Evaluation of killed infectious bronchitis vaccine from different commercial strains

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Abstract

This study was conducted to evaluate multivalent inactivated infectious bronchitis (IB) vaccine from the commercial IB (H120, Ma5 and 4\91) strains on the Abs response (titre) in chicks reared up to 40 days. Two hundred and fifty broilers (Ross breed) at one day old were divided randomly into 5 groups (50 chicks of each group) diet and drinking water offered ad libitium, experimental groups vaccinated as follows: Group A: Vaccinated with 0.2 ml inactivated IBV vaccine. Group B: Vaccinated with 0.3 ml inactivated IBV vaccine. Group C: Vaccinated with 0.5 ml inactivated IBV vaccine. Group D: Vaccinated with 0.25 ml inactivated ND+IB (Commercial). Group E: Control group. All groups except the control group vaccinated with IBV at 4 days old via subcutaneous route, and vaccinated with attenuated ND+IB (Volvac®) at one day old via ocular route, and vaccinated with attenuated IBD (Ceva®) at 14 days old via drinking water route, and vaccinated with live ND (*La sota* strain) (Boehringer®) at 10 and 20 days old via drinking water route. All groups were challenged with local virulent IBV isolate (Variant 2 and QX) at 28 days old. Morbidity and mortality were recorded in all the challenged birds. Blood samples were collected from jugular vein at (7, 14, 21, 28, 35 and 42) and transported to the laboratory for serum separation which were used in the ELISA and HI test to determine the antibody titer against IBV. Statistical analysis showed that ELISA and heamagglutination inhibition (HI) antibody titer of IB are antigen specific after challenge with local isolated of IBV (QX and Variant2) strains, group C were significantly ($P \leq 0.05$) higher than other groups and control group.

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Key word: Infectious bronchitis disease, killed vaccine, broilers, HI, ELISA.

تقييم لقاح التهاب القصبات المعدي المقتول من العتر اللقاحية التجارية

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الخلاصة

اجريت هذه الدراسة لتقييم لقاح مقتول من عدة عتر تجارية لمرض التهاب القصبات الهوائية المعدي (Ma5, IBH120 4/91) على معيار الاستجابة المناعية لمرض التهاب القصبات الهوائية المعدي في الافراخ المرباة لغاية اربعين يوم. 250 فرخة بعمر يوم واحد سلالة Ross حيث قسمت عشوائيا الى خمس مجاميع كل مجموعة خمسين فرخة، العلف والماء اعطت بشكل حر، المجموعة A: لقحت باللقاح التجريبي بجرعة 0.2 مل تحت الجلد، المجموعة B: لقحت باللقاح التجريبي بجرعة 0.3 مل تحت الجلد، المجموعة C: لقحت باللقاح التجريبي بجرعة 0.5 مل تحت الجلد، المجموعة D: لقحت باللقاح التجاري بجرعة 0.25 مل تحت الجلد، المجموعة E: لم تلقح باي لقاح واعتبرت مجموعة سيطرة. كل المجاميع لقحت بلقاح التهاب القصبات المعدي بعمر 4 ايام من العمر تحت الجلد، ولقحت باللقاح الحي المضعف المزدوج من النيوكاسل والتهاب القصيات المعدي بعمر يوم واحد عن طريق التقطير بالعين، ولقحت باللقاح الحي المضعف لمرض التهاب جراب فابريشيا المعدي بعمر 14يوم عن طريق ماء الشرب، ولقحت باللقاح الحي لمرض النيوكاسل عترة لاسوتا بعمر 10 و20 يوم من العمر عن طريق ماء الشرب. جميع المجاميع عرضت الى فحص التحدي باستخدام عزلتين من مرض التهاب القصبات المعدي

الحقلية الضارية (QX and Variant2) بعمر 28 يوم من العمر وسجلت العلامات السريرية ونسبة الهلاكات خلال مدة التحدي لكل المجاميع. جمعت عينات الدم من الوريد الوداجي بعمر (7، 14، 21، 28، 35 و42) ونقلت الى المختبر لفصل المصل وتقدير مستوى الاجسام المناعية ضد مرض التهاب القصبات المعدي بواسطة فحص الاليزا وفحص اثباط اتلازن الدموي. واظهرت نتائج التحليل الاحصائي لمستوى معيار الاجسام المناعية ضد فايروس التهاب القصبات المعدي لفحص الاليزا واثباط التلازن الدموي ان المجموعة C تفوقت معنويا بمستوى ($P<0.05$) مقارنة ببقية المجاميع ومجموعة السيطرة.

الكلمات المفتاحية: لقاح التهاب القصبات المعدي، المقتول من العتر اللقاحية التجارية، الدواجن، الاليزا، اثباط التلازن الدموي.

Introduction

Poultry industry play important role in feed security in the world and is regarded important source of meat according to saif *et al.*, (1), two imported and dangerous disease causes high economical loses, in broiler and layers, these are infectious bronchitis (IB) and air sacculitis (AS), in different area of the world (2). Both diseases have the ability to induce respiratory sign and reduced egg production in layers and the interaction of *E. coli* and IB was studied by Shalish and Zahid, (3), they found a relationship between these two disease. The severity of IB and high incidence of outbreaks is due to the ability of IB virus for mutagenic activity to produce new serotypes (4). In Iraq the new serotypes were isolated and sequenced as QX and Variant2 these two strains were studied by Zahid and Keshwan (5), this study had confirmed the severity of variant2 in Iraq as the main cause for mortality in broiler with nephropathogenic lesion. Out-breaks of IB frequently occurs in the field in many countries because the virus has a tendency of frequent mutations and many variants strain with changes in the genome have been identified (6). Controlling and prevention of the IBV by using several types of vaccines, both live and inactivated vaccines are employed for mmunization against IB. Hasan and Zahid, (7) Proved that immunization by using a IBV live vaccine prepared from three vaccinal strains of IBV (H120, Ma5 and 4/91) gave protection against challenge with IBV (QX and Variant2). Accordingly, the protection afforded by vaccines strains against field isolates must be assessed. The main interest of serotyping IBV is to determine the prevalence of IBV strains in the field and to compare them with the IBV strains used in the vaccination programs, since the heterologous protection can break through the immunity induced by vaccines, make it difficult to establish effective vaccination control. The objective of the present study was to investigate the efficacy of a trivalent killed vaccine from vaccinal strains (H120, Ma5 and 4/91). The immune response following vaccination as well as protection to challenge was evaluated.

Materials and Methods

Two hundred and fifty broiler chicks (Breed: Rose 308, Origin: Belgium) were brought in good condition from AL-Anwar Hatchery-Baghdad. The chicks weight at hatching was with an average of 39.3 gm, they were divided randomly into 5 groups, each group contained 50 birds in poultry field distance 8 × 4 m divided into five groups by plastic barriers and the ground mattress with wood sheavers litter and supplemented all management requirements as poultry hygiene standardization. Lighting and ventilation were controlled according to Allam (8). Experimental groups vaccinated as follows: Group A: Vaccinated with 0.2 ml inactivated IBV vaccine. Group B: Vaccinated with 0.3 ml inactivated IBV vaccine. Group C: Vaccinated with 0.5 ml inactivated IBV vaccine. Group D: Vaccinated with 0.25 ml inactivated ND+IB (Commercial). Group E: Control group. The groups A, B, C were vaccinated with IBV vaccine was prepared by passage on chicken embryo and tissue culture. All groups

except the control group vaccinated with IBV at 4 days old via subcutaneous route, and vaccinated with attenuated ND+IB (Volvac®) at one day old via ocular route, and vaccinated with attenuated IBD (Ceva®) at 14 days old via drinking water route, and vaccinated with live ND (La sota strain) (Boehringer®) at 10 and 20 days old via drinking water route. All groups were challenged with local virulent IBV isolate (Variant 2 and QX) at 28 days old. The blood samples were collected from jugular vein at (7, 14, 21, 28, 35 and 42) to evaluate the antibody titre against IBV disease by ELISA and HI test according to (9). All the challenged birds were observed daily for 6-10 days post challenge, morbidity (respiratory and nervous signs) and/or mortality were recorded (10). The Statistical Analysis System- SAS (11) was used to clarify the effect of different factors in parameters study. Least significant difference-LSD multiple range test was used to significantly compare between means in this study.

Results and Discussion

The result showed that the maternal immunity (M.I) of 18-serum sample out of 250 one-day old chicks (before division into groups) as shown by ELISA and HI test was (15671.54±1654) and f(398.4±33.6) respectively. This result was in agreement with Hamal *et al*., (12), who demonstrated that there are several serotypes of IBV reached the reproductive system of chickens (hens vaccinated by IB attenuated vaccine at early breeding stages followed by oil emulsion at 18 weeks) resulted in activation of the mucosal immunity in the reproductive tract causing direct release of IBV antibodies into the eggs. During the development of the reproductive tract of the hens, some of stimulated lymphocytes localized in the lamina propria of the oviduct and in the stroma of the ovary. Antibodies produced locally in these organs usually represent a significant source of the transferred antibody to the eggs (13). These findings agree with data obtained by Gharaibeh *et al*. (14) who found that the IBV had the second highest transfer rate (maternal Ab titer) among ten broiler diseases (chicken anemia virus, infectious bursal disease virus, laryngotracheitis virus, *Mycoplasma gallisepticum* , *Mycoplasma synoviae*, Newcastle disease virus, and reovirus) at 37 weeks of stocks breeder. The same authors found that the protection at seven days were less than 30%. This is in agreement with Hamal *et al.*, (12) who observed that MDA decreased substantially at day 7, and were no longer detected at day 14. Table 1. summarizes the Abs titers of chicks vaccinated with live vaccine ND+IB (Volvac®) at one day old via ocular route and inactivated IBV vaccine in different doses. Sera were collected after 7 days up to 42 days and subjected to ELISA test (mean ± SD) for the determination of Abs titers. At the age of 28 days, all groups were challenged with local virulent IBV (QX and variant) virus strains with a dose of $10^{5.3}$ ELD50 and $10^{6.0}$ ELD_{50} respectively. The results showed that the Mabs in Group E (control) was persisted to an optimum levels up to 14 days and not detected at 21 days. After challenge with virulent IBV QX strain at (28 days) the Abs increased at 42 to 14938.6±213.3 compared to virulent IBV variant strain was recorded higher Abs titre 18002.9±248 than challenge with QX strain. Groups (A, B, C and D) showed significantly higher ($p<0.05$) Abs titers in comparison with the control group (E), (Tab 4.7). From day 7 to day 21 the Abs titers gradually decreased in group (A to E). However, at days 28 group C (experimental vaccine in dose 0.5 ml) had significantly higher Abs titers followed by group B (in dose 0.3 ml), group A (in dose 0.2 ml) and group E (commercial vaccine) in comparison with control group. At the age of 35 days (after challenge with QX strian), group C (in dose 0.5 ml) had significantly higher ($p<0.05$) Abs titers compared with group (B, C and D). At the age of 42 days, group C significantly differe ($p<0.05$) from B and A (7498.9±195.6, 6168.7±213.8 and 5346.3±196.1) respectively, in comparison with commercial vaccine in group D which was (5060.9±201.2) However, no significant difference was recorded

between group A and D. while the results of Abs titre at day 35 after challenge with IBV variant strain, group C was recorded highest Abs titre among others vaccinated groups also at day 42 group C significantly differe ($p<0.05$) from B and A (5865.4±308, 4488.7±246.3 and 4070.9±166.7) respectively, in comparison with commercial vaccine in group D which was (2431.8±111.6). Comparing the result of experiment and commercial IBV vaccine at the age of 42 days (slaughter age) demonstrated that experimental killed vaccine group C and B (in dose 0.5 and 0.3 ml) induced significantly higher Abs titers as compared with commercial killed vaccine group D in dose (0.25 ml) while, group A (in dose 0.2 ml) of experimental vaccine gave Approach results of Abs titre with commercial vaccine in group D.

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

Day	Group A	Group B	Group C	Group D	Group E	LSD
7	6709.3±250.8 A	7115.6±418.4 A	7018.8±206.4 A	6817.3±210.5 A	7136.2±350 A	851.52
14	2111.1±62.2 A	2139.6±107.1 A	2207.2±126.5 A	2008.6±84.3 A	1722.4±89.7 B	274.63
21	1071.2±32.5 BC	1236.8±62.7 B	1445.8±56.8 A	1222.1±41.7 C	523±78.5 D	161.76
28	2258.1±125.8 C	3090.5±99.4 B	3961.8±202.2 A	2269.3±138.6 C	183.4±40.8 D	376.56
QX 35*	1873.5±55.3 C	2163.8±74.6 B	2544.6±120.1 A	1546±87 D	138.9±34.1 E	227.03
V 35*	1557.6±89.1 B	1899.8±62.9 A	2059.6±54.4 A	1072.3±41.8 C	138.9±34.1 D	169.81
QX 42#	5346.3±196.1 D	6168.7±213.8 C	7498.9±195.6 B	5060.9±201.2 D	14938.6±213.3 A	581.24
V 42#	4070.9±166.7 C	4488.7±246.3 C	5865.4±308 B	2431.8±111.6 D	18002.9±248 A	645.81

QX 35*: Ab titre against IBV at 35 days after challenge with (QX) strain. V 35*: Ab titre against IBV at 35 days after challenge with (Variant2) strain.

QX 42#: Ab titre against IBV at 42 days after challenge with (QX) strain. V 42#: Ab titre against IBV at 42 days after challenge with (Variant2)

Group A: Vaccinated with 0.2 ml inactivated IBV vaccine at 4 days old via subcutaneous route.
Group B: Vaccinated with 0.3 ml inactivated IBV vaccine at 4 days old via subcutaneous route.
Group C: Vaccinated with 0.5 ml inactivated IBV vaccine at 4 days old via subcutaneous route.
Group D: Vaccinated with 0.25 ml inactivated ND+IB (Commercial) at 4 days old via subcutaneous route.
Group E: Control group.

The results of (Table 2.) revealed no significant differences ($P>0.05$) in antibody titers among vaccinated groups at 7 and 14 days. A significant ($P \leq 0.05$) increased antibody titers against IBV virus in blood serum samples of the group C at days 28 group C (experimental vaccine in dose 0.5 ml) had significantly higher Abs titers followed by group B (in dose 0.3 ml), group A (in dose 0.2 ml) and group E (commercial vaccine) in comparison with control group. At the age of 35 days (after challenge with QX strain), group C (in dose 0.5 ml) had significantly higher ($p<0.05$) Abs titers compared with group (B, A and D). At the age of 42 days, group C significantly differe ($p<0.05$) from B and A (192±19.8, 156.8±16.8 and 134.4±14.9) respectively, in comparison with commercial vaccine in group D which was (128±16.5) However, no significant difference was recorded between group A and D. while the results of Abs titre at day 35 after challenge with IBV variant strain, showed that, group C recorded highest Abs titre among others vaccinated groups also at day 42 group C significantly differe ($p<0.05$) from B and A (149.7±18.1, 114.4±19.3 and 102.4±19.5) respectively, in comparison with commercial vaccine in group D which was (61.5±11.9). In addition, the control group (E) recorded a significant ($P\leq0.05$) increased antibody titers as compared with vaccinal groups at (42) days old which was

(378.9±18.9) after challenge IBV (QX) strain while (457.3±21) after challenge wit IBV (variant) strain.

Table (2) 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
7	169.6±19.1 A	179.2±20.9 A	179.2±20.9 A	172.8±23.4 A	181.9±42.2 A	81.711
14	52.8±5.8 A	54.4±9.6 A	56±9.9 A	51.2±10.6 A	43.2±10.9 A	27.234
21	31.3±1.8A B	32±1.4 AB	35.6±1.7 A	27.2±2.4 B	6.4±0.6 C	4.94
28	57.6±9.3 B	78.6±13.9 AB	100.6±14 A	57.6±9.3 B	4.6±0.6 C	30.25
QX 35*	48±5.3A B	54.4±9.6 AB	65.4±11.3 A	38.4±4.2 B	3.6±0.7 C	20.85
V 35*	39.6±4.2 AB	48±10.6 AB	52.6±4.8 A	27.2±4.8 B	3.6±0.7 C	17.02
QX 42#	134.4±14.9 C	156.8±16.8 BC	192±19.8 B	128±16.5 C	378.9±18.9 A	94.82
V 42#	102.4±19.5 BC	114.4±19.3 B	149.7±18.1 B	61.5±11.9 C	457.3±21 A	52.06

QX 35*: Ab titre against IBV at 35 days after challenge with (QX) strain. V 35*: Ab titre against IBV at 35 days after challenge with (Variant2) strain.

QX 42#: Ab titre against IBV at 42 days after challenge with (QX) strain. V 42#: Ab titre against IBV at 42 days after challenge with (Variant2)

Group A: Vaccinated with 0.2 ml inactivated IBV vaccine at 4 days old via subcutaneous route.
Group B: Vaccinated with 0.3 ml inactivated IBV vaccine at 4 days old via subcutaneous route.
Group C: Vaccinated with 0.5 ml inactivated IBV vaccine at 4 days old via subcutaneous route.
Group D: Vaccinated with 0.25 ml inactivated ND+IB (Commercial) at 4 days old via subcutaneous route.
Group E: Control group.

The results of (Tab. 1, 2) referred that there are no significant differences (P>0.05) in antibody titers among vaccinated groups and control at 7 and 14 days old which may be due to interferance between the maternal antibody with the vaccine strain. These findings in this study were in agreement with Gharaibeh *et al.,* (14) and Zahid *et al.,* (15) who mentioned that the maternal antibody titers may interfere with the response to the first and second vaccine stimulus promoting neutralization of the first vaccination and a different response at the second one, according to high or low maternal antibodies. The increase in Abs titre at 21 and 28 days age return to immune response of inactivated vaccine these results are in agreement with Alexander, (16) who confirmed that the inactivated vaccine which was used in early age of chicks led to elevation immune response with progress of the time especially after 14-21 days of vaccination, also, Grimes, (17) mentioned that the inactivated vaccine needed for long time about 21 days to reach a high level of antibody production. also this is in agreement with Tzvetkov *et al.,* (18) and Ali *et al.,* (19) who mentioned the inactivated oil emulsion vaccine are not adversely affected by maternal immunity as live vaccine because the oil adjuvant acts as stimulus of defense mechanism and disperse antigen slowly. In these circumstances, there is a progressive stimulation of the active immunity while the passive immunity declines and the immune system reaches full competence. The results of this study showed a significant decrease in antibody titre in day 35 this is due to the challenge with local isolate of IBV at day 28, generally low antibody titre post challenge with local IBV isolate agree to the findings described by (20, 21), who showed that a low antibody titre when infected with variant IBV strains. While the group C recorded high protection in comparison with other vaccinated groups after challenge. The results agree with Kapczynski, (22) who mentioned the positive relationship between high level dose of vaccine and antibody titers, then chicks will protect after challenge test, therefore, the

group C and B group showed a significant (P≤0.05) increased antibody titers as compared with other vaccinated groups of inactivated vaccine, may be due to increase in the dose of antigen in the prepared vaccine (two strains of IBV) than commercial vaccine (one strain) whereas the killed organism do not replicate in vaccinated birds or spread between birds in the flock, these finding agreed with finding of (23, 24). The results of the current studies showed high Abs titre in vaccinated group after challenge these finding were in agreement with the results of Okino.,*et al* (25) who mentioned the effect of tracheal memory humoral immune responses mediated by lachrymal IgG and IgA anti-IBV antibodies, at one and five days post-infection, in the mucosa against infection with local IBV strain. Fig. (1) Summarized the development of the clinical signs and mortalities during 14 days post challenge with local IBV (QX) isolate at 28 days of age. The mortality was recorded dialy and represented as total number of dead chicken in each group. The result of the present study confirmed that vaccinating groups group (C, B and A) with experimental vaccine in different doses gave lowest morbidity percent (20, 30 and 50) respectively, on the other hand the mortality percentage which was (0, 10 and 30) in comparison with vaccinating group D that's received commercial vaccine in dose 0.25 ml recorded medium percentage (morbidity 60 and mortality 30) while the control group registered the highest percentage in (morbidity 100 and mortality 50).

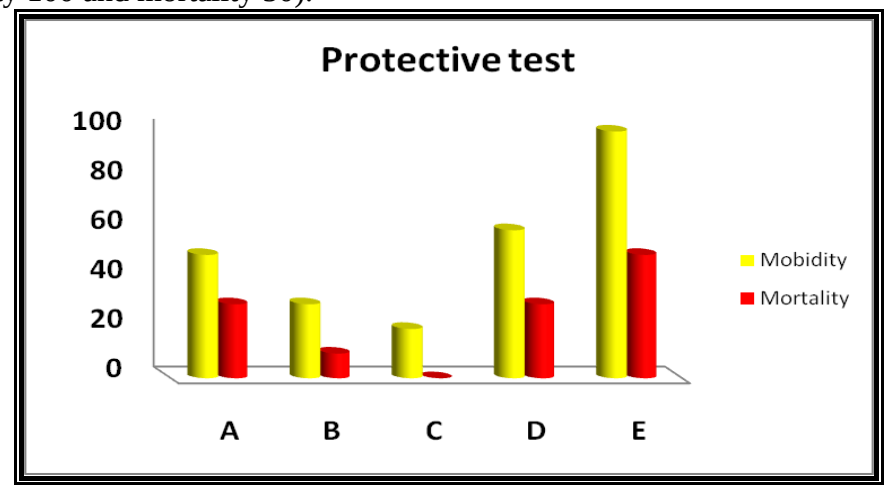

Fig. (1) Showed the protective levels induced by different types of vaccine and doses in broilers chickens (Total morbidity and mortality) after challenge with IBV virulent local QX (ELD_{50} $10^{5.3}$) at 28 days of age

Data in Fig. (2) showed that the morbidity rate in group C post challenge with local IBV variant isolate at 28 days of age, had a significant protective level (P<0.05) rate (30%) as compared with group (B, A, D and E) (40, 60, 70 and 100%) respectively, while the mortality rate in group C recorded the lowest (20%), this rate was significantly lower than (P<0.05) group (B, A, D and E) (30, 40, 50 and 70%) respectively.

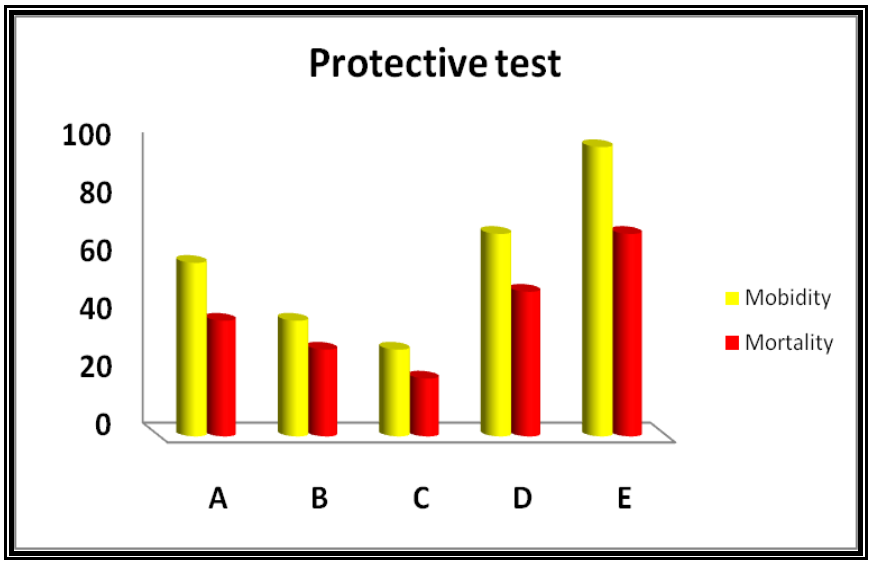

Fig. (2) showed the development of clinical signs and mortalities post challenge with local IBV variant (ELD_{50} 10^6) at 28 days of age

These results in experimental infection were in agreement with (26 and 27) who inoculated the virulent virus at (14) days old, after two days all chicks showed respiratory signs, depression, ruffled feathers and the lesion of dead chicks characterized by swollen kidney and severe urate deposition. Mortality may occur in young chicks due to respiratory or kidney infection (28). The vaccine used provided only little protection against them. This result is in agreement with finding that reports of in vivo cross protection often declines with decreasing S1 sequence homology between vaccinal strain and strains used in challenge (29). The combination of these three strains MA5, IBH120 and 4/91 gave higher levels of cross-protection to variant-2 heterologous strain and this in agreement with Terregino *et al.*, (30) who referred to the combination of some strains such as Mass and Conn or Mass and JMK which produce higher levels of cross-protection to some heterogeneous strain. Also De Wit and Van de Sande, (31) suggested that Ma5 can be used incombination with IB 4/91 vaccine and inactivated vaccine for broad protection against different IB serotypes. Mase *et al.*, (32) Reported that specific protection against IBV type, IB 4/91 or IB D274 vaccine are used when combined with Ma5 and IB multi vaccines, they provide broad protection and this in agreement with this study. Also Cook *et al.*, (33), referred to the use of heterologous vaccine strains, broadened the protection spectrum. These results were in agreement with (34), who referred that vaccination may induce mild signs of IB disease and disappear, also these findings were in agreement with Terregino *et al.*, (30) who suggested that the bivalent IB vaccine was considered the best one on giving the highest antibody titer. Also group A showed mild clinical signs and the morbidity rate was 50% with mortality rate 20%.

Reference

1. Saif, Y. M.; Barnes, H. J.; Glisson, J. R.; Fadly, A. M.; McDougald, L. R. & Swayne, D. E. (2008). Diseases of Poultry. 12th Ed., Iowa State Univ. Press. Blackwell Publishing Company, Iowa, USA. PP. 117-129.
2. Cavanagh, D. & Gelb, J. (2008). Infectious bronchitis. In: Saif YM, editor. Diseases of poultry, 12th Ed. Ames (IA): Wiley-Blackwell Publishing. PP. 101-120.
3. Shalish, A. H.; Zahid, A. H. & Almremdly, H. A. (2014). Effects of E. coli infection on immune response against IB vaccine in broilers. 1st scientific conference of AL-Qasim Green Unversity 26-27 Nov. PP. 114-127.

4. Cavanagh, D. & Naqi, N. S. (2003). Infectious bronchitis, PP. 101-119. In B. H. J. Saif Y.M., Glisson J.R., Fadly A.M., McDougald L.R., Swayne D.E (ed.), Diseases of poultry, 11th ed. Iowa State University Press.
5. Zahid, A. H. & Keshwan, H. A. (2014). Preparation and evaluation of killed Bivalent vaccine using locally strains of infectious bronchitis (QX) and Avian influenza virus. 2nd seientific conference of Faculty of Vet. Med. Uni. Of Kufa 12-13 Feb. PP. 42-47.
6. Susan, S. M.; Salama, E. & Ahmed, A. (2012). Efficacy of some living classical and variant infectious bronchitis vaccines against local variant isolated from Egypt. Nature and Science, 10 (12): 292-299.
7. Hassan, A. H. & Zahid, A. H. (2015). Preparation and evaluation of mulivallent live attenuated infectious bronchitis vaccine from commercial vicinal strains. The Iraqi Journal of Veterinary Medicine, 37(1):109-114.
8. Allam, S. (1975). Breeding and Management of Poultry. 2nd Ed. Cairo-Egypt. PP. 32-38.
9. Synbiotic® Corporation. (2005). Newcastle disease virus antibody test kit. Proflock R. plus. Item No 96-95 33. Frontera San Diego, CA 92127. U.S. Vet LIC No312.
10. OIE (Office International des Epizooties) (2008). Avian infectious bronchitis. In: "Manual of Standards for Diagnostic Tests and Vaccines" 5th Ed., Paris, PP. 1-18.
11. SAS. (2012). Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.
12. Hamal, K. R.; Burgess, S. C.; Pevzner, I. Y. & Erf, G. F. (2006). Maternal antibody transfer from dams to their egg yolks, egg whites, and chicks in meat lines of chickens. Poult. Sci., 85:1364-1372.
13. Sharma, J. M. (2003). The Avian Immune System. 11th ed. Saif, Y. M.; Barnes, H. J.; Glisson, J. R.; Fadly, A. M.; McDougald, L. R. and Swayne, D. E. ed. Iowa State Press, Ames. Poult. Dis., 5: 5-16.
14. Gharaibeh, S.; Mahmoud, K. & Al-Natour, M. (2008). Immunology, Health and Diseases Field Evaluation of Maternal Antibody Transfer to a Group of Pathogens in Meat-Type Chickens Poult Sci., 87:1550-1555.
15. Zahid, A. H.; Kadhum, L. I. & Al-Safy, A. K. (2011). Interfeance of infectious bronchitis vaccine (MA5 and 4/91) strains on the immune response of Newcastle and avian influenza vaccines in male layers. Iraqi J. Agrc. (Special Issue), 16: 2.
16. Alexander, D. J. (2003). Newcastle disease other avian paramyxoviruses and pneumovirus infections. In Saif, J.M., Barnes, H.J., Glisson, J. R., Fadly, A. M., Mcdougald, L. R., Swayne, D. E. (ed.), Diseases of Poultry. Iowa State University Press, Ames, PP. 63-87.
17. Grimes, S. E. (2002). A basic Laboratory Manual for the small-scale production and testing of 1-2 Newcastle disease vaccine. Australian Center for International Agricultural Research.
18. Tzvetkov, P.; Genova, K. & Djambazova, N. (2000). Preparation of trivalent inactivated vaccine against Newcastle disease, infectious bursal disease and infectious bronchitis in chickens. Experimental Pathology and Parasitology, Bulgarian Academy of Sciences.
19. Ali, S.; Arshad, M.; Siddique, M. & Ashraf, M. (2007). Preparation and Evaluation of Vitamin Adjuvanted Oil Emulsified Infectious Bronchitis Experimental Vaccine. Pakistan Vet. J., 27(4): 159-162.
20. Gough, R.; Cox, W.; Welchman, D. D.; Worthington, K. J. & Jones, R. (2008). Chinese QX strain of infectious bronchitis virus isolated in the UK. Veterinary Record, 162: 99-100.

21.Mahgoub, K. M.; Bassiouni, A. A.; Afify, M. A. & Rabie, N. S. (2010). The Prevalence of Infectious Bronchitis (IB) Outbreaks in Some Chicken Farms. I. Spotlight on the Status of IB Outbraks in Some Chicken Flocks. J. Am. Sci., 6 (9): 57-70.

22. Kapczynski, D. R.; Hilt, D. A.; Shapiro, D.; Sellers, H. S. & Jackwood, M. W. (2003). Protection of chickens from infectious bronchitis by in ovo and intramuscular vaccination with a DNA vaccine expressing the S1 glycoprotein. Avian Dis, 47: 272-285.

23. Mondal, S. P. & Naqi, S. A. (2001). Maternal antibody to infectious bronchitis virus: its role in protection against infection and development of active immunity to vaccine .Vet. Immunol. Immunopathol., 79: 31-40.

24. Ladman, B.; Pope, C.; Ziegler, A.; Swieczkowski, T.; Callahan, J.; Davison, S. & Gelb J. (2002). Protection of chickens after live and inactivated virus vaccination against challenge with nephropathogenic infectious bronchitis virus PA/Wolgemuth/98. Avian Dis., 46: 938-944.

25. Okino, C. H.; Alessi, A. C.; Montassier, M. F. S.; Rosa, A. J. M.; Wang, X. & Montassier, H. J. (2013). Humoral and Cell-Mediated Immune Responses to Different Doses of Attenuated Vaccine Against Avian Infectious Bronchitis Virus. Viral. Immunol., 26: 259-267.

26. Zhou, J. Y.; Zhang, D. Y.; Ye, J. X. & Cheng, L. Q. (2004). Characterization of an avian infectious bronchitis virus isolated in China from chickens with nephritis. J. Vet. Med., 51: 147-152.

27. Zahid, A. H.; Kadhum, L. I. & Al-Alwany, T. K. (2012). Immunosuppressive properties of infectious bronchitis vaccine 4/91 strain against different programs and method of vaccination against Newcastle disease in broilers, Al-Qadisiya J. Vet. Med. Sci. of 5th conference 21-22 Nov., Vol.11: No.3.

28. Ahmed, Z.; Naeem, K. & Hameed, A. (2007). Detection and Seroprevalence of Infectious Bronchitis Virus Strains in Commercial Poultry in Pakistan, Poult. Sci., 86:1329-1335.

29. Gelb, J. Jr.; Weisman, Y.; Ladman, B. S. & Meir, R. (2005). S1 gene characteristics and efficacy of vaccination against infectious bronchitis virus field isolates from the United States and Israel (1996 to 2000). Avian Pathol.,34:194-203.

30. Terregino, G.; Toffan, A. & Serna, M. (2008). Pathogenicity of a QX strain of infectious bronchitis virus in specific pathogen free and commercial broiler chicken and evaluation of protection induced by vaccination program based on the Ma5 and 4/91 serotypes. Avian Pathol., 37 (5): 487-493.

31. De Wit, J. J. & Van de Sande, H. (2009). Efficacy of combined vaccines at day of hatch against a D388 challenge in SPF and commercial chickens. In: Heffels-Redmann, U. and Kaleta, E.F. (Eds.) *Proceedings of the 5Th International Symposium on Corona and Pneumoviruses and Complicating Pathogens*, PP. 177-182. Rauischholzhausen, Germany.

32.Mase, M.; Inoue, T.; Yamaguchi, S. & Imada, T. (2008). Existence of Avian IBV with a European- prevalent 4/91 genotype in Japan. J. Vet. Med. Sci., 70: 1341-1344.

33. Cook, J.; Orbell, S.; Woods, M. & Huggins, M. (1999). Breadth of protection of the respiratory tract provided by different live-attenuated infectious bronchitis vaccines against challenge with infectious bronchitis viruses of heterologous serotypes. Avian Pathol., 28: 477-485.

34. Matthijs, M. G.; Van Eck, J. H.; Landman, W. J. & Stegeman, J. A. (2003). Ability of Massachusetts-type infectious bronchitis virus to increase coli-bacillosis susceptibility in commercial broilers: a comparison between vaccine and virulent virus. Avian Pathol., 32: 473-481.