads) replaced with endodermal cells and histocytes and remnant normal lymphocytes within the cortical area of the bursal follicles (white arrowheads). **B2-b:** Bursa of Fabricius of infected bird (G2) sacrificed at 7th dpc showing marked atrophy of the bursal follicles associated with germinal histocytic cells proliferation (white arrowheads) and severe interstitial fibrosis (black arrowheads). **B3-a:** Bursa of Fabricius of G3 bird sacrificed at 7th dpc showing mild necrosis of the cortical area (white arrowheads), marked necrosis of the germinal center (black arrowheads) with pronounced interstitial fibrosis. **B4-a:** Bursa of Fabricius of G4 bird sacrificed at 4th dpc showing mild lymphoid necrosis of the cortical area of the follicle (white arrowhead) and with severe vacuolar degenerative changes of the germinal center (black arrowhead). **B4-b:** Bursa of Fabricius of G4 bird sacrificed at 7th dpc showing decreased necrosis within the lymphoid follicle either within cortical or germinal (black arrowheads) and with marked interstitial fibrosis. **B5-a:** Bursa of Fabricius of G5 bird sacrificed at 4th dpc showing moderate degree of lymphoid necrosis of the cortical (white arrowhead) and the germinal center (black arrowhead) of the follicle. **B5-b:** Bursa of Fabricius of G5 bird sacrificed at 7th dpc showing decreased necrosis within the lymphoid follicle (black arrowheads) and marked decreased interstitial fibrosis (white arrowhead). **S1-a:** Spleen of control bird (G1) showing normal white and red pulps (W and R, respectively) (arrowhead indicates normal lymphoid cells within the white pulp). **S2-a:** Spleen of infected bird (G2) sacrificed at 4th dpc showing marked lymphoid necrosis of the white pulp (arrowhead). **S2-b:** Spleen of infected bird (G2) sacrificed at 7th dpc showing marked lymphoid cells depletion (white arrowhead) associated with marked histocytic cells proliferation distributed all over the white pulp (black arrowheads). **S3-a:** Spleen of G3 bird sacrificed at 7th dpc showing congestion of the red pulp and mild degree of lymphoid depletion of white pulp with starry-sky appearance (white arrowhead). **S4-a:** Spleen of G4 bird sacrificed at 4th dpc showing mild degree of lymphoid depletion (arrowhead). **S4-b:** Spleen of G4 bird sacrificed at 7th dpc showing marked lymphoid hyperplasia of the white pulp (arrowhead). **S5-a:** Spleen of G5 bird sacrificed at 4th dpc showing normal white pulp cells (arrowhead indicates lymphoid cells). **S5-b:** Spleen of G5 bird sacrificed at 7th pdc showing hyperplasia of the lymphoid cells within the white pulp.

4. Discussion

Antibody production is a complicated biological process. Literature recommendations and guidelines may not always be monitored, as procedures and protocols may need to be modified depending on the antigen. A single injection may be sufficient for some purposes, but generally higher antibody yields are obtained with a series of injections **(Cruickshank et al., 1968)**. To select a species for polyclonal antibody production, consideration should be given to the amount of antiserum required, the evolutionary relationship between the recipient and donor of the antigen, and the characteristics of the antibody produced by the recipient. Many vertebrate species have been used over the years, such as domestic animals, rabbits, small laboratory rodents and chickens **(Carpenter, 1975)**. Rabbits are the most used species because they are convenient in size, easy to bleed and handle, have a relatively long lifespan, and produce reasonable amounts of antisera. Also, they are free from antibodies against avian viruses. In our study, we produced and evaluated the protective efficacy of rabbit anti-IBDV IgG against IBD infection in broilers by measuring the percentage of morbidity, mortality and virus shedding. Here, we used rabbits because they are not susceptible to IBDV and is easy in handling and give large amount of IgG. The antibody titers produced against IBDV in rabbit serum samples measured by PHA increased during the experiment after a series of injections. It began from zero in the first immunization then increased to 2^2 then 2^4 till reaching peak 2^{10} two weeks after the last injection (5th injection). The PHA antibody titer in the rabbits of control groups was zero throughout the study.

Live highly virulent IBDV was emulsified with equal volume of adjuvant (MONTANIDE™ ISA 71 VG) to increase the immune response and subsequently increasing the antibody yield. **Kaeberle (1986)** observed that combining antigen with adjuvant generally resulted in much less antigen being used and significantly increased antibody titers compared to antigen without adjuvant. The antibody formation is enhanced by the use of various adjuvants as it is supposed that prolonged exposure of the antigen to immune system, protect it from degradation and stimulate the immune system efficiently **(Jennings, 1995)**.

In this study, the last antibody titer 2 weeks after the last injection was 2^{10} but it declined to 2^8 after antibody purification by ammonium sulphate. Similar observation was recorded by **Hassl et al., (1987)** who indicated that antibody titer was declined about one fifth of its original titer after purification. Ammonium sulfate precipitation is one of the most widely used methods for large-scale and laboratory scale protein purification and fractionation, separating proteins by altering their solubility in the presence of high salt concentrations **(Steinbuch and Audran 1969)**.

The antibody titers were measured in each challenged chicken group by PHA test to detect the maternal-derived antibody (MDAb) at

12th day, which was found to be 2^1, indicating that the chicks were susceptible to possible IBDV infection. Various field and experimental studies have focused on the importance of determining the optimal timing of vaccination based on the half-life and variability of the MDAb and the ability of vaccine strains to break through the MDAb **(Rautenschlein et al., 2005; Block et al., 2007)**. The half-life of maternal antibodies to IBDV is 3-5 days **(Eterradossi and Saif, 2020)**. Therefore, to determine the age at which a chick is susceptible to IBD, the exact antibody titer should be known. Antibody titer should not be less than 1:64 before chickens be efficiently vaccinated **(Eterradossi and Saif, 2020)**. Titer of log 6 (1:64) is thought to be protective and confer specific immunity **(Eterradossi and Saif, 2020)**. In our study, Groups (4) and (5) were passively immunized with 12 ml of 2^8 PHA titer / bird of purified IgG that was calculated by trial as **Ezeibe et al., (2013)** to reach PHA titer of 2^6 in chickens (protective titer for IBD in chicken by PHA) and this titer protect the challenged chicks, this protection was indicated in the absence of mortality and decreased severity of clinical sings and histopathological changes and also decreased virus shedding. According to **Maas et al. (2001),** circulating antibody levels of up to 7 (log2) or 1092 can alter mortality from vvIBD virus, and according to **Eterradossi and Saif (2020)** birds with antibody titers <1000 or 10 (log2) allowed colonization of mild virus parenterally inoculated at one-day old.

After challenging, the birds were monitored twice daily till the end of experimental period. It showed that in G2 (control positive), mortality was 30%. On postmortem examination, it showed thigh muscle hemorrhage with edematous bursa. No mortality was recorded in G3, they only showed symptoms of IBD infection like off feed, restlessness, and anorexia, that was similarly observed by **Eterradossi and Saif (2020)**. Similarly, the birds that received passive immune serum 24 hrs before (G4), and 24 h after (G5) challenge showed no mortality and morbidity was observed only as mild to moderate clinical signs. Similar observation was observed by **Rabbi et al., (2001)** who studied the passive immunization against Infectious Bursal Disease in chicks but using IgY.

In general, the vaccines are useful in preventing IBD because they could induce humoral and cellular immune responses **(Maas et al. 2001)**. In our study, chickens in group 3 (G3) were vaccinated with live attenuated intermediate plus IBDV vaccine at 13th day old after measuring IBDV-maternal derived antibody that was 2^1 then the antibody titer was measured again by PHA and ELISA at 35th day old (day 1 of virus inoculation). PHA titer for group 3 was $2^{8.3}$ at first day of challenge, which exceeded the protective titer so protected G3 against virus challenge then declined at 3rd day of challenge to 2^6 possibly due to virus neutralization then increased again after further development of the immune response to the virus challenge. A gradual increase in mean antibody titers was observed in the non-vaccinated control group. In contrast, one week after challenge, the vaccinated group showed a modest reduction in mean antibody titers compared to vaccinated, non-challenged groups. This decrease in antibody titers in vaccinated flocks may be due to neutralization of antibodies by field challenge viruses in these vaccinated flocks. These results were consistent with several previous studies that showed the presence of interference between high levels of maternally derived antibodies (MDAb) at vaccination with the vaccines used **(Alam et al., 2002; Rautenschlein et al., 2005)**. Evaluation of the optimal timing of vaccination is therefore important **(Moraes et al., 2005).** The 'intermediate' and 'intermediate plus' or 'hot' IBD vaccines have much greater efficacy and are able to penetrate higher levels of maternal antibodies but induce moderate to severe bursal lesions and response may cause immunosuppression **(Mazariegos et al., 1990; Tsukamoto et al., 1995; AlMufarrej. 2014)**. They may not fully protect chickens from infection with vvIBDV strains **(Kumar et al., 2000)**. In this study, no mortality was recorded in the vaccinated group, whereas a 30% mortality (3/10) was recorded within 7 days after challenge in the non-vaccinated group challenged at 35 days of age. Severe bursa atrophy with severe macroscopic and microscopic lesions was observed 3 and 7 days after challenge in the non-vaccinated control group. This is consistent with **Jung, A. (2006.)** who found that broilers vaccinated with an intermediate strain compared with non-vaccinated broilers was protected against mortality, morbidity, and the development of severe bursa lesions, in which 100% of birds vaccinated with highly virulent IBDV developed a lesion score of 3 or higher. In a previous research study by **Abdel-Alim and Saif (2001),** they found in commercial 1-day-old broilers vaccinated at a high dose of 10^4 EID50/chicken that the virus was detected in bursa only at 7- and 14-days post-inoculation. On the other hand, when vaccinated with a low dose of 10^3 EID50/chicken, the virus was detected only at 14 days post-inoculation. In our study, the cloacal swabs samples were collected at 3-, 7- and 10-days post challenge for detection of the virus shedding by RT-PCR. The virus shedding prolonged to day 10 post challenge in control positive group and to day 7 in vaccinated and IgG treated group indicating that the vaccination and hyper-immune serum treatment were effective in decreasing virus shedding and reducing the course of the disease. The presence of histopathological lesions in the bursa confirmed the pathogenicity of the field IBD virus used.

Sediq et al., (2019) observed that the intermediate plus vaccine (228E) produced high ELISA antibody titers with vvIBDV at 2 weeks post-vaccination and 1-week post-challenge. Here, ELISA antibody titers for vaccinated group (G3) at 35th days old was 2943 and increased to 12347 at day 10 of challenge. **Jakka et al., (2014)** found that the Intermediate-Plus vaccine induced high protective antibody titers with rapid onset, an increase in the CD8+T cell population, and a concomitant decrease in the CD4+T cell population, providing protection against pathogenic strains. Therefore, the intermediate plus vaccine is recommended for use in disease-endemic areas to prevent IBDV infection. In our study, G3 showed mild clinical signs that started at the 3rd dpc and increased to moderate signs by 4th dpc, then complete subsiding of all signs at 8th dpc. The mortality rate was 0%. PM examination showed hemorrhages in the thigh and breast muscles. **Sedeik et al., (2019)** reported that birds infected with IBDV showed depression and whitish diarrhea on days 2-7, birds vaccinated with doses of 228E (intermediate plus vaccine) showed 10% mortality, with gelatinous exudates, swollen bursa, bleeding in thigh muscle, nephritis, and swollen spleen. Histological observations in the challenged untreated group showed a marked decrease in lymphocytes, depletion of lymphoid follicles and necrosis in the spleen, depletion, and atrophy of lymphocytes in the bursa were observed. **Stoute et al., (2013)** reported lymphocyte depletion and reduced immune responses in IBDV-infected chickens. **Chansiripornchai and Sasipreeyajan (2009)** stated that the virulence of IBDV spreads to extra-bursal lymphoid organs such as the spleen. vvIBDV challenge significantly increased the splenic index in non-vaccinated birds 7 days after challenge. Similar results were reported by **Suliman et al., (2017)**. Moreover, it induced diffuse lymphocytic depletion and coagulative necrosis. In this study, the birds in the control positive group had severe hemorrhages in thigh, bursa, thymus and breast muscles and swollen kidney.

G4 and G5 had the same pattern of the nature and start of the clinical signs, morbidity, and mortality as well as the PM lesions as G3 indicating that the passive antiviral therapy administered in the form of IgG had the same effects exerted by the vaccine in the challenged birds. Further, the lack of adverse clinical signs or mortalities in G4 and G5 indicates the safety of the use of the rabbit IgG in chickens. Moreover, the pre-existence of the immunoglobulins in G3 and G4 correlated well with the protection of the disease in challenged birds. However, in G5, the delayed administration of the immunoglobulin did not seem to be a weakness point. This may possibly indicate a therapeutic window of 3 days following infection for IBDV control. Furthermore, this may possibly indicate that the chicken IgY and the rabbit IgG produced by vaccination behaved similarly regarding their effects on IBDV control in chicks.

Despite the interesting results in this study, several limitations may possibly hamper the clinical and commercial application of IgG for controlling vvIBD in Egypt such as the difficulty of identifying the exact timing of infection and disease under field conditions and so the possible delayed application of IgG could be ineffective and still needs testing, the possible mixed viral infections, the stress of catching diseased birds during the infectious bursal disease coarse, and the quantities of IgG required for commercial use in comparison to the available vaccines. In addition, the information regarding IgG therapy in animals is quite

scarce. Moreover, the pharmacokinetics and pharmacodynamics of the IgG in the veterinary field is not fully understood.

5. Conclusion

In conclusion, our results showed that IgG raised in rabbits against IBDV can control the infection in chickens experimentally, and as early as it is administrated, it decreased the virus shedding. Future study is currently under investigation to clarify the role of administration of IgG as a prophylaxis against vvIBD challenge, as therapy during the disease course and possibly addressing the different limitations associated with IgG therapy in the veterinary field as well as understanding the pharmacokinetics and pharmacodynamics of passively administered IgG in animals.

Conflict of interest: No conflict of interest.

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