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Research Article
Production and Evaluation of the Immuno-protective Efficacy of the Immunoglobulins IgY-antibodies Prepared Against Infectious Bursal Disease

[1,2]Moussa I. Mohamed, [1]Khalid S. Al-Maary, [1]Turki M. Dawoud, [1]Ayman S. Mubarak, [2,3]Ashgan M. Hessain and [2]Kh. F. Mohamed

[1]Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia
[2]Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Egypt
[3]Department of Health Science, College of Applied Studies and Community Service, King Saud University, Saudi Arabia

Abstract

Background: Infectious Bursal Disease (IBD) is a highly contagious disease characterize by severe damage of the bursa of fabricious and immuuosupperssion. The current study is aimed to evaluate the protective efficiency of IBDV specific IgY-antibodies prepared against the disease. **Materials and Methods:** Twenty white Leghorn laying hens were immunized with Infectious Bursal Disease Virus (IBDV) vaccine. Blood samples and eggs were collected simultaneously from laying hens before immunization and every 2 weeks after primary immunization. The collected eggs were used for separation of the yolk and extraction of IgY-antibodies by ammonium sulphate-caprylic acid method. **Results:** The mean $\log_{10}$ antibody titer of the serum samples showed significant increase after 2 weeks of immunization and reached its maximum level after 6-8 weeks from the primary immunization. Egg yolk IgY-antibodies level increased after 4 weeks of immunization and reached its maximum level after 8-10 weeks of immunization. Evaluation of the protective value of IBDV-specific IgY-antibodies revealed reduction in morbidity and mortality rate in challenged chickens 15 and 10%, respectively as compared with 90% morbidity and 40% mortality in non-immunized challenged chickens. In chicken group actively immunized with IBDV vaccine (live and inactivated), the morbidity and mortality rates were reduced to 10 and 5%, respectively, following challenge with virulent IBDV. However, when IBDV specific IgY-antibodies were used simultaneously with IBDV vaccines the morbidity and mortality rates were reduced to zero. **Conclusion:** The IBDV specific IgY-antibodies can be used simultaneously with IBDV vaccines for controlling the disease as the morbidity and mortality rates can be reduced to zero.

Key words: Infectious bursal disease, immunization, laying hens, IgY-antibodies, vaccine, ELISA

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Corresponding Author: Moussa I. Mohamed, Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia Tel: 00966560749553 Fax: 00966-114036600

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Infectious Bursal Disease (IBD) is a highly contagious disease affecting chickens characterized by severe damage of the bursa of fabricious and immuuosupperssion as discussed by Sampaio *et al.*[1] and Vera *et al.*[2]. During the last decade, outbreaks of an acute IBD with high mortality occurred in commercial broiler pullet flocks throughout Egypt as discussed by Abdel-Alim *et al.*[3] and Wang *et al.*[4]. The disease causes severe economic losses in poultry industry due to high mortality. Passive antibody immunization, in addition to innovative vaccine technology may have a far-reaching impact on control of IBD. Antibodies presently available for passive immunization purposes belong to either the mammalian or the avian species. The species chosen for the antibody production, however have usually been mammals. This is probably based on tradition because avian antibodies have been recognized for several decades and offer many advantages over the mammalian ones[5-7].

Avian IgG is transported selectively and receptor-depended through the follicular epithelium of the ovary from the serum of the hen to the egg yolk as discussed by Vera *et al.*[2]. In this study, passive immunization is transferred from hen to the embryo[8,9]. Due to its origin and some biochemical differences from mammalian IgY, avian IgG in the egg yolk is called IgY by Sampaio *et al.*[1]. The advantages of the immunoglobulin from the egg yolk of immunized hens have been pointed out repeatedly. The amount of antibodies gained from 1 hen is several times higher than a rabbit's equivalent[5,10], allowing reduction of the number of experimental animals. This as well as, the isolation of IgY from the egg yolk prevents animal suffering[11]. In addition, purchase and maintenance costs of a chicken are lower than those of rabbit's ones[10,11]. Therefore, the aim of the present investigation was to elucidate the effect of use of caprylic acid on purification of IgY and removal of non-immunoglobulin proteins and evaluation of protective efficiency of IBDV-specific IgY against IBD.

MATERIALS AND METHODS

Chicken: A total of 20 white laying Leghorn hens (7 months old and apparently health) were kept under good hygienic condition with food and water ad labium in a layer housing system at the College of Pharmacy, King Saud University. The hens were used for the production of serum and yolk IgY-polyclonal antibodies against infectious bursal disease virus.

At the same time, 100 broiler 1 day old chicks were used for evaluation of the protective value of specific IgY-antibodies prepared against IBDV. The chicks were housed in clean disinfected rooms with food and water ad labtium.

Vaccines: Two commercially available IBDV vaccines (Rhone-Merieux, France) were used in this study. The vaccines were Live Gumboral CT vaccine, (Batch No. 83L/34) and inactivated Gumboriffa vaccine (Batch No. 83L/59596j.

Challenge virus: Virulent IBDV field isolate that had been propagated in susceptible chicks was used for challenge (105 BID50/bird) and evaluation of protection.

Immunization of chickens with IBDV vaccine: It was carried out according to Cao *et al.*[12]. A total of 20 laying hens were primed on day zero by Gumboral CT vaccine (live vaccine, recommended to be used by ocular route). Two drops on the eye of each bird. The 1st booster dose was given 2 week after the 1st priming dose using Gomboriffa vaccine (IBD vaccine in oil adjuvant). The hens were repeatedly boostered (3 times) with the inactivated vaccine at 2 weeks intervals. Blood samples and eggs were simultaneously collected before immunization and at 2 weeks intervals after each immunization.

Extraction of IgY-antibodies: The IgY-antibodies were extracted from the egg yolk of immunized hen by ammonium sulphate-caprylic acid method according to Moussa *et al.*[10].

Determination of total protein content: Using Biuret method according to Gao *et al.*[7].

Enzyme linked immunosorbent assay (ELISA): The serum samples and the IgY-antibody preparations were tested by ELISA as developed by Gao *et al.*[7] and adapted for use with egg yolk-derived antibodies by Moussa *et al.*[10].

Evaluation of the efficacy of the IgY-antibodies prepared against IBDV vaccine in protection of commercial broilers against IBDV infection: A total of 100 commercial broiler chicks were used for evaluation of the protective value of IgY-antibodies prepared against IBDV vaccine. The chicks were divided into five groups, the 1st group received Gumboral CT (Live vaccine) at 7 and 21 days of age (104 CCID50/bird orally) and Gumboriffa inactivated vaccine at 8 days of age (0.5 mL^{-1} by intramuscular infection) according to the manufacturer procedures. The 2nd group received the IgY-antibodies (0.5 mL^{-1} chick) of diluted IgY that contained 2500-3500 ELISA antibody titre orally 1 week before the challenge. The 3rd group was vaccinated with live

vaccine at 7 and 21 days of age and inactivated vaccine at 8 days old followed by oral dose of IgY-antibodies (2500-3500 ELISA antibody titre/0.5 mL^{-1} PBS) 1 week before challenge. The 4th and 5th groups were left as a positive and negative control groups, respectively.

Challenge study: At 35 days of age, the chickens of the first four groups were challenged through the eye drop route by 105 EID50/bird of the field IBD strain according to Bublot *et al.*[13]. The 5th group was left as a negative control group. Mortality and morbidity rates during the study and after challenge were recorded.

RESULTS AND DISCUSSION

In chicken, IgG is the major serum antibodies. It is transported to eggs in a manner similar to the placental transfer of IgG in mammals to be stored ill the egg yolk[7,8]. The concentration of IgG in the yolk, more commonly called IgY is higher than that in serum[1,2]. At the same time, chickens store high contents of IgY-antibodies in the yolk and are considered to be efficient antibody producers[3-5]. The present study was planned to separate the IgY-antibodies by ammonium sulphate-caprylic acid method and to evaluate the immunizing potentials or polyclonal IgY-antibodies extracted from the egg yolk of chickens immunized with IBDY vaccine. Monitor of the total protein content in serum samples collected from hens immunized with IBDV vaccines showed significant increase ($p<0.001$) 2 weeks after the primary immunization (Table 1, Fig. 1). Boostering induced both increase and maintenance of the levels of total protein in the examined serum samples. This increase continued up to the end of observation period. The immunization dependent increase in the total proteins of serum can be attributed to the increased production of immunoglobulin's and other immune-regulatory proteins by immune-component cells. These results agree with those reported by Moussa *et al.*[10] and Chalghoumi *et al.*[11].

The mean value of the total protein content of the IgY preparations extracted by ammonium sulphate-caprylic acid method. Table 2 and Fig. 1 showed that there was no significant increase in the total protein content of IgY-antibody preparation when measured 2 weeks after the primary immunization. Significant increase in the total protein content was 1st reported 4 weeks from primary immunization (2 weeks after the 2nd booster dose). The total protein content reached 0.64 ± 0.021 g dL^{-1} in IgY-antibody preparations as compared with a pre-immunization level of 0.37 ± 0.040. The maximum level of the total protein content was detected at 10 weeks from the primary immunization (2 weeks after the last booster dose). In concern with anti-IBDV antibodies in serum samples and 1gY extracts from IBDV immunized hens. Table 1 significant increase in the ELISA antibody titres ($p<0.001$) were measured after 2 and 4 weeks, respectively. It is necessary to denote that the antibodies found in serum following primary immunization are primarily of IgM nature, which is followed by IgG release at

Table 1: Comparison between the antibody titer in the serum samples and the extracted IgY- antibodies at different time intervals

Period (Days)	Immunization of laying hens with IBDV	Antibody titer in the serum samples*	IgY-antibody titer in the extracted egg yolk*
0	Before immunization	1.16 ± 1.60	1.06 ± 0.00
14	14 days after the immunization	1.42 ± 0.116	1.17 ± 0.16
28	14 days after the 1st booster dose	2.85 ± 0.126	2.64 ± 0.16
42	14 days after the 2nd booster dose	3.44 ± 0.160	3.68 ± 0.165
56	14 days after the 2nd booster dose	3.68 ± 0.164	3.88 ± 0.162
70	14 days after the 2nd booster dose	3.22 ± 0.126	3.68 ± 0.164
84	-	2.48 ± 0.166	3.44 ± 0.165
98	-	2.22 ± 0.164	3.24 ± 0.165

*Mean $\log_{10}$ antibody titer $X\pm SD_n$, SD_n: Standard deviation

Table 2: Effect of IBDV-specific IgY-antibodies on morbidity and mortality rates in IBDV vaccinated and non vaccinated broiler chickens challenged with virulent IBDV strain at 35 days of age

				Morbidity		Mortality		
Groups	IBDV vaccines (Live and inactivated)	IBDV specific IgY	Challenge	No.*	(%)	No.**	(%)	Survival rate (%)
1	+	-	+	2/20	10	1/20	5	95
2	-	+	+	3/20	15	2/20	10	90
3	+	+	+	0/20	0	0/20	0	100
4	-	-	+	18/20	90	8/20	40	60
5	-	-		0/20	0	0/20	0	100

*Number of birds showing signs/total number of examined birds, **Number of death/ total number of examined birds

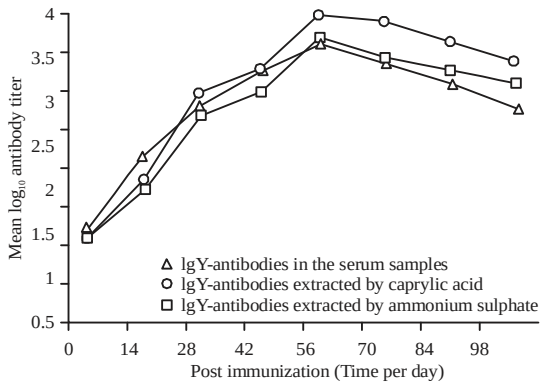

Fig. 1: Comparison between the antibodies ELISA titers in serum samples and in IgY-antibody preparations from hens immunized with IBDV vaccines at different time intervals post immunization

the end of the 1st 2 weeks[1]. This might explain why the level of specific antibodies in serum samples following the primary immunization (IgM and IgG) is usually higher than that in the egg yolk, which contains only the 1gG class or antibodies.

The antibody titres of the tested serum samples reached its maximum level after 6-8 weeks, but in the extracted IgY preparation, the maximum level was recorded after 10 weeks, where the antibody titre reached a level higher than that of the tested serum samples. This can be attributed to the time consumed for transfer and concentration of IgG in the yolk. These results confirm the capability of chickens to store high content of IgY in the yolk and to be considered as efficient antibody producers[8].

Evaluation or the protective value of the JBDV-specific IgY-antibodies Table 2, unimmunized challenged chickens (group 4) shower's morbidity and mortality rates of 90 and 40%, respectively, at 36-48 h post challenge. Chickens in group (1) that were vaccinated with live and inactivated IBDV vaccines and challenged showed 10% morbidity and 5% mortalities at the 4th clay post challenge. However, in the unvaccinated-challenged chicken group (2) that was passively immunized with IgY-preparations 15% morbidity and 10% mortalities were recorded. Chickens that were immunized actively with IBDV vaccine and passively with IBDV-specific IgY extracts showed neither clinical signs nor mortalities. No mortalities or morbidity were seen in the negative control chicken grout[5]. The reported results indicated the ability of IBDV-specific IgY-antibodies to control infectious bursal disease in chicken. The present study, confirm the conclusion of Wu *et al.*[5], Karlsson *et al.*[6] and Gao *et al.*[7]. They stated that the oral adminstration of IBDV-IgY antibodies for chichens could sigantly decrease the clinical symptoms in the passively immunized chichens. Morever, Malik *et al.*[14] and Ko and Ahn[15] rcommended the use of specific IgY-antibodies for prevention and controlling the infectionwith infectious bursal disease. The repeated immunization of chickens with the oil adjuvant IBDV vaccine simulatenousally increased the antibody titer against the virus in the serum samples and in the extracted IgY-prepartion. At the same time, passively immunized chickens with IgY-prepartions at 4 weeks old showed 85% recovery with mild clinical manifestation after infection with IBDV vaccine. The antibody titer in the extrated IgY were higher than that of the serum samples at each time post immunization, which confirm the conclusion of Malik *et al.*[14].

From the results of the present study, laying hens are considered highly cost-effective. Sources of antigen specific polyclonal antibodies as compared with mammals traditionally used for such purpose. Also, due to the phylogenetic difference between avian and mammalian species, the use of IgY in immunological assays is associated with increased sensitivity and specificity. Thus, egg yolk antibodies may replace mammalian antibodies in the future both as immunotherapeutic and imrnunodiagnostic tool[10,16] and disease prevention[17,18].

CONCLUSION

Evaluation of the protective value of IBDV-specific IgY-antibodies revealed reduction in morbidity and mortality rate in challenged chickens 15 and 10%, respectively as compared with 90% morbidity and 40% mortality in non-immunized challenged chickens. However, when IBDV specific IgY-antibodies were used simultaneously with IBDV vaccines the morbidity and mortality rates were reduced to zero.

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REFERENCES

1. Sampaio, L.C.L., M.D. Baldissera, T.H. Grando, L.T. Gressler and D.M. Capeleto *et al.*, 2014. Production, purification and therapeutic potential of egg yolk antibodies for treating *Trypanosoma evansi* infection. Avian Vet. Parasitol., 204: 96-103.
2. Vera, F., M.I. Craig, V. Olivera, F. Rojas, G. Konig, A. Pereda and A. Vagnozzi, 2015. Molecular characterization of infectious bursal disease virus (IBDV) isolated in Argentina indicates a regional lineage. Arch. Virol., 160: 1909-1921.

3. Abdel-Alim, G.A., M.H.H. Awaad and Y.M. Saif, 2003. Characterization of Egyptian field strains of infectious bursal disease virus. Avian Dis., 47: 1452-1457.
4. Wang, C.Z., H.M. Mo, Y.L. Cheng, L. Wang, Z.W. Jiang, W.Q. Liu and Y.L. Li, 2007. [Production and identification of chicken egg yolk antibodies against soluble egg antigen of *Schistosoma japonicum*]. Chin. J. Parasitol. Parasitic Dis., 25: 193-197, (In Chinese).
5. Wu, C.C., P. Rubinelli and T.L. Lin, 2007. Molecular detection and differentiation of infectious bursal disease virus. Avian Dis., 51: 515-526.
6. Karlsson, M., H. Kollberg and A. Larsson, 2004. Chicken IgY: Utilizing the evolutionary advantage. World Poult. Sci. J., 60: 341-347.
7. Gao, J., Y.C. Zhou and Y.F. Huang, 2008. [Purification and clinical application of egg yolk immunoglobulins]. Natl. J. Androl., 14: 166-170, (In Chinese).
8. Moussa, I.M., A.M. Hessan, A.M. Aleisa, A.A. Al-Arfaj, M.M. Salem-Bekhit and S.A. Alrejai, 2012. Protective efficacy of immunoglobulins Y prepared against *Cerastes cerastes* snake venom in the Kingdom of Saudi Arabia. Saudi Med. J., 33: 846-851.
9. Zhang, J., J. Tanha, T. Hirama, N.H. Khieu and R. To *et al*., 2004. Pentamerization of single-domain antibodies from phage libraries: A novel strategy for the rapid generation of high-avidity antibody reagents. J. Mol. Biol., 335: 49-56.
10. Moussa, I.M., A.M. Hessain, A.A. Al-Arfaj, K.M. Farouk and S.A. Selim, 2015. Immunological properties of anti *Naja haje arabica* (The Arabian Cobra) snake venom antibodies prepared in chicken. Int. J. Pharmacol., 11: 956-959.
11. Chalghoumi, R., A. Thewis, D. Portetelle and Y. Beckers, 2008. Production of hen egg yolk immunoglobulins simultaneously directed against *Salmonella* enteritidis and *Salmonella* typhimurium in the same egg yolk. Poult. Sci., 87: 32-40.
12. Cao, Y.C., Q.C. Shi, J.Y. Ma, Q.M. Xie and Y.Z. Bi, 2005. Vaccination against very virulent infectious bursal disease virus using recombinant T4 bacteriophage displaying viral protein VP2. Acta Biochimica et Biophysica Sinica, 37: 657-664.
13. Bublot, M., N. Pritchard, F.X. Le Gros and S. Goutebroze, 2007. Use of a vectored vaccine against infectious bursal disease of chickens in the face of high-titred maternally derived antibody. J. Compar. Pathol., 137: S81-S84.
14. Malik, M.W., N. Ayub and I.Z. Qureshi, 2006. Passive immunization using purified IgYs against infectious bursal disease of chickens in Pakistan. J. Vet. Sci., 7: 43-46.
15. Ko, K.Y. and D.U. Ahn, 2007. Preparation of immunoglobulin Y from egg yolk using ammonium sulfate precipitation and ion exchange chromatography. Poult. Sci., 86: 400-407.
16. Dubie, T., S. Yimer, M. Adugna and T. Sisay, 2014. The potential application of avian egg antibodies with emphasis on immunotherapeutic and immunodiagnostic purpose. Adv. Res. J. Biochem. Biotechnol., 1: 18-30.
17. You, J., Y. Xu, H. Li, L. Wang and F. Wu *et al*., 2014. Chicken egg yolk immunoglobulin (IgY) developed against fusion protein LTB-STa-STb neutralizes the toxicity of *Escherichia coli* heat stable enterotoxins. J. Applied Microbiol., 117: 320-328.
18. Li, X., L. Wang, Y. Zhen, S. Li and Y. Xu, 2015. Chicken egg yolk antibodies (IgY) as non-antibiotic production enhancers for use in swine production: A review. J. Anim. Sci. Biotechnol., Vol. 6. 10.1186/s40104-015-0038-8