

EVALUATION OF INTRAYOLK SAC INOCULATION OF INFECTIOUS BURSAL DISEASE VACCINE ON IMMUNE RESPONSES IN NEWLY HATCHED BROILER CHICKS

Harith A. Najem

Department of Pathology and Poultry Diseases , College of Veterinary Medicine ,
University of Basra, Basra,Iraq.

(Received 18 December 2017 ,Accepted 22 January 2018)

Keywords:Ross broiler,IBD, lymphoid organ

Corresponding author E.mail:harithvet2007@gmail.com

ABSTRACT

A total of 60 one-day old Ross broiler chicks were used in this study. The birds were divided into four groups. The first group A was vaccinated against IBD with a Cevac IBD L vaccine at one-day old by intra-yolk sac method (IYS) with a dose of 0.5ml using a (1ml) syringe, whereas the second group B was vaccinated against IBD with the same vaccine at 14 days of age by drinking water method (DW). The third group C and fourth group D were considered as a positive and negative control respectively . On day 35, the birds were weight and killed to collect blood samples and lymphoid organs were removed and weight to study the effects of (IYS) rout on body weight , lymphoid organ weights and the humeral immune response to IBD vaccine which measured by ELISA test. The results showed significant differences at ($P \leq 0.05$) in body weight of group A, in addition significant ($p < 0.05$) increase in the all lymphoid organs weight , Bursa of fabricius (BF) was $1.643^a \pm 0.066$ in group comparison with other groups which had been recorded $1.102^b \pm 0.022$, $0.850^c \pm 0.067$ in group B and C respectively. Group B indicated that was a significant decrease at ($p < 0.05$) in the weight of of spleen which was $1.102^b \pm 0.022$ in comparison to control groups. With regard to serological test, bird from group A recorded higher Ab titer $24038^a \pm 685$ compared to those of other groups , and this increment was statically significant ($p < 0.05$), however both group A and B revealed a

significant differences($p < 0.05$) from the control groups. Generally the results indicated that intra-yolk sac method was highly effective rout of IBD vaccine administration.

INTRODUCTION

Infectious bursa disease (IBD), also known as Gumboro disease, is caused by a virus that is a member of the genus *Avibirnavirus* (family *Birnaviridae*). IBD is one of the major causes of economic loss to the poultry industry. This is mainly due to mortality, immunosuppressive state and loss in body weight of surviving birds. Prophylaxis is the best way to control the disease, especially vaccination, therefore poultry vaccines are widely applied to prevent and control contagious poultry diseases. Their use in poultry production is aimed to avoiding or minimizing the emergence of clinical disease at farm level, thus increasing production (1). Type of vaccine and rout of vaccination are effect on level of protection against infection. Commercial vaccine are administered to broilers in different ways, subcutaneously (SC) with an automatic injection, in ovo at 17 or 18 day of embryonic development, by spray vaccination of newly hatched birds, and by spray or in the (DW) at 7 to 12 days of age. In addition, a new technique, Inrayolk sac vaccination (IYS) has been introduced (2). Vaccination still remains the most effective method of prevention and control of IBD alongside good biosecurity measures (3).

Inrayolk sac vaccination route in broiler chicks is unusual route of administration and it is differ from accepted method .Therefore, this work aimed to investigate its immune efficacy of IBD vaccine through administration by Inrayolk sac of one-day old chicks

MATERIALS AND METHODS

Chickens:

A total of 60 one-day old Ross broiler chicks delivered from Saif Hatchery in Baghdad Province were used in this study. The chicks raised in an isolated cages in the

experimental house of the Department of Pathology and Poultry Diseases, College of Veterinary Medicine, Basra University under strict hygienic and standard management Conditions. Pellet feed and water were supplied *ad-libitum* during the interval of the experiment. They were reared for thirty five days.

Vaccine Strain:

CEVAC@ IBD L vaccine which contains the Winter field 2512 strain of Infectious Bursal Disease virus in live, freeze dried form was used in this study.

Experimental design:

One-day old Ross broiler chicks were randomly divided into four groups (A, B, C and D) with fifteen birds in each group. Group A were vaccinated with IBD vaccine at one-day - old by Intra-yolk sac method .The vaccines were reconstituted in distilled water to obtain one field dose in 0.5 ml, each 0.1 ml of the vaccine containing at least 10^7 and given individually by intra-yolk sac method using a (1 ml) syringe (2) . Chickens in group B were vaccinated against IBD at 14 days of age, by one dose was given orally to each chicken by syringe. Whereas chicks group C which was administrated through Intra-yolk sac with 0.5ml normal saline and acted as positive control, and chicks in group D were served as negative control group.

Monitoring of chicken:

Clinical monitoring of the chicken along the study period , to determine the effects of the vaccine . Mortality percentage was major parameter to monitoring chicken clinically.

Relative body weight and weight of lymphoid organs:

On day 35 of age, five birds were selected randomly each group and individually weighed and slaughtered. (BF), spleen and thymus were immediately removed and weighed in order to determine the effect of vaccine on these organs. (4).

Mean of body weight was estimated to demonstrate the effect of vaccine on body weight of vaccinated birds (5).

Serological examination:

At day of hatching blood samples from five chicks were collected after decapitation to determine maternal antibodies to IBD, and on day 35, five birds from each group were killed to collect blood. The blood samples were collected from birds, immediately transferred into sterile test tube and allowed to clot at room temperature for separation of serum, and then frozen at -20°C until the serological tests were performed. A commercial ELISA kit (BioCheck company, Holland) was performed according to manufactures direction to determine Ab levels against IBD vaccine (6).

Statistical analysis:

Data was performed in the bases of one-way analysis in variance (*SPSS* program version 19) depending on the experimental design; the significant ($P \leq 0.05$) differences were determined using least significant differences (7).

RESULTS

The vaccination of chicken have effects, as positive known as 'herd immunity' and negative effect known as post vaccine reaction . One of negative effects of vaccination is a mortality .

Table (1): Mortality rate (%) along study period.

Method of vaccination	No. of birds	No. dead birds	Mortality %
Intrayolk sac	15	1	6.6
Drinking water	15	2	13
Positive Control	15	3	20
Negative control	15	1	6.6

Table (1) show the result of clinical monitoring of chickens during experiment which was exhibited the mortality rate of all group along the period of four group. The

data revealed 6.6% in group A whereas 13 % in group B , 20% and 6.6% in group C and D respectively . The number of dead birds was higher in group C in comparison to the other group, but there was no statistical difference between all group at($p < 0.05$) level

Table2:Relative body weight and weight of lymphoid organs at 35 days of age.(means \pm SD)

Method of vaccination	Body weight(g)	Bursa of Fabricius(g)	Spleen(g)	Thymus(g)
Intrayolk sac	1008 \pm 46.8 a	1.643 \pm 0.066 a	1.204 \pm 0.023 a	0.517 \pm 0.032 a
Drinking water	904 \pm 24.9 b	1.102 \pm 0.022 b	1.210 \pm 0.031 a	0.352 \pm 0.035 c
Positive Control	989 \pm 34.11 b	0.850 \pm 0.067 c	0.795 \pm 0.036 b	0.458 \pm 0.042 b
Negative control	998 \pm 36.4 a	0.868 \pm 0.074 c	0.823 \pm 0.038 b	0.467 \pm 0.030 b

*Different letters vertically refers to presence a significant differences between groups. ($P < 0.05$).

N=5 sample in each group.

The parameter which have been concerned in this study were body weight, lymphoid organs weights and serological testing. Body weight of 5 birds at one day of age was 43.85g.

Table 2, revealed that the mean of body weight of group A which have been vaccinated by (IYS) was 1008^a \pm 46.8 these result significant difference at $P < 0.05$ compared with other groups especially group C 989^a \pm 34.11. Compared with control groups the mean values of group B, which have been vaccinated by (DW) 904^b \pm 24.9 were significantly decreased at ($P \leq 0.05$). Although of numerically difference in the results of control groups C 989 a \pm 34.11 and D 998 a \pm 36.4, compared with group A there was no significant difference at $P < 0.05$.

The mean of lymphoid organs weight included (BF) ,spleen and thymus in the table (2) of group A was 1.643^a \pm 0.066 , 1.204^a \pm 0.023 and 0.517^a \pm 0.032 respectively

that indicated there was a significant difference ($p<0.05$) in the weight of all lymphoid organs of vaccinated chickens (IYS) compared with other groups.

Group B indicated there was a significant increased at ($p<0.05$) in the weight of (BF) $1.102^b \pm 0.022$ and in the mean values weight of thymus $0.352^c \pm 0.035$ but the weight of spleen of same was recorded $1.102^b \pm 0.022$ significant decreased at ($p<0.05$) in comparison to control groups.

Table (3): ELISA Antibody titers against IBD vaccine (Mean \pm SD) at 35days of age

Method of vaccination	Age of vaccination	Mean
Intrayolk sac	1	24038 ^a \pm 685
Drinking water	14	20602 ^a \pm 452
Positive Control	-	12968 ^b \pm 421
Negative control	-	1312 ^b \pm 368

*Different letters vertically refers to presence a significant differences between groups. ($P<0.05$).

N=5 sample in each group.

The mean of Maternal Derived Antibodies (MDA) of 5 birds which was tested at the first day of age by ELISA was 24197. The antibody titer of IBDV vaccine at 35day of age which was tested by ELISA and have been shown in table (3), indicated that group A which vaccinated by Intra-yolk sac route was 24038^a \pm 685 this indicated higher antibody titer and 20602^a \pm 452 in group B which was vaccinated by drinking water method, all this result were a significant difference ($p<0.05$) compared with control groups which were 12968^b \pm 421 and 1312^b \pm 368 respectively. Although the numerical differences between group (A) and group B but there are no significant differences.

DISCUSSION

The effects of vaccine on clinical state of birds until the end of the experiment, which indicated by mortality rate shown in table (1). The mortality rate of the (IYS) vaccinated chickens were lower than the birds of other group C which inoculated with normal saline. This result was in agreement with that of (2) who found that the

vaccinated by (IYS) have no effect on mortality rate. Result of vaccinated chickens by (DW) indicated some numerical increased in mortality rate, in group B may be attributed to the effect of IBD vaccine on chickens as stress factor and this might be due to the type of vaccine, strain of chickens or might be due to the systemic reaction of the vaccine in case of drinking method of vaccination. . This result was in agreement with that of (8) who mention that the vaccination provides excellent protection against mortality after a natural infection. The positive control group C was higher mortality rate than (IYS) vaccinated group. This attributed to cross reaction with IBD vaccine virus from other groups.

Table 2, revealed that the mean of body weight of group A which have been vaccinated by (IYS) was significant increased at ($P<0.05$) compared with other groups especially group B . This result demonstrated that I day old broiler chicks vaccinated by (IYS) method against IBD have not negative effect on body weight . On other hand vaccination by (DW) produced lower body weight than non vaccinated control. This might be due to the stress produced by handling during vaccination process. The result in agreement with (10) who reported that decrease the body weight of vaccinated group with IBD vaccine . The result of the present study was in disagreement with (11) , who stated that the mean body weight gains for each week between vaccinated and non vaccinated groups were not significantly differed ($p>0.05$).

The mean weights of lymphoid organs (BF), spleen and thymus which show in table (2) there was a significant difference ($p<0.05$) in the weight of all lymphoid organs of group A compared with other groups. This good indicated referred to activity process of vaccine especially the chickens without clinical signs of IBD . These results were in agreement with those of (12), who reported that the weights of bursa Fabricius, spleen and thymus were found to be higher in non vaccinated broilers as compared to the vaccinated broilers.

Group B indicated there was a significant increased at ($p<0.05$) in the weight of (BF) and in the mean values weight of thymus. Our finding weight of (BF) and weight of thymus disagreement (13) who explained that reduction of lymphoid organ weight by the lower degree of attenuation of vaccines, in which the virus was capable to destroy the

lymphocytes, leading to reduction in their size. This differences may be attributed to stain of vaccine which used in experimental .

On the same group weight of spleen was recorded significant decreased at ($p<0.05$) compared with control groups. These results were in line with those of (10) who reported that the weight of lymphoid organs were decreased after IBD vaccination at 14th day of age. (14) also reported significant decreased in size of spleen due to sub-clinical of (IBD) .

The antibody titer of the present study as shown in table (3) , indicated that the titer was increased after vaccination in both groups in comparison to the control groups. This result was in agreement with those of (15) who reported that antibody titers were increased from 21st to 28th day post vaccination , ,Also (16) mention that post hatch vaccinated poults had significantly better compared to the non vaccinated birds during same period. On the other hand the control group , the titer was gradually declined from 1st to 35th days. This result in line with (17) who mention that the Passive Immunity has relatively short duration, commonly 1-2 weeks and generally less than 4 weeks and its function is to protect young chicks during a period (first few weeks) when their immune system is not fully developed to proper react to an early challenge.

The present study also revealed that (IYS) method of vaccination produced higher antibody titer in comparison to that of drinking water. (IYS) route of viral vaccines administration has not been yet experienced therefore there is no information in respect to its effect on lymphoid organs and antibody titers. Its effect on mortality and BW are the only information's has been found

CONCLUSION

In conclusion, (IYS) route was found to be effective in respect to immune response due to direct delivery of vaccine into the yolk sac of newly hatched chicks Finally, if (IYS) method is automated, a single operator can vaccinate a large number of birds due to labor-saving.

RECOMMENDATION

A comprehensive study should be conducted on (IYS) method of administration included clinical post – mortem changes and histopathology in addition to the effect of this method on yolk sac absorption.

تقييم حقن لقاح التهاب غدة فابريشيوس في كيس المح على الاستجابة المناعية في الأفراخ الحديثة الفقس

حارث عبد الله نجم

فرع الأمراض وإمراض الدواجن ، كلية الطب البشري ، جامعة البصرة، البصرة، العراق.

الخلاصة

استخدمت في هذه الدراسة ٦٠ من أفراخ الدجاج اللاحم بعمر يوم واحد. تم تقسيم الطيور إلى أربع مجاميع المجموعه ١٥ طير لكل مجموعه ، المجموعه الاولى لقت بعمر يوم واحد بلقاح التهاب جراب فابريشيوس بطريقة الحقن في كيس المح بجرعه ٠.٣ مل ، بينما المجموعه الثانية لقت بماء الشرب بالتجريب بعمر ١٤ يوم بلقاح حي بينما المجموعه الثالثة حقنت بمحلول متعادل في كيس المح بعمر يوم واحد واستخدمت كمجموعه سيطره ايجابية بينما المجموعه الرابعه كانت كمجموعه سيطرة سلبية وبعمر ٣٥ يوم وزنت الطيور وتم قتلها و جمع عينات الدم للدراسة تأثير التلقيح بطريقة الحقن بكيس المح على وزن الجسم ، ووزن الاعضاء اللمفاوية غدة فابريشيوس ، التوتة و الطحال والاستجابة المناعية باستخدام الاليزا بينت النتائج وجود فروقات معنوية للمجموعه (اي) لوزن الجسم ووزن كل الاعضاء اللمفاوية وكان وزن الجراب 0.066 ± 1.743 . مقارنة مع المجاميع الاخرى التي سجلت 1.102 ± 0.022 و 0.067 ± 0.850 ل مجموعه اي ومجموعه سي على التوالي ، مجموعه بي بينت هناك انخفاض معنوي عند مستوى اقل من ٠.٥ في وزن الطحال الذي كان 0.022 ± 1.02 مقارنة مع مجاميع السيطره

نتائج الاليزا للمجموعه اي سجلت ارتفاع في مستوى الاضداد 240.39 ± 685 مقارنة مع المجموعات الاخرى وهذه الزيادة كانت معنوية مقارنة مع مجموعات السيطرة وبصورة عامة اثبتت النتائج ان طريقة التلقيح بكيس المح كانت فعالة للتلقيح ضد مرض التهاب جراب فابريشيوس

REFERENCE

- 1- Marangon ,S. and Busani , L. 2006. The use of vaccination in poultry production . Rev. sci. tech. Off. inÈ. Epiz . 265-274.

- 2-**Thaxton J.P. and Vizzier- Thaxton** 2005. Intra-yolk sac delivery of viral vaccines to broiler. *J. Appl. Res.* 14:582-587.
- 3-**DeWit, J.J. and Baxeuciale, W.** 2013. Gumboro . [www. Gumboro. com](http://www.Gumboro.com). Assessed on 3/03/2013, 11 .56pm.
- 4-**Dawood, 1. M AL-Mayah.** 2012. Serological and histological Evaluation of Some Commercial Infectious Bursal Disease Vaccines in Broilers. Thesis submitted Department of Pathology and Poultry Diseases, College of Veterinary Medicine, Basra University, Basra, Iraq.
- 5-**Angani, M. T. ; Abdu, P. A. ; Sa'idu, L. and Kazeem, H. M.** 2014. Comparison of Bursal and Body Weights in Cockerels Vaccinated With a Live and two Killed Vaccines Against Infectious Bursal Disease Virus *J.Vet. Adv .* 4(6): 576-581 .
- 6- **Ali ,E.J .**2014 .Comparative study between some additives on immune response of Infectious Bursal Disease vaccine in broiler feed with aflatoxin- contaminated poisons. *I.J.S.N.,* Vol.5: 113-120 .
- 7- **Steel, R.G.; and Terrie, J.H.**1980. Principle and procedures of statistics. A biometrical approach. 2nd Ed. McGraw-Hill Book Company. New York, USA.
- 8- **Boven , M.V; Bouma,A; Fabri,T.H.; Katsma,E.; Hartog,L. and Koch,G.** 2008. Herd immunity to Newcastle disease virus in poultry by vaccination. *J. Avi. Path.* 37(1).
- 9- **Chamblee, T. N.; Brake, J. D.; Schultz, C. D. and Thaxton, J. P.**1992. Yolk sac absorption and initiation of growth in broilers. *Poult. Sci.* 71:1811–1816
- 10-**Al-mayah, A.S. and Abu Tabeekh, M.A.** 2010. Investigation on Bursa Fabricius and Body Weights in Broiler and Local Chicks Vaccinated with Two Types of Infectious Bursal Disease Vaccines. *Inter. J. Pout. Sci.* 9 (5): 464-467.
- 11- **Bolis, D.A., Paganini, F.J. ; Simon, V.A.; Zuanaze, M. Scanavini, N.H.; Correa, A. and Ito, N.** 2003. Gumboro Disease: Evaluation of serological and

anatomopathological responses in vaccinated broiler chickens challenged with very virulent virus strain. *Brazil. J. Poult. Sci.*, 5: 137-146.

12- Hair-Bejo, M.; Ng, M.K. and Ng, H.Y. 2004. Day old vaccination against infectious bursal disease in broiler chickens. *Int. J. Poult. Sci.*, 3: 124-128.

13- Nishizawa, M., A.C. Paulillo¹, A. Bernardino, A.C. Alessi¹, S. Sayd, L.S.N. Okada and L. Doretto Junior, 2007. Evaluation of anatomo-pathological, serological, immunological responses and protection in broilers vaccinated with live infectious bursal disease vaccines. *Arq. Inst. Biol. Sao Paulo.*, 74: 219-226.

14- Latif, I. k. ; Majed, H. M. and Sahar, H. 2014 . Determine the weight of thymus, bursa of Fabricius and spleen and its ratio to body weight in some diseases of broilers. *MRVSA.* 3(1), 8-14.

15- Bhattacharyya ,A.; Majumdar, S.; Bhanja, S.K.; Mandal , A.B.; Dash , B.B. and Kadam,M. 2016. Comparative Response to Breeder Vaccination, in Ovo Vaccination and Post Hatch Vaccination against Newcastle Disease on the Neonatal Growth Performance of Turkey Poults. *Inter. J. of Sci. Env. and Tech.*, Vol. 5, No 2, 366 – 373.

16- Bhattacharyya, A., Majumdar, S., Bhanja, S.K., Mandal, A.B. and Dash, B.B. 2013. Comparative response to maternal vaccination, in ovo vaccination and post hatch vaccination on the neonatal immunocompetent traits of turkey poults. *Indian J. Anim. Sci.*, 3: 16–19.

17- Roberto, S.2008. Passive immunity : part 1. *Ceva Animal Health Asia Issue No.18.*