Assessment of the Immunogenic Potential of the Infectious Bursal Disease Vaccine Virus (G-61) in Broiler Chicks

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Abstract

Using of numerous methods of vaccination of broiler chicks leading to variation in the average of immune response that elicited against Gumboro vaccine viruses. The study aimed to determine the effect of the immunogenicity of the live attenuated infectious bursal disease (IBD) vaccine virus (G-61 strain) in Broiler Chicks. A total of (160) chicks were divided into four equal groups namely A, B, C and D (40 chicks per group). The chicks of groups(A, B and C) were vaccinated with live attenuated IBD(G-61) vaccine at (14) days old, via the aerosol, intranasal and drinking water routes respectively while chicks of group D were left without vaccination as a control. An indirect enzyme linked immunosorbent assay (ELISA) was used to detect the antibodies titres against infectious bursal disease (IBD) vaccine virus in sera of chicks at (28) days of age. At (29) day of age, chicks of all groups were challenged by using of a virulent IBDV. At (40) day of age, the levels of antibodies against infectious bursal disease virus have been measured by indirect ELISA. The results obtained showed that higher levels of antibodies were noted when the vaccine administered via aerosol route as compared to the intranasal and drinking water. Following challenge of vaccinated chicks, the protection rates noted are correlated to the levels of antibodies elicited. It is concluded that the choice of approach to achieve higher and protective immune status against infectious bursal disease viruses in chicks is to apply IBDV (G-61) vaccine strain in broiler chicks via the aerosol route.

Key words: IBDV, antibody titre, aersol, vaccination methods.

الخلاصة

استعمال طرق عديدة للتلقيح ضد مرض الكمبورو في دجاج اللحم يؤدي الى تباين في معدل الاستجابة المناعية المتحفزة ضد فايروسات مرض الكمبورو .هدفت الدراسة تحديد تأثير القابلية المناعية ضد لقاح مرض التهاب جراب فابريشيا الخمجي الحي المضعف (عترة G-61) في فروج اللحم.تم تقسيم إجمالي الأفراخ وعددها (160) طير الى اربعة مجاميع متساوية سُمِيتْ ب (D,C,B,A) وبواقع (40) طير لكل مجموعة. تم تلقيح أفراخ المجاميع (C,B,A) بلقاح مرض التهاب جراب فابريشيا الخمجي الحي المضعف (عترة G-61) بطريقة الضبوب , والتقطير بالمنخرين , وبطريقة ماء الشرب على التوالي بينما تُرِكَتْ أفراخ المجموعة (D) بدون تلقيح وعُدَتْ كمجموعة سيطرة . أُستُخدِمَ أختبار الانزيم المناعي الممتز غير المباشر للتحري عن معيار الاضداد ضد فايروسات التهاب جراب فابريشيا الخمجي في مصول الدجاج عند عمر (28) يوم. أُعطِيَتْ جرعة التحدي باستخدام فايروسات مرض التهاب جراب فابريشيا الخمجي الضارية الى جميع أفراخ التجربة عند عمر (29) يوم وتم قياس معيار الاضداد ضد فايروسات التهاب جراب فابريشيا الخمجي في مصولها باستخدام أختبار الانزيم المناعي الممتز غير المباشر عند عمر (40) يوم.أظهرت النتائج أعلى معيار من الاضداد المتحفزة ضد فايروسات التهاب جراب فابريشيا الخمجي كان في مصول الدجاج الملقح بطريقة الضبوب مقارنةً بالدجاج الملقح بطريقة بالمنخرين ثم بطريقة ماء الشرب.وبينت النتائج أنّ معدل الحماية للدجاج الملقح بلقاح مرض التهاب جراب فابريشيا الخمجي الحي المضعف (عترة G-61) بعد أعطائهِ جرعة التحدي مرتبطٌ بمستويات الاضداد المتحفزة ضد فايروسات التهاب جراب فابريشيا الخمجي. نستنتج من ذلك , أنّ أفضل طريقة للحصول على أعلى مستوى من الحصانة المناعية ضد فايروسات مرض التهاب جراب فابريشيا الخمجي في فروج اللحم الملقح بلقاح مرض التهاب جراب فابريشيا الخمجي الحي المضعف (عترة G-61) كان بطريقة الضبوب.

Introduction

Infectious bursal disease virus (IBDV) primarily affects the bursa of Fabricius in young birds resulting in impaired immunological capabilities [1,2]. The disease is responsible for high mortality in 3 to 4 week-old chicks, but adult birds remained clinically less affected [3]. The control of the disease mainly through proper immunization as well as maintaining a good

hygienic environment [4].Many virus strains had been used as vaccines and classified into mild, intermediate and hot vaccines [5]. Intermediate vaccines were proved to be immunogenic without residual pathogenic effects on the vaccinated chicks [6,7, 8]. The enzyme linked immunosorbed assay (ELISA) is more sensitive, specific and reproducible in detecting antibodies against IBD virus [9,10].

Materials and Methods

Experiment Design

One hundred and ninety chicks were used in this study .They were obtained as one day old from Iraqi Company for production and marketing of meats, Al-Khalis poultry fields. Thirty chicks were selected randomly and blood samples collected by sacrificing of these chicks at one day of age for demonstration of indirect ELISA in order to measure the derived maternal antibody titer against infectious bursal disease virus in their sera.

The rest chicks were divided into four equal groups namely A, B, C and D (40 chicks per group). The chicks in the groups A, B and C were vaccinated with live attenuated IBD (G-61) vaccine at (14) days old, via the aerosol, intranasal and drinking water routes respectively while chicks in group D were left without vaccination as a control. At (28 days old), blood samples were collected. At (29 days old), chicks were challenged using a virulent IBDV. At (40) day of age, six blood samples were collected randomly from chicks of treated groups plus control group by puncture of heart for demonstration of indirect ELISA in order to measure the antibody titer against infectious bursal disease virus in their sera.

Collection of bursae samples

Infected bursae were collected from an outbreak of infectious bursal disease at a local poultry farms in Diyala governorate. Complete history of outbreak was taken. These samples were stored at −20 °C till used.

Field virus isolation and purification

A 10% (w/v) suspension of infected bursae was made by chopping and grinding them in sterilized pestle and mortar with sterilized sand after the method of Reddy *et al* [11]. The suspension was made in phosphate buffered saline (PBS) containing antibiotics (100 IU

penicillin-G/ml and 50 µg gentamicin sulfate/ml). This suspension was later centrifuged at 5000 r/min for 20 min and the supernatant was collected. The supernatant fluid was mixed with chloroform (1:1, v/v) in centrifuge tubes and centrifuged at 5000 r/min for 20 min. Three distinct layers were obtained: top layer containing virus, middle one containing bursal tissue debris and bottom layer was containing chloroform. The clear supernatant was collected in sterilized screw capped test tubes.

Titration of virus

The embryo lethal dose for (ELD_{50}) for the virulent infectious bursal disease virus were determined by inoculation of the virus in the incubated chicks emberyoes at 9 day-of-age embryos after making of series of ten fold serial dilution. Fertile eggs with 9 day-of-age embryos were used and injected with virus through chorioallantoic membrane (CAM) route. Ten fold serial dilutions of the virus suspension were performed from 10−1 to 10−10 in sterile normal saline and each dilution was injected in a batch of five eggs. The eggs were inoculated with each dilution at rate of 0.1 ml per egg. Ten eggs were inoculated with sterile saline and were kept as control. After inoculation all the eggs were sealed with melted wax and were re-incubated at 37.5 °C. Inoculated eggs were candled daily. Mortality during first 24 hours was discounted as non-specific. After 96 hours the eggs were opened and embryos were checked for lesions.

Methods of vaccine application

For administration of the vaccine in drinking water (DW), the vaccine was dissolved in an amount of water which should be consumed by the birds within approximately two hours. When using the aerosol method of vaccination, the vaccine was dissolved in a quantity of water equal to 1000 doses per liter and spread as a coarse spray evenly over the birds at a distant of 30-40 cm. For the intranasal (I/N) route of vaccination, the vaccine was dissolved in physiological saline solution (usually 30ml per 1000 doses) and administrated by means of a standardized dropper by which drop should be applied intranasally.

Statistical analysis

The two-way analysis of variance (ANOVA) and Leatest Significant Differences were used to determine the differences among groups of data obtained.

Results

Results of values of antibodies titer against IBDV of indirect ELISA test to at (28) day of age.

The results of table (1) revealed that there were statistically significant differences (P<0.05) in the mean values of titer of antibodies against (IBDV) among the following treated groups (A, B and C) in compared with control group at (28) day of age. The results showed that there was significant variations (P<0.05) in the mean values of titer of antibodies against (IBDV) between groups (A and C) whereas no significant differences (P<0.05) in the mean values of titer of antibodies against (IBDV) between groups (A and B) at (28) day of age as shown in table (1).

Table (1): Values of Antibody titer of indirect ELISA test against (IBDV) at (28) day of age.

Group	Mean	S .E	L .S .D
Group A	a 12347.1	± 1792	2742.16
Group B	a 10020	± 1889	
Group C	7525 b	± 1370	
Group D	c 2735.1	± 498	

Values are mean ± SE "Standard Error". L.S.D means Leatest Significant Differences. The letters that differ vertically indicate to statistical significantly variations (P<0.05).

Results of values of antibodies titer against IBDV of indirect ELISA test to at (40) day of age.

The results showed that there were an important statistically significant variations (P<0.05) in the mean values of titer of antibodies against (IBDV) among the treated groups (A, B and C) respectively in comparsion with control group at (40) day of age as shown in table (2). The recorded results in table (2) revealed that there was a significant increasing (P<0.05) in the mean values of titer of antibodies against (IBDV) of group "A" in compared with groups (B and C) respectively at (40) day of age. Control group gave the lowest mean values of titer of antibodies against (IBDV) in comparsion with all the treated groups at (28and40) day of age.

Table (2): Values of Antibody titer of indirect ELISA test against (IBDV) at (40) day of age

Group	Mean	S .E	L .S .D
Group A	a 17565.5	± 1770	2192.4
Group B	b 10993.3	± 1130	
Group C	c 6208.3	± 1133	
Group D	0 d	± 0	

Values are mean ± SE "Standard Error" .Values followed by different letters on the table are significantly different (P<0.05).

L.S.D means Leatest Significant Differences.

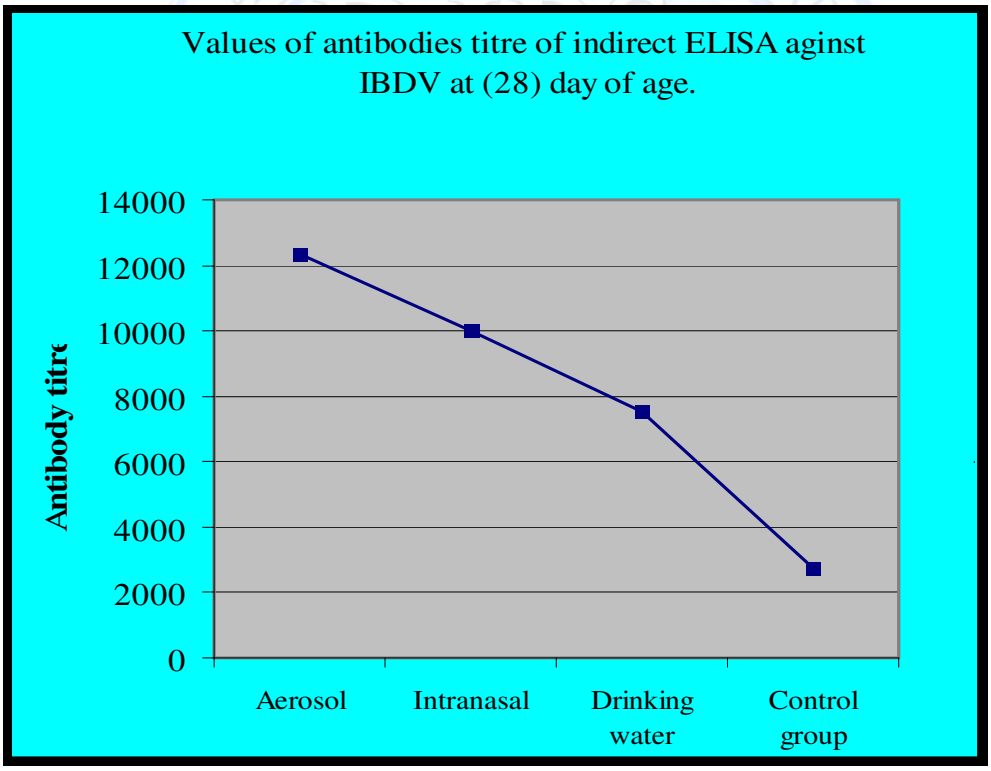

Figure (1): Values of Antibody titer of indirect ELISA test against (IBDV) at (28) day of age.

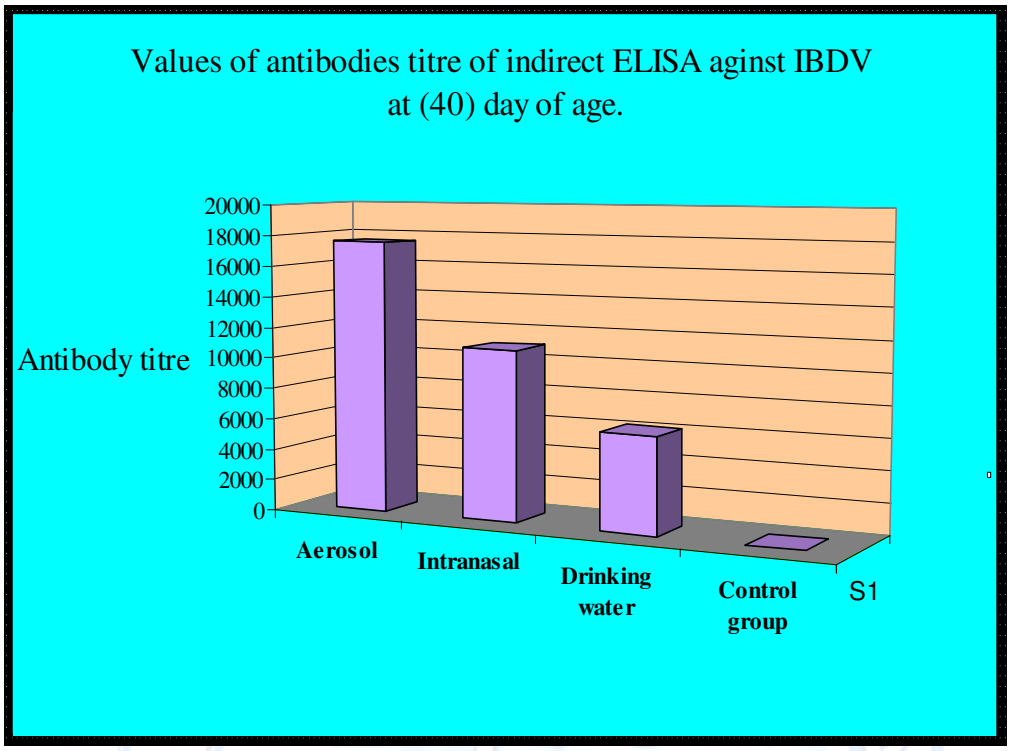

Figure (2): Values of antibodies titer against IBDV of indirect ELISA test at (40) day of age

Discussion

The antibody responses against IBD vaccine (G-61 strain) as detected in chicks by indirect ELISA are shown in tables 1 and 2 respectively. Using indirect ELISA, a significantly ($p < 0.05$) higher serologic response was found among chicks vaccinated via the aerosol followed by DW and the least response when chicks vaccinated via I/N. When indirect ELISA technique was employed to measure the antibody levels to the vaccine, it was observed that significantly ($p<0.05$) higher antibodies levels were obtained when aerosol route was used as compared to intranasal and drinking water (Table 1and 2). Following challenge of vaccinated birds with the virulent IBDV, the protection rates of the data obtained were in the groups of birds vaccinated via the aerosol, intranasal and drinking water respectively. The serologic response to the intermediate vaccine of IDB (G-61), administered via three commonly used routes of application, was assessed in the present study.

The protective potential of the immunogenicity of the IDB (G-61) vaccine and correlation of that protection to the antibody responses measured by indirect ELISA tests was also targeted in this study. It is interestingly that the highest response to the vaccine was observed

when given via aerosol route. The ability of this route to elicit high levels of antibody responses to avian viruses was previously confirmed by [12]. The recorded results in table (2) revealed that there was a significant increasing ($P<0.05$) in the mean values of titer of antibodies against (IBDV) of chickens of group of aerosol in compared with chickens of groups intranasal and drinking water respectively at (40) day of age. This promotes the vaccine as a good immunizing agent as these sites are not major sites for the virus replication. The virus was proved specifically replicating in the lymphoid [13] tissues especially those of the bursa of Fabricius. The protection rates obtained following challenge of chicks with the virulent virus strain were observed to correlate to the antibody responses induced by them. This confirmed the potential role of antibody in protection against the IBDV infection, the fact that recently confirmed by Hassan [14,15].

Conclusions

In conclusion, the intermediate IBD vaccine (G-61 strain) proved highly immunogenic and protective response when administered in chickens via aerosol route and indirect ELISA is a better serologic technique to monitor that potential of the virus.

References

1. Asi, Y. and A.S. Iyisan. Comparison of enzyme linked immunosorbent assay with the quantitative agar gel precipitation test for detection antibodies for infectious bursal disease in broilers. Pendik Hastakikari Merkez Arastirma (1992).Vol 22: 107.

2. Das S., N.N. Barman, A. Gohain, K. Sharma and D.K. Karma. Infectious bursal disease virus infection inducing immunosuppression in NDV vaccinated broiler chicken. Indian Journal of Animal Sciences (1996).Vol (8): 753-754.

3. Farooq, M., F.R. Durrani, N. Imran, Z. Durrani and N. Chand. Prevalence and economic losses due to infectious bursal disease in broilers in Mirpur and Kolti districts of Kashmir. International Journal of Poultry Science (2003). Vol (4): 267-270.

4. Haddad, E.E., C.E. Whitfill, A.P. Avakian, C.A. Ricks, P.D. Andrews, J.A. Thoma and P.S. Wakenell. Efficacy of a novel infectious bursal disease immune complex vaccine in broiler chickens. Avian Diseases (1997). Vol 41: 882-889.

5. Kumar Amiya, A.T. Roa and N.K. Parhi. An indirect ELISA for detection of antibodies in chicks infected with infectious bursal disease virus. Indian Veterinary Journal (1984). Vol 68:1109-12.

6. Marquardt, W.W., R.B. Johnson, W.F. Odenwald and B.A. Schlotthober. An indirect enzyme linked assay for measuring antibodies in chickens infected with infectious bursal disease virus. Avian Diseases (1980).Vol 24: 375-385.

7. Michele Guittet, N., H. Eterradossi, J.P. Le Coq, H. Lecoq and J.P. Picault. Quality control of infectious bursal disease vaccines. Proceeding of the Second International Symposium on Infectious Bursal Disease (IBD) and Chicken Infectious Anemia. (1994). (CIA) 162-170. Rauischholzhausen Germany.

8. Rao, V.N. and K.P.N. Rao. Immunodepression to Ranikhet disease vaccination due to infectious bursal disease virus in chicken. Indian Veterinary Journal (1992). Vol 69: 686-689.

9. Rosales, A.G., P. Villagas, P.D. Lukert, O.J. Fletcher M.A. Mohammed and J. Brown. Pathogenicity of recent isolates of infectious bursal disease virus in specific pathogen free chickens: Protection conferred by an intermediate vaccine strain. Avian Diseases (1989). Vol 33: 729-734.

10. Sahar, M.O., A.S. Ali and E. Mahasin. Residual pathogenic effects of infectious bursal disease vaccines containing intermediate and hot strains of the virus in broiler chickens. International Journal of Poultry Science (2004).Vol (6): 415-418.

11. Reddy, Y.K., Koteeswaren, A., Dorairajan, N., 1977. Pattern of infectious bursal disease in commercial white Leghorn chicken. *Indian Vet. J.*, 74:1019-1021.

12. Giambrone, J.J. and T.L. Hathcock. Efficacy of coarse-spray administration of a reovirus vaccine in young chickens. Avian Diseases (1991).Vol (1): Page 204.

13. Weimann, J. and S.B. Hitchner. Virus neutralization versus agar-gel precipitation test for detecting serological response to infectious bursal disease virus. Avian Diseases (1997).Vol 22: 598-603.

14. Tabidi, M.H., A. Makkawi, E. Mahasin and A.S. Ali. The serological responses in chicks vaccinated with combined or single for infectious bursal disease and Newcastle disease vaccines. Journal of Animal and Veterinary Advances (2004).Vol 3: 9-12.

15. Hassan, M.K., Very virulent infectious bursal disease virus in Egypt. Epidemiology. Isolation and Immunogenicity of classic vaccine. Veterinary Research communication. (2004) 28 (4) 347-356.