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Comparison Between Immunoglobulins IgY and the Vaccine for Prevention of Infectious Bursal Disease in Chickens

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Abstract: This study was designed to compare between the efficacy of immunoglobuline IgY and the vaccine in the prevention of broiler chickens against infectious bursal disease virus (IBDV) infection. In this study 18-week-old white Leghorn laying chickens were received live intermediate strain IBDV vaccine (D78) followed by a booster doses of inactivated oil adjuvanted IBDV vaccine. The eggs of the hens were used for the separation of yolk polyclonal IgY. Enzyme Linked Immuno Sorbent Assay (ELISA) was used to determine the titer of antibodies in the serum and yolk. To evaluate the efficacy of the vaccine and IgY preparation against IBDV infection, day-old Hubbard broiler chicks were equally divided into 5 groups. The first group received live intermediate IBDV vaccine (D78), the second group was given IgY preparation, the third group received both the vaccine and IgY and the fourth group was kept as the control challenged. Chickens in the first, second, third and fourth group were challenged by the virulent field IBDV strain. Chickens of the fifth group were kept as blank control (not vaccinated, not IgY treated and not challenged). Morbidity and mortality rates, post mortem lesions, the bursa/body weight (B/BW) ratios and the histopathological examination of the bursae were investigated as criteria for evaluation. In conclusion, the vaccine and IgY were relatively equally effective but their combination was superior in prevention of IBDV infection in broiler chickens.

Key words: IBD · Yolk IgY · Immunoglobulins · Vaccines · Control

INTRODUCTION

Infectious bursal disease (IBD) is a highly contagious viral disease of chickens characterized by severe immunosuppression resulting in great bursal damage [1]. Outbreaks of IBD virus (IBDV) cause devastating losses in both broiler and layer flocks [2, 3]. Attempts to prevent IBDV infection is based mainly on using of vaccines parallel with application of strict biosecurity measures in the farm. Intermediate live IBDV vaccines are effective in stimulation of active immune response even in the presence of high maternal antibody titers [4]. In spite of intensive application of the different types of IBDV vaccines, IBD is still considered as a major production problem affecting poultry farms.

The effect of immunoglobulins IgY in competing different bacterial pathogens in avian species was evaluated with successful results. It had been used for controlling of *Salmonella enteritidis* in chickens and duckings [5-9], prophylaxis and therapy of *Campylabacter jejuni* infected chickens [10], reduction of colonization of *Clostridium perfringens* in broiler chickens [11], suppression of the colonization of *Salmonella typhimurium*, *Escherichia coli* (*E. coli*) and *C. jejuni* in laying hens [12], inducing resistance of broiler chickens to *E. coli* respiratory tract infection [13] and controlling of *E. coli* infection in rabbits [14]. Recently, hyperimmune IgY antibodies passively provide significant protection against avian coccidiosis in newly hatched birds [15].

Using immunoglobulins IgY in ailment of IBDV is not fully studied, therefore, this work was planned to compare between the efficacy of immunoglobulins IgY and the vaccine in the protection against IBDV infection in broiler chickens

MATERIALS AND METHODS

Experimental Chickens

Layer Chickens: A total of 10, 18-week-old white Leghorn laying chickens that obtained from a commercial source with good health conditions and known history of

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vaccination were used for production of eggs. The eggs were used for preparation of egg yolk polyclonal IgY antibodies against IBDV vaccine.

Broiler Chickens: Two hundreds day-old Hubbard broiler chicks that obtained from a commercial hatchery were used to compare between the efficacy of specific IgY antibodies and the vaccine against IBDV.

Layer and broiler chickens were kept in separated thorough cleaned and disinfected houses under complete hygienic measures and the feed and water were given *adlibitum.*

Vaccines of IBDV

Inactivated Oil Adjuvanted Vaccine: Inactivated IBDV vaccine of Rhone-Merieax, France (batch No. 1005F22) was used to immunize layer hens intramuscularly (I/M) for production of IBDV specific polyclonal IgY antibodies.

Freeze Dried Live Intermediate Vaccine (D78): Nobilis Gumboro D78 vaccine (intermediate strain), Intervet International, B. V. Boxmeer, Holand (batch No. 09623Hjo1) was used. The vaccine contains live IBDV strain D78: = $\log^{4.0}$ tissue culture infectivity dose ($TCID_{50}$). It was used orally to prime layer chickens for production of polyclonal IgY and in broiler chickens to compare efficacy with immunoglobulins IgY and the vaccine.

The Challenge IBDV: Virulent field IBDV strain was obtained kindly from Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt. The virus was used for challenge of broiler chickens [10^5 embryo infective dose fifty (EID_{50})/bird] through eye drop instillation [16]. Also, that antigen was used in the serological detection of the antibody titer of IBDV vaccine in layer chickens.

Experimental Design

The 1st Experiment: The design modified by Sriram and Yogeeswaran [17] was followed. Ten, 18-week-old white Leghorn laying hickens were received live intermediate strain IBDV vaccine (D78) orally on day 0 and then followed by a booster dose of inactivated oil adjuvanted IBDV vaccine (I/M) on day 14. The hens were repeatedly boosterd 3 times with the inactivated IBDV vaccine at 2 weeks intervals. During that period the eggs as well as the sera of the hens were collected at the different intervals and used for the separation of yolk IgY and for serological detection of antibodies, respectively.

The 2nd Experiment: A total of 200 hundreds, day-old Hubbard broiler chicks were equally divided into 5 groups, each consist of 40 birds. Each bird in the first group received orally live intermediate IBDV vaccine (D78) $\log^{4.0}$ ($TCID_{50}$) at 7 and 14 days old according to the manufacturing directions. Birds of the second group were given orally phosphate buffer saline diluted IgY (0.5 ml/bird) at 28 days of age. Chicken in the third group received both live intermediate IBDV vaccine (D78) $\log^{4.0}$ ($TCID_{50}$) orally at 7 and 14 days old and diluted IgY (0.5 ml/bird) at 28 days old. The fourth group was kept as control positive (only IBDV challenged, non vaccinated and non IgY treated group). Chickens in the first, second, third and fourth group were challenged at 35 days of age by the virulent field IBDV strain (10^5 EID_{50}/bird) through eye drop instillation method. Birds of the fifth group were kept as blank control negative without challenge, vaccination or IgY treatment. All the groups were kept for observation till the end of the study (45 days of age).

Collection of Eggs and Serum Samples: The eggs of layer chickens were collected just before IBDV vaccine immunization and at the different intervals of immunization. At the same time, the blood samples were collected from the wing veins of those birds at the same previous intervals, left to clot, centrifuged and the serum samples were separated for further serological tests.

Extraction of IgY Antibodies from the Egg Yolk of IBDV Immunized Hens: Extraction of IgY polyclonal antibodies from the egg yolk of immunized hens was done using saturated ammonium sulphate precipitation method as described by Akita and Nakai [18].

Sterility Test: A loopful of the hyperimmunised yolk was inoculated in sterilized nutrient broth (Difco, 5 ml/ vial). The vials were examined for bacterial growth after 48 hours of incubation at 37°C. No growth indicated that the yolk was sterile.

Enzyme Linked Immuno Sorbent Assay (ELISA) Test: This test was used to estimate the immune response of layer chickens to IBDV vaccine either in their sera or in IgY preparation. It was done according to the method of Silim and Venne [19].

Parameters Used for Evaluation of the Protective Efficacy of Both the Immunoglobulins IgY and IBDV Vaccine in Broiler Chickens

Morbidity and Mortality Rates, Post Mortem Lesions and the Survival Rate: All broiler chickens in the five groups were observed daily after challenge (35 days old) for 10 days till the end of observation period (45 days old). Clinical signs, morbidity and mortality rates were

recorded. Dead as well as sacrificed birds at 5 and 10 days post challenge were examined for IBDV gross lesions (bursal odema or haemorrhages, muscular and/or proventricular haemorrhages and nephrosis) as described by Ley *et al.* [20].

The Bursa/ Body Weight (B/BW) Ratios: At 5 and 10 days post IBDV challenge, 10 birds from each group was weight then sacrificed and the bursae of fabricus of them were weight to determine (B/BW) ratios. The bursa/body weight ratio = bursal weight/body weight [21].

Histopathological Examination: The bursae of fabricus were collected from dead and sacrificed chickens and subjected to histopathological examination [22]. Changes in bursa tissues were subjectively graded as normal (0), mild (1), moderate (2), severe (3) and very severe (4) according to Hair-Bejo *et al.* [23] as a modified scoring for previously established method.

Statistical Analysis of the Data: The collected data were tested using the method of Snedecor and Cochran [24].

RESULTS

The used extracted yolk immunoglobulins IgY was sterile as it didn't show any bacterial growth after inoculation in nutrient broth media.

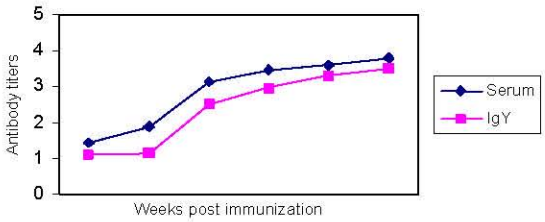

Fig. 1: Comparison between the mean IBDV vaccine antibody titer in the serum of immunized layer chickens and in immunoglobulins IgY using ELISA test

Results of table 1 and figure 1 summarized the comparison between the mean IBDV vaccine antibody titer in the serum of immunized layer chickens and in immunoglobulins IgY using ELISA test. In the serum samples, there were significant ($p<0.001$) gradual increase in the mean antibody titer (mean $\log_{10}$) to reach its peak at 2 weeks after 4th immunization at the different time intervals (3.81 ±0.11) when compared with the control (preimmunization) (1.45±0.10). Regarding that mean antibody titer in IgY preparation, the titer was not significantly increased 2 weeks after priming immunization (1.15±0.01) in comparison with preimmunization (1.11±0.01) but it showed gradual and significant ($p<0.001$) increase till it reached the maximamum (3.50±0.12) at 8 weeks of immunization (2 weeks following the 4th booster dose).

Table 1: Comparison between the mean IBDV vaccine antibody titer in the serum of immunized layer chickens and in immunoglobulins IgY using ELISA test

	Mean $\log_{10}$ antibody titer	
Immunization intervals	Serum	IgY
Control (Pre vaccination)	1.45±0.10	1.11±0.01
2 weeks after living vaccine (Priming)	1.92* ±0.15	1.15±0.01
2 weeks after inactivated vaccine (1st booster)	3.12* ±0.17	2.51*±0.14
2 weeks after 2nd booster inactivated vaccine	3.49* ±0.14	2.99*±0.15
2 weeks after 3rd booster inactivated vaccine	3.60* ±0.13	3.31*±0.16
2 weeks after 4th booster inactivated vaccine	3.81* ±0.11	3.50*±0.12

* Significant difference ($p<0.001$) compared with control (pre-vaccination)

Table 2: The morbidity and mortality rates, post mortem lesions and the survival rate of IBDV vaccinated, IgY treated, vaccinated and IgY treated as well as control broiler chicken groups after IBDV challenge.

							Survival rate	
Group No.	Live IBDV vaccine	IBDV specific IgY	IBDV challenge	Morbidity rate	Mortality rate	P/M IBDV lesions %	Morbidity rate	Mortality rate
1	+	-	+	8/40 (20%)	2/40 (5%)	10/40 (25%)	32/40 (80%)	38/40 (95%)
2	-	+	+	10/40 (25%)	4/40 (10%)	10/40 (25%)	30/40 (75%)	36/40 (90%)
3	+	+	+	0/40 (0%)	0/40 (0%)	0/40 (0%)	40/40 (100%)	40/40 (100%)
4	-	-	+	36/40 (90%)	20/40 (50%)	40/40 (100%)	4/40 (10%)	20/40 (50%)
5	-	-	-	0/40 (0%)	0/40 (0%)	0/40 (0%)	40/40 (100%)	40/40 (100%)

-= Negative += Positive
Morbidity rate= The number of birds with signs/total number of birds X100
Mortality rate= The number of dead birds /total number of birds X100
P/M= Post mortem
Dead and sacrificed birds at 5 and 10 days post challenge were examined for IBDV gross lesions (bursal odema or haemorrhages, muscular and/or proventricular haemorrhages and nephrosis).

Table 3: The mean bursa to body weight (B/BW) ratios of IBDV vaccinated, IgY treated, vaccinated and IgY treated as well as control broiler chicken groups post IBDV challenge.

				B/BW ratios	
Group No.	Live IBDV vaccine	IBDV specific IgY	IBDV challenge	5 days PC	10 days PC
1	+	-	+	2.34[b]	1.99[b]
2	-	+	+	2.41[b]	2.10[b]
3	+	+	+	3.77[a]	3.45[a]
4	-	-	+	0.85[c]	0.59[c]
5	-	-	-	3.91[a]	3.70[a]

-= Negative += Positive PC= Post challenge

[a-c] Figures sharing common superscripts are not significantly different ($p<0.05$).

Table 4: Histopathological examination of the bursae of fabricus of IBDV vaccinated, IgY treated, vaccinated and IgY treated as well as control broiler chicken groups post IBDV challenge.

				Histopathological alterations	
Group No.	Live IBDV vaccine	IBDV specific IgY	IBDV challenge	Lymphoid depletion	Haemorrhages
1	+	-	+	2	1
2	-	+	+	1	0
3	+	+	+	0	0
4	-	-	+	4	4
5	-	-	-	0	0

Changes in bursa tissues were subjectively graded as normal (0), mild (1), moderate (2), severe (3) and very severe (4) according to Hair-Bejo *et al.*, 2004

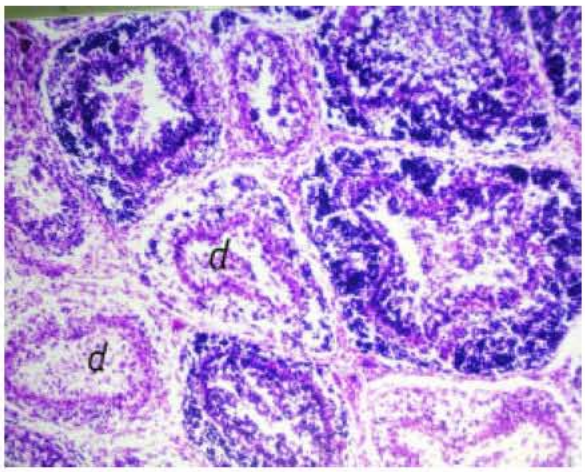

Fig. 2: Bursa of fabricus of group (1)- vaccinated with live IBDV vaccine and challenged with the virus. There was moderate depletion in some follicles (d) while others were intact (f) (H&E X40).

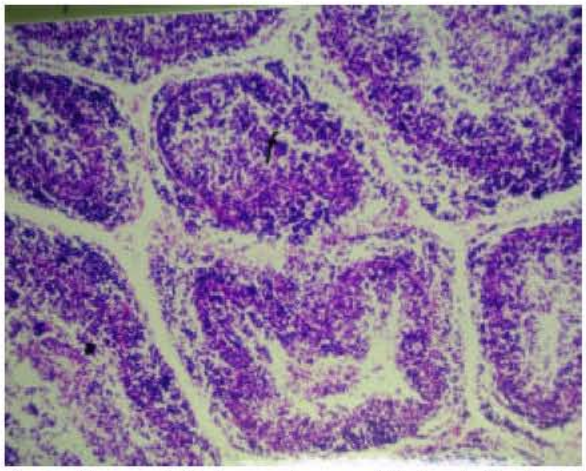

Fig. 3: Bursa of fabricus of group (2) –treated with IgY preparation and challenged with the virus. There was mild depletion in the central portion of the follicles (f) (H&E X40).

The results of morbidity and mortality rates, post mortem lesions and the survival rate of the vaccinated, IgY treated, vaccinated and IgY treated as well as control broiler chicken groups were seen in table 2. No IBDV clinical signs, mortalities or gross lesions were observed in the blank control group and in the vaccinated with IgY treated group. Clinical signs of depression, off food and perfuse watery diarrhea were observed in challenged groups at the 2nd day post challenge. The morbidity rate was 90% in control positive chickens, while it was 20% in the vaccinated birds and 25% in IgY treated ones. Birds showed mortalities at the 4th day post challenge. The mortality rate was 50% in control positive challenged birds, reduced to reach 5 and 10% in the vaccinated and

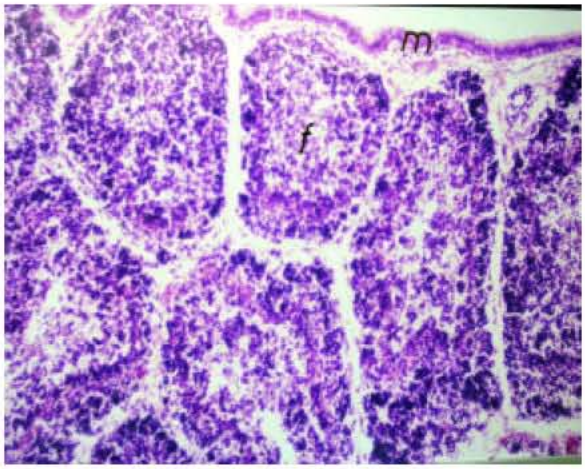

Fig. 4: Bursa of fabricus of group (3)- vaccinated with live IBDV vaccine, treated with IgY preparation and challenged with the virus. There was normal histological structure of the mucosal lining epithelium (m) and lymphoid follicles (f) (H&E X40).

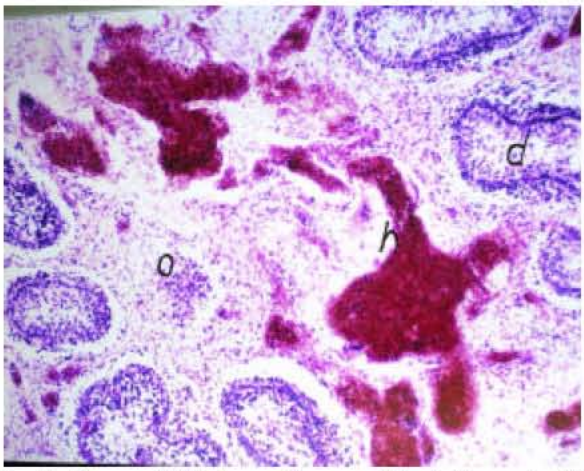

Fig. 5: Bursa of fabricus of group (4) challenged with IBDV. There were severe oedema (o) and focal haemorrhges (h) between depletated follicles (d) (H&E X40).

in IgY treated chickens, respectively. Specific lesions of IBDV (bursal odema or haemorrhages, muscular and/or proventricular haemorrhages and nephrosis) were recorded in dead and sacrificed birds at 5 and 10 days post challenge. These lesions percentage was the same 25% in both vaccinated and in IgY treated broilers, reached to 100% in control positive birds.

Table 3 showed the mean bursa to body weight (B/BW) ratios of IBDV vaccinated, IgY treated, vaccinated and IgY treated and control broiler chicken groups 5 and 10 days post IBDV challenge. The data revealed

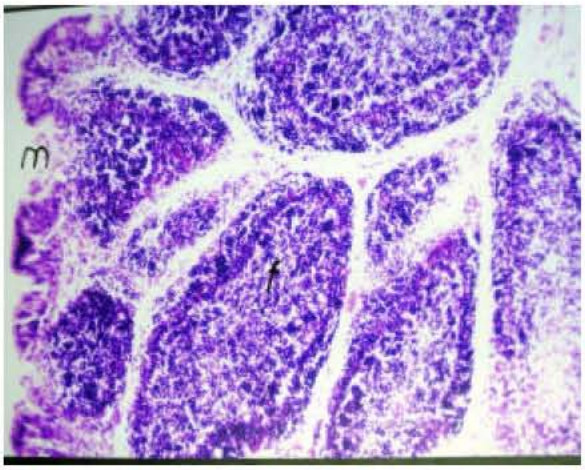

Fig. 6: Bursa of fabricus of group (5) not vaccinated, not treated or challenged. There was normal histological structure of the mucosal lining epithelium (m) and lymphoid follicles (f) (H&E X40).

significant ($p<0.05$) differences in (B/BW) ratios between the vaccinated and IgY treated birds and those of control groups. The highest (B/BW) ratios were observed in the blank control broilers as well as vaccinated with IgY treated group. The lowest (B/BW) ratios were present in control positive birds. There was no significant ($p<0.05$) difference in the ratios between IBDV vaccinated and IgY treated groups but both of them were significantly ($p<0.05$) different from the control positive group.

The results of histopathological examination of bursae of fabricus of broiler chickens were illustrated in figures 2-6 and table 4. Figure 2 indicated that IBDV vaccinated and challenged birds showed depletion of some of the lymphoid follicles while other were intact. Figure 3 revealed mild depletion in the lymphoid follicles in IgY treated and challenged chickens. There was no histopathological alteration in the lymphoid follicles (Figure 4) in the vaccinated and IgY treated group. Bursae of the control positive birds showed oedema with focal haemorrhages in between the degenerated and depleted lymphoid follicles at the lamina propria of the mucosal layer (Figure 5). Blank control negative group indicated no histopathological alterations in the bursae (Figure 6).

DISCUSSION

The IBD caused by birna virus is considered as a formidable disease of poultry that causes great economic losses all over the world and severe consternation to the poultry farmers. Vaccination of broiler chickens with live

mild or intermediate strains of IBDV vaccine is still the main tool to prevent the infection. Although using of intermediate strains of IBDV as a vaccine is effective, but they have some disadvantages as they may retain their virulence and induce pathological lesions in the bursae [25]. Therefore, IBD is common even in the vaccinated flocks.

Another control strategy of IBDV rather than the vaccine has been investigated recently. Using of specific IBDV egg yolk IgY immunoglobulin was studied in some reports [26-31] and proved its success in the prevention or control of IBDV.

Repeated immunization of layer hens with IBDV vaccine revealed marked and good ELISA antibody titer in the sera and so immunoglobulin IgY. Such result confirmed the findings of Marquardt *et al.* [32] and Briggs *et al.* [33]. Moreover, Muhammad *et al.* [34] found that chicken layers primed with oil based IBDV vaccine at age of 13 weeks and boosted with the same vaccine at 15 weeks of age showed high titer of yolk agar gel precipitating antibodies against IBDV at 21 and 28 weeks of age, also IBDV infected broilers (28 days old) when passively immunized with the yolk induced 80% recovery while all untreated birds died. Furthermore, Malik *et al.* [35] implicated that when specific hyper-immune polyclonal antibodies against IBDV were inoculated in layer chickens produced antibody titers significantly higher in yolk than serum using ELISA. Other approach for passive immunization of birds with IgY against IBDV infection was the inoculation of this specific immunoglobulins in the ova for ensuring of maternally-transmitted immunity to day old chicks [36].

Administration of IBDV vaccine and yolk IgY alone helped in reduction of morbidity and mortality rates of broiler chickens after challenge with IBDV. Moreover, combination of them succeeded in complete recovery without losses when compared with positive control. The role of IBDV vaccine for protection of broiler chickens against the infection was investigated by many researchers [37 - 41] with similar observations. Babiker and Tawfeeg [42] demonstrated that oral vaccination of broiler chickens with living intermediate IBDV vaccine (D78) induced only 12% mortalities in comparison with 32% in control group. Administration of immunoglobulins IgY preparation for control IBDV was studied by Aly *et al.* [28], Ahmed *et al.* [29] and Yousif *et al.* [43] who demonstrated that when IBDV hyperimmune yolk was administered to IBDV-exposed layer chickens, significant decrease in the mortality rate with milder symptoms in the exposed-treated group compared to the control exposed-ntreated group. As well, Moustafa [31] and Malik *et al.* [35] detected absence of both signs and deaths in IBDV infected broiler chickens after giving IgY orally when compared to control birds.

When considering (B/BW) ratios, the highest ratios were seen in groups treated IBDV vaccine and IgY preparation after challenge while the lowest ratios were observed in the only challenged group. This observation was accord with that reported previously by Zouelfakar *et al.* [44] and Babiker and Tawfeeg [42] who used intermediate live strains IBDV vaccine in protection of broilers against virulent IBDV infection.

The histopathological alterations of the bursa of fabricus were severe in IBDV challenged, none vaccinated or IgY treated group, became milder in the vaccinated and treated group till it completely became as normal structure in the blank control negative as well as vaccinated and treated challenged birds. Amer *et al.* [45] recorded similar findings after vaccination of broilers with intermediate live IBD vaccine.

There were many hypotheses considering the mode of action of IgY against bacterial infections especially the enteric ones in mammals and birds [46]. Agglutination might be one mediator of growth inhibition or strict hindrance of two Fab arms of IgY precludes the cross-linking of bacteria [47] or the binding of antibodies to certain components on the bacterial surface as outer membrane protein, lipopolysaccharide, flagella and fimbriae which lead to the impairment of the biological functions of them that play an essential role in the bacterial growth [48] and attachment to the intestinal cells [49]. Unfortunately, the mechanism of IgY for competing IBDV was not studied, so another studies will be needed in the future to explain this point.

Indeed, IgY technology offers great future opportunities for designing prophylactic strategies and it is becoming a more interesting alternative to control important thereating diseases in poultry.

From the above mentioned, it could be concluded that both the IBDV vaccine and the specific polyclonal immunoglobulines IgY were relatively equally effective in prevention of IBDV infection in broiler chickens, however, the combination of them is more effective in complete disease prevention.

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