EFFICACY OF INFECTIOUS BRONCHITIS DISEASE VACCINES AS MEASURED BY VIRAL SHED AFTER VIRULENT CHALLENGE IN BROILER

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ABSTRACT

Infectious bronchitis threatens the poultry industry throughout the world, The control of IB of the big problems in the world because of the wide variations in serotypes and development in the virulence of strains from time to time, and nature is very contagious, the rapid evolution in the specific tissue tropism and recombinants because of the synchronization of infection with different virus types and the use of live vaccines.found that the IB virus which is isolated from the recent outbreak is the same serotype but the difference genotype compared with the strains of current vaccine. Previous studies have indicated that the broiler vaccination with inactivated vaccines showed significantly less virus shed if challenge with the homologous vaccines (same genotype viruses) as compared with birds that vaccination genetically heterologous vaccines. The current study compared the extent of protection resulting from vaccination with live Commercial vaccines(Volvac® IB Mass MLV, Poulvac® IB Primer (D274), Avipro® IB M48 and mixed vaccine from (Volvac®, Poulvac® and Avipro®)). Vaccinates werechallenged with virulent field isolate (Variant2) strain.

Weekly post-vaccination, collected serum for analytical knowledge of the amount of antibodies using hemagglutiation inhibition test against all vaccine antigens used in the experiment after challenge with field virulent (Variant2 isolate), examine the birds daily to monitor the morbidity and mortality rates in selected periods for shedding virus by real time PCRto detect and quantitate the IBV viral copy number from clinical samples.After challenge with (Variant2) birdsvaccinated with mixed vaccine revealed less shedding virus compared to (Volvac®, Poulvac® and Avipro®)-vaccinated birdsboth separately.Genotypic differences between the vaccines and the challenge virus do not reduce the ability of vaccines to protect against the disease, but genotypic similarities reduce the virus shed and limiting its spread. The use of same

genetically advanced vaccines and expected to provide the best protection against the challenge with virulent field IB strains and limit the spread of poultry farming.

INTRODUCTION

Infectious bronchitis disease is the prototype of Coronaviridae, acute respiratory and contagious disease in the chicken Saif et al., (1). IB disease is characterized by respiratory signs of gasping, coughing, sneezing, tracheal rales and nasal secretions. While in young chicken's characterized by severe respiratory distress, either in laying hens are signs of respiratory distress and record a drop in egg production with loss of internal and shell quality of eggs (2). There are many strains of IBV have a high affinity for kidney infection (Nephropathogenic strains) and accompanied by a high rate of mortality in infected birds (3).IB disease outbreak could occur in vaccinated flocks as a result of the loss of cross-protection against antigens unrelated serotypes and variant strains of the virus (4). A recent study by Gelb et al., (5), proved that there is a significant immunological differences between strains and this explains the lack of cross-protection between IBV strains. In other studies proved that some vaccines as possible to stimulate protection against some field IBV infection (6). Some researches pointed that occurs in function of genetic diversity of variant strains origin (7). Many investigations reported to why the vaccination programs inefficiency to antigenic diversity of different IB strains because of possession of this disease on the genetic recombination phenomenon or the ability of the virus to cause mutation and generate new strains (8). Emerged and spread variant IBV strains from vaccinated flocks despite the use of many strains of IBV as live attenuated vaccines (9). Real time polymerase chain reaction RT-PCR assay is rapid and sensitive test was used method for the detection of nucleic acid and used widely in the detection of pathogens and quantification copy number of nucleic acid and analysis of gene expression (10). Therefore, the protection offered by IBV strains vaccines against IBV field isolates must be evaluation. The main purpose of serotyping of IBV strains is to determine the prevalence the field IBV strains and compared with IBV strains used in the vaccination programs accordingly, the heterologous protection can break the protection resulting from the vaccine, and this is what makes it difficult to establish effective control of vaccination.The aim of this study was to determine the effectiveness of live vaccines for various IBV strains of

commercial vaccines (Vovac®, Poulvac® and Avipro®) after which it is evaluating the immune response after vaccination and protection arising after the challenge.

MATERIALS AND METHODS

Experimental design:

Two hundred fifty broiler chicks were bought (breed: Rose 308, Origin: Belgian) in good condition from the hatchery Alpiedr- Baghdad. And divided randomly into five groups, each group containing fifty chicks.(A to E).The birds were primed with attenuated live IB vaccine at one days and repeated at 10 days oldVia intraocular injection.The birds of group A were maintained as non-vaccinated control.Group B were administered (Volvac® IB Mass MLV $10^{2.5}$ EID_{50}) vaccine.Group C were administered (Poulvac® IB Primer (D274) 10^{3} EID_{50}) vaccine.Group D were administered (Avipro® IB M48 10^{2} EID_{50}) vaccine.Group E were administered (mixed) vaccine.All groups offered at 28-days challenge with local virulent IBV strain (Variant2). Collected blood samples from the jugular vein at (7, 14, 21, 28, 35 and 42) to measure the antibodies titre against the IB disease by HI according to (11). Challenge birds were monitored daily for 10 days after challenge, to record the morbidity (respiratory and neurological signs) as well as the mortality rate.

RNA isolation and Real Time RT-qPCR:

The total RNA oftracheal, kidney tissue and fecal samples after 2 and day post challenge at 28 days old were extracted withTRIZOL Reagent® (Invitrogen, USA) and then cDNAswere obtained in the RT with a Superscript III Kit(Invitrogen, USA), as described previously. The Cdnasamples were submitted to real time quantitive PCR forthe absolute quantification of viral load, and thistechnique was conducted as recommended Okino*et al*.(12), except that the primers described by Wang andTsai (6) were used in place of HV+ and HV- primers. A linear regression was determined plotting the logarithmic values of the number of copy of plasmid DNA containing the insert of gene S1 against the cycle in other organs.threshold (Ct) values, in order to convert the Ct valuesfrom tissue samples into S1 gene copy number (12).

Challenge test:

Virulent local IBV strain (Variant2) used in the challenge in titration (100 ELD_{50} $10^{4.0}$) identified according to Reed and Muench (13). All challenge birds observed

daily for 10 days after challenge, to monitor the morbidity (respiratory and neurological symptoms) and mortality rate.

Statistical Analysis:

To illustrate the effect of different factors in the parameter of study used SAS system (14). To learn significant comparison between the means in this study used least significant difference (LSD) in multiple ranges.

RESULTS

Immunity against IB by HI test

This study aimed to evaluate the efficiency of the commercial IB vaccines with the best vaccination program gives the highest protection post challenge with virulent field IBV isolate (Variant2). (Table.1) showed birds vaccinated with different types of IB commercial vaccines induced high levels of antibodies titre.At one day old, non significant difference (P>0.05) among Ab titer of groups A, B, C, D and E (Table 1). It indicates correct randomization of experimental birds in the five groups. At day (7, 14 and 21) Therefore, all vaccinated groups (B, C, D and E) showed a significant difference at level (P <0.05) in antibodies titre as compared to control group(A).At 42 post-challenge with (Variant2 strain), the differences among Ab titer of all the groups were significant (P< 0.05); the highest titres were recorded in group E, followed by group C, B and D.Whereas the difference between the titres of groups B and D was non-significant (P>0.05).

Table 1. Antibody titers against IB measured by HI test of different groups (Mean ± SE) in different times

Day	Group A	Group B	Group C	Group D	Group E	LSD
1	31.6±0.9 a	30.2±0.8 a	31±0.8a	30±0.8a	31±0.7a	3.46
7	20.2±0.4 e	42.6±0.7 c	50.8±0.8b	39.8±0.4d	58.4±0.6a	2.77
14	15.4±0.5 e	77.2±0.5 c	84.4±1.01b	67±0.5d	96±0.7a	2.84
21	10.8±0.7 e	92±1.07 c	102.8±1.8b	82.8±0.7d	130.4±2.1a	5.91
28*	4.2±0.2 e	129.4±1.9 c	140.8±1.4b	112.2±1.7d	182±1.6a	6.29
35	20.4±1.21 d	64.4±1.27 c	82±1.62b	58±1.8c	199.4±2.2a	7.08
42	430.2±5.6 a	87.4±1.2 d	130.8±2.1c	81.6±1.4d	297±1.8b	12.24

*: challenge with (Variant2) at 28 days
Group A: Control group.
Group B: Vaccinated with IBV (Volvac® IB Mass MLV strain) vaccine Via intraocular injection.
Group C: Vaccinated withIBV (Poulvac® IB Primer (D274)strain) vaccine Via intraocular injection.
Group D: Vaccinated with IBV (Avipro® IB M48strain) vaccine Via intraocular injection.
Group E: Vaccinated with IBVmixed from (Volvac®, Poulvac® and Avipro®) vaccine Via intraocular injection.

Post challenge viral load distribution:

The results of viral load distribution of different tissues (trachea and kidney) and fecal samples in chickens vaccinated with live vaccine in different IBV strains and challenged with local IBV virulent Variant2 strain (100 ELD_{50} $10^{4.0}$) at 28 days of age are listed in Tables 2, 3 and 4. The results of the viral load at 2 and 4 days post challenge showed a high significant difference ($P<0.05$) between the 5 groups at 2 days, group A showed the height viral load (viral shedding) as compared with group Eshowed the most lowest ($P<0.05$) viral load followed by group (C, B and D).At 4 days post challenge the same trends were recorded in the five groups with significantly higher ($P<0.05$) viral load within and between the five groups. However, group A rank in the first place followed by groups (C, B and D) in the second and third rank respectively in compared with group E was recoded less viral shedding.

Table 2. Distribution of viral load (RT-PCR, means ± SE) of the trachea tissue.

Groups	Post challenge at 28 days old	
	2 days	4 days
A	3286.4±58.3**a**	6584.4±265.7**a**
B	548.4±5.2**b**	1695±18.01**b**
C	333±7.3**c**	1065.6±24.03**c**
D	602.6±18.8**b**	1906±13.7**b**
E	228±3.6**c**	464.4±19.7**d**
LSD	115.8	500.67

Number of samples=5.
-The different small letters refer to significant differences between different columns (P<0.05)

Table 3. Distribution of viral load (RT-PCR, means ± SE) of the kidney tissue.

Groups	Post challenge at 28 days old	
	2 days	4 days
A	3810±217.3**a**	6879.4±183.2**a**
B	1614.8±30.4**b**	1850.4±26.7b **c**
C	1066.8±29.7**c**	1575.2±35.9**c**
D	1901.2±24.8**b**	2099.4±43.1**b**
E	783.8±14.9**c**	1199.4±31.8**d**
LSD	416.37	365.6

Number of samples=5.
-The different small letters refer to significant differences between different columns (P<0.05)

Table 4. Distribution of viral load (RT-PCR, means ± SE) of the fecal samples.

Groups	Post challenge at 28 days old	
	2 days	4 days
A	5497.4±197.2**a**	9834±234.5**a**
B	1266.2±35.3**b**	1669.8±13**b**
C	956.4±21.3**c**	1246±23.7**d**
D	1576.6±40.2**bc**	1867.6±42.1b **c**
E	676.8±14.5**d**	1088.8±26.3**d**
LSD	384.16	449.87

Number of samples=5.
-The different small letters refer to significant differences between different columns (P<0.05)

Protection test

The data in Table 5. showed that the morbidity rate in group Ehad significant lower (P<0.05) rate (15)% followed groups (C, B and D) were recorded (20, 25 and 35)% respectively as compared with group Awas recorded 100%. While the no mortality rate was recorded in vaccinated groups except in groups (B and D)were recorded (5 and 8)% respectively, in compared with group A (control group) was recorded (100%) mortality rate.

Table 5. The protective levels

Groups	Morbidity %	Mortality %
A	100**a**	100**a**
B	25**b**	5**b**
C	20 **bc**	0**b**
D	35 **c**	8**b**
E	15 **b**	0**b**

*Number of chicks groups= 250

DISCUSSION

In the current study, evaluating the efficiency of different commercial IB vaccines after challenge virulent field IBV (Variant2)strain. Used in this study three parameters to assess the resulting protection after challenge represented by clinical protection depending on the morbidity and mortality rate, and immune protection through determined the antibodietitres for each antigen from vaccinal strains using HI test and viral protection in the trachea, kidney and feces as sites for the IBV replication. Increase HI antibody at day (7, 14 and 21) returns to the immune generated response after vaccination, these finding agreed with Nakamura et al., (15), whom found that the increase in (IgM, IgA and IgG) antibodies begin in gradually after vaccination with live attenuated IB vaccines, starting after the first week of the first vaccination.Thompson et al., (16), found variation in antibodies ratios (IgM, IgA and IgG) After examining the tracheal mucous membranes of vaccinated bird with live attenuated IB vaccines, where 70% of the samples containing IgA and 52% and 56% of the samples containing (IgM and IgG) respectively, due to the ability of the virus to cause cell mediated immunity able to prohibit any virus attack. Ignjatovic et al., (17), proved that the spray vaccination of live attenuated vaccine helps to make rapid localized immunity with large amounts of IgA antibodies. In chicken IgM, IgG and IgA produced as part of the immune response (18). The present study recorded a rise in the antibodies titres in days 14 and 21 after the second vaccination (booster doses) at 10 days of age, these finding agreed with De Wit et al., (19), found that large amounts of memory cells stimulated to produce additional quantities of antibodies after the second vaccination with same antigen. After the sixth day of the vaccination antibodies detected in the vaccination site and in the blood up to the highest levels after 21-28 days of vaccination (20). In the present study there found a significant decrease in antibodies levels at 35 day and this goes back to the challenge with virulent IBV strain at 28 days and in general decline occurrence in antibodies as a result of the challenge variant IBV were similar these results with (21), which proved

that the challenge with variant IBV strain caused a significant decrease in the levels of antibodies due to variation in S1 antigen. While Group E recorded a high-protection level compared with other vaccinated groups these results are consistent with Okino et al., (12), who reported that the obtained protection after vaccination with haemagglutinating IBV-strain is the best and highest protection, which are obtained from non -haemagglutinating IBV-strain whenever there is any serotyping convergence between vaccinal and challenge strains was the best immune. The rise in Abs levels at 42-day, especially in Group E has been interpreted by Thompson et al., (17),Who reported that the significant elevation in immune antibodies at level ($P < 0.05$) after challenge in vaccinated birds compared it's level in control group (non-vaccinated birds) is the result recognition the virus by the immune system (there are more than 11 site Epitopes) of spike glycoprotein, which lies on the IBV envelope is specific to stimulating antibodies. Results of Viral load revealed to decrease viral shedding after challenge in group E compared to vaccinated groups, especially in the trachea compared to kidney and feces these results agreed with Pei et al., (22), who proved that the challenge with IBV isolate antigen identically with vaccinal strains give less viral shedding compared to variant strains as well as reported (23) the challenge with nephropathogenic IBV strains have little effect on the trachea in comparison with kidney tissue and the results agreed with the study (24) who reported less protection can be obtained against (nephropathogenic IBV strains) and less viral shedding is vaccination by using similar genetically strains.So that variations in the amino acids composition located in different regions of the S1 glycoprotein as a result of the change of the IBV tissue tropism, virulence and antigenicity all these main strategy of IBV to escape from mechanical defense of the host (25). In addition to the less virus shedding in the environment, the vaccinated birds against IBV able to resist the challenge, which requires large amounts of the virus to become infected birds (26).

CONCLUSION

Our studies show that virus shed can becontrolled by mixed vaccines that are more genetically similar to the challenge virus.

قياس كفاءة لقاحات التهاب الشعب الهوائي المعدي بواسطة الطرح الفايروسي بعد التحدي بالضاري في الدجاج اللاحم

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الخلاصـــــــــة

يهدد مرض التهاب الشعب الهوائية المعدي صناعة الدواجن في جميع أنحاء العالم، وتعد السيطرة على هذا المرض من المشاكل الكبيرة في العالم بسبب الاختلافات الواسعة في الأنماط المصلية التطور في ضراوة السلالات من وقت لآخر، والطبيعة السارية للغاية، والتطور السريع الفية الفايروس للنسيج بسبب تزامن عدوى مع مختلف أنواع الفيروسات واستخدام لقاحات حية. وجدت أن فيروس IB الذي عزل من الاندلاع الأخير هو نفس النمط المصلي إلا أن الاختلاف في النمط الجيني مقارنة مع سلالات اللقاح الحالي. وقد أشارت دراسات سابقة إلى أن التلقيح الدجاج اللاحم باللقاحات المعطل أظهر أقل طرح للفايروس اذا كان التحدي مع لقاحات متجانسة (الفيروسات من نفس النمط الجيني) بالمقارنة مع الطيور التي لقحت باللقاحات المغاير وراثيا. وقارنت الدراسة الحالية على مدى الحماية الناتجة من التلقيح باللقاحات التجارية الحية (Volvac® IB Mass MLV, Poulvac® IB Primer (D274), Avipro® IB M48 and mixed vaccine (Volvac®, Poulvac® and Avipro®)). وقد تم التحدي مع العزلة الحقلية الضارية (Variant2). اسبوعيا، جمع مصل الدم لمعرفة كمية الأجسام المضادة باستخدام الاختبار التثبيطيhemagglutiation ضد جميع مستضدات الفايروس اللقاحي المستخدم في التجربة بعد التحدي مع العزلة الحقلية الضارية (Variant2)، تفحص الطيور يوميا لمراقبة معدلات الاصابة والوفيات في فترات مختارة لمعرفة طرح الفيروس باستخدام PCR للكشف عن كمية اعداد نسخ IBV في العينات السريرية. بعد التحدي مع (Variant2) الطيور الملقحة بلقاح المختلطة كشف أقل طرح للفايروس مقارنة بالطيور الملقحة (Volvac®، Poulvac® وAvipro®) كل على حدة. الاختلافات الوراثية بين الفايروس اللقاحي وفيروس التحدي لا تقلل من قدرة اللقاحات للوقاية من المرض، ولكن التشابه الوراثي يقلل من طرح الفايروس والحد من انتشاره. استخدام اللقاحات المتطورة وراثيا، من المتوقع أن توفر أفضل حماية ضد التحدي مع سلالات مرض التهاب الشعب الهوائي المعدي الحقلي الضارية والحد من انتشارها في تربية الدواجن.

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