

Preparation and evaluation of multivalent infectious bronchitis vaccine from commercial vaccine strains

H. A. Hammadi

Abdul Ameer H. Zahid

Coll. of Vet. Med./ Univ. of AL-Qadisiya

Coll. of Vet. Med./ Univ. of Baghdad

email: hassanhamadi66@yahoo.com

(Received 14 October 2014, Accepted 15 December 2014)

Abstract

This study was designed to prepare and evaluate multivalent live attenuated (IBV) vaccine (lyophilized seed) from the commercial IBH120, 4/91 and MA5 strains in ALKindy Company for Drug and Vaccines Production, to reduce economical losses and increase the broad protection against infectious bronchitis disease in chickens. The viruses inoculated via chorioallantoic cavity route with 0.1 ml at 9-11 days embryonated eggs obtained from unvaccinated layers against IBV, and were passaged for several times, then three equal doses of the strains were mixed, and the embryonated eggs were inoculated with 0.1 ml of the mixed vaccine. After 72 hours the allantoic fluids (Vaccine) were collected. The results of the infective dose of the new vaccine were $10^{3.2}$ EID₅₀ /Bird. This multivalent live attenuated IB vaccine was tested for (safety, purity, potency, and sterility test) according to the international protocol steps of vaccine production. Efficacy of multivalent vaccine was evaluated (depending on ELISA, HI, and challenge test) by using 160 one day old (Ross 308) chicks divided into four equal groups (40 chicks of each group) reared in well isolated places. Group (A) was vaccinated with three doses (0.3 ml) of multivalent live attenuated IB vaccine, group (B) was vaccinated with two doses (0.2 ml), group (C) was vaccinated with one dose (0.1 ml) of the multivalent live attenuated IB vaccine via ocular route at the 8th day of age, and group (D) was leaved without vaccination as a control group. Blood was collected at (1, 8, 14, 21, 28, 35, 42) days for ELISA test. The mean titer of maternal immunity at day one of age was 4849.8 ± 1161.8 , decreased to 69.9 ± 35.92 at 8th day of age before vaccination. The serum of control group was seen avuncular from IB antibodies at the 14th day of age. The experiment showed a significant differences ($p < 0.05$) titers resulting from use of different doses (0.3 ml, 0.2 ml and 0.1 ml) at age of 14 days, it were 255.30 ± 15.00 , 736.60 ± 94.00 and 545.50 ± 17.00 respectively in comparison with zero in control group. The challenge test carried out for different groups with (IB- field isolated Variant 2 strain 10^5 EID₅₀ / bird which was supplied from Veterinary Directorate) at 28 days old with 0.5 ml through ocular route. Ten birds from each group were taken randomly. According to the clinical signs, morbidity and mortality rate, gross lesion, and the antibody titer, the results carried out that protection rate in group B was higher than others groups, then group C and group A respectively, the test revealed significant differences ($p < 0.05$) between the vaccinated groups, and also between vaccinated groups and control group. This study was concluded the possibility of production IB multivalent live attenuated vaccine (broad protection vaccine) using different IB Vaccine strains in chickens.

Key words: Infectious bronchitis, vaccine, preparation, evaluation.

تحضير وتقييم لقاح التهاب القصبات الحي المضعف المتعدد من العتر اللقاحية التجارية

عبد الأمير حسين زاهد
كلية الطب البيطري/جامعة بغداد

حسن علي حمادي
كلية الطب البيطري/جامعة القادسية

الخلاصة

اجريت هذه الدراسة لتحضير وتقييم لقاح حي مضعف متعدد لمرض التهاب القصبات في الدواجن من العتر التجارية (MA5, 4/91, IBH120) في شركة الكندي لإنتاج اللقاحات والأدوية البيطرية لتقليل الخسائر الاقتصادية من خلال

الحصول على طيف واسع من الحماية ضد مرض التهاب القصبات المعدي في الدواجن. تم الحقن عن طريق كيس اللقائي المشيمي في اجنة الافراخ بعمر (9-11) يوم حيث مرر لعدة مرات ، (تم جلب البيض المجنن من امهات غير ملقحة ضد مرض التهاب القصبات المعدي). ثم اخذت جرعة متساوية من كل عترة لقاحية ومزجت ومن ثم حقن البيض المجنن 0.1 مل من المزيج وحضن وبعد 72 ساعة جمع سائل اللانتيوس (اللقاح) ، أجريت معايرة الفايروس وكان معيار اللقاح المنتج $EID_{50} / Bird$ $10^{3.2}$. خضع اللقاح لعدة اختبارات شملت اختبارات الامان والنقاوة وكفاءة اللقاح وفقا للخطوات المتبعة في البروتوكول العالمي لإنتاج اللقاحات ، وتم تقييم كفاءة اللقاح اعتمادا على فحص HI و ELISA واختبار التحدي حيث تم استخدام مئة وستون فرخ دجاج لحم (Ross 308) بعمر يوم واحد حيث قسمت الى أربعة مجاميع متساوية ، رببت في أماكن معزولة واعتمادا على فحص الأليزا لقحت المجاميع الثلاثة الأولى (A,B,C) بعمر ثمانية أيام باللقاح الحي المضعف المتعدد عن طريق التقطير بالعين اذ لقحت المجموعة الأولى (A) بثلاثة جرعة (0.3 ml) ولقحت المجموعة الثانية (B) بجرعتين (0.2 ml) ولقحت المجموعة الثالثة (C) بجرعة واحدة (0.1 ml) في حين تركت المجموعة الرابعة (D) بدون تلقيح واعتبرت مجموعة سيطرة. تم جمع الدم بعمر (1, 8, 14, 21, 28, 35, 42) يوم لعمل فحص الاليزا، اذ كان معيار المناعة الامية (4849.8±1161.8) بعمر يوم واحد وتضائل حتى وصل بعمر ثمانية أيام الى (69.9±35.92) قبل التلقيح بلقاح التهاب الشعب الهوائية الحي المضعف المتعدد المنتج. ووفقا لفحص الأليزا كانت مجموعة السيطرة بعمر 14 يوم خالية من الاجسام المناعية الامية. ان التجربة أظهرت ان المعيار الناتج من استخدام ثلاثة جرعة وجرعتان وجرعة واحدة من اللقاح هو على التوالي (255.30±15.00 736.60±94.00 545.50±17.00) وبالمقارنة مع مجموعة السيطرة ظهرت اختلافات مهمة احصائيا عند مستوى اختبار ($p<0.05$). اجري اختبار التحدي بعمر 28 يوم باستخدام العترة الحقلية $EID_{50} / Bird$ Variant 2 strain 10^5 والمجهزة من الهيئة العامة للبيطرة ، بالتقطير بالعينين 0.5 ml اذ سحبت من كل مجموعة عشرة طيور بشكل عشوائي وتمت مراقبة العلامات السريرية ونسبة الاصابة ونسبة الهلاك لكل مجموعة وسجلت الافات العيانية وتم قياس معيار الأجسام المناعية. وأظهرت النتائج ان افضل حماية تحققت لمجموعة B تلتهها مجموعة C ثم جاءت بالمرتبة الثالثة مجموعة A اذ لوحظت فروق معنوية في مستوى المعيار المناعي بين المجاميع الملقحة ذاتها، وبين كل مجموعة من المجاميع الملقحة ومجموعة السيطرة عند مستوى احتمال ($p<0.05$). كما أظهرت هذه الدراسة إمكانية انتاج لقاحات حية مضعة متعددة باستخدام عترة لقاحية من فايروس التهاب القصبات في الدواجن.

الكلمات المفتاحية: التهاب القصبات المعدي ، اللقاح ، تحضير اللقاح ، تقييم اللقاح.

Introduction

Infectious bronchitis virus (IBV) causes a highly contagious disease in chickens (1). The disease cause significant economic losses throughout the world and is able to spread very rapidly in non-protected birds (2). The respiratory tract is mainly affected causing tracheal rales, sneezing, coughing, reduced weight gain, high morbidity and mortality particularly in broilers. The urogenital tract may also affected, resulting in interstitial nephritis and visceral gout. Several tests including virus isolation, virus neutralization, hemagglutination inhibition, and ELISA have been employed to monitor the existing different IBV serotypes (3). (4) reported that the control of IB currently relies on vaccination with live attenuated and inactivated vaccines. In Iraq infectious bronchitis cause a major problem for poultry industry which found in flocks of layers and broilers (5). Also (6) isolated the IBV from suspected cases of broiler chickens in Mosul. (7) detected the IBV during many outbreaks occurred in Baghdad by ELISA. experimental infection has been conducted by (8) for virus isolation and identification in broilers at Kerbela governorate. (9) isolated

the virus and gave genotype and characterization of IBV from broilers farm in Kurdistan-Iraq which was suffering from respiratory signs and nephropathological lesions with high mortality. These farms were vaccinated with (4/91 and Ma5) vaccine strains and they isolated strains (4/91 and Sul\01\09).

Materials and methods

Materials

1-Three Nobilis® (MA5, IBH120, and 4/91) strains (Intervet Company), as lyophilized seed vaccine strain transported to AL-Kindy Company for Drug and Vaccines Production. 2-Fertilized eggs used for propagation of live IBV strains were obtained from the agricultural research center (Abou-Greeb), which collected from unvaccinated chickens, and then transported to AL-Kindy Company for Drug and Vaccines Production. The abnormal and misshaped eggs were excluded, and incubated after graded. At the tenth day candled and sterilized with 70% alcohol and inoculated through a pinhole made at the broad end of eggs with 0.2 ml / egg, after dissolved vaccine vials by

phosphate buffer solution PBS PH (7-7.2) (used eight eggs for each vaccine strain, and four eggs was inoculated with 0.2ml of PBS as a control positive and four eggs without inoculation as a negative control). Immediately after the inoculation, the site was sealed with sterile paraffin wax and re-incubated for 48 hour, candled twice daily and discarded the dead embryos within 24 hour post inoculum, then collected the allantoic fluids. Repeat passage of the virus in embryonated eggs and titration of the viruses were made, then mixed equal doses of IBH120, 4/91 and MA5 then passage the mixture in embryonated eggs, and titration the product then examined it's (sterility, safety, and potency) according to the standard international protocol before used as alive vaccine (10).

3-Volvac[®] IBD MLV (Intervet): live virus vaccine, D 78 and 228E.

4-Volvac[®] ND (Intervet): live virus vaccine, Clon 30 and ND Lasota.

Broiler chicks

One hundred-sixty (Ross-308) broilers chicks from AL-kawther Hatchery at one day old were divided randomly into four isolated groups (A, B, C, and D (Control)) (40 chicks of each group) housed in the poultry house

(the control group rear in the distance region), while the challenge test was carried out in the central animal house of the avian pathology department / College of Veterinary Medicine / Baghdad University. Preparation of the poultry house according to (11). The blood samples were collected directly from the heart at the first day of age to determine the maternal immunity. The vaccination programs, the route of administration and the day of vaccination was applied (Table 1). At the 8th day of age blood samples from each group were collected for detection the Ab titer of IB pre-vaccination. Then vaccination the first three groups (A,B,C) with the new IB vaccine products with different doses (Table 2) while control group housed without vaccination and under strict hygienic measures.

Challenge test

Ten birds of each group including the control group were used. The challenged test was carried out with 0.5 ml of the virulent IB virus (field Variant 2 Strain each dose contained 10^5 ELD₅₀ / bird) given via ocular route. All the birds remain under observation for ten days post challenge to observe development of the clinical signs of the disease.

Table (1): Vaccination program of experimental groups.

Groups	Strain	Age / day	Route of administration
A,B,C and D (control)	ND/clon30	6	Via drinking water
	ND/Lasota	18	Via drinking water
	IBD/D78	10	Via drinking water
	IBD/D228E	22	Via drinking water

Table (2): Number of doses used in vaccination programs.

group	Age of Birds	Doses and route of administration
A	8 days	3 doses of $10^{3.2}$ EID ₅₀ IB Vaccine/eye drops
B		2 doses of $10^{3.2}$ EID ₅₀ IB Vaccine/eye drops
C		1doses of $10^{3.2}$ EID ₅₀ IB Vaccine/eye drops

Results

According to the doses, the morbidity and mortality rates post challenge test distributed on days were seen in (Table 3). The clinical signs represented by respiratory signs started gradually at the second day post challenge test, ruffled feathers, depression, rales, wet eye, gasping and conjunctivitis with different degrees especially in control group, where the morbidity and mortality reached 100%, while the birds in B and C groups showed very mild clinical signs for short time, and

the morbidity rate was 30% in both groups, but the mortality rate was zero in group B and 10% in group C. The ELISA antibody titers was increased gradually from 1st week post vaccination till record the highest level at the 3rd week in group (B) which received two doses of multivalent vaccine. Also there was statistically significant difference ($P < 0.05$) between vaccinated groups and the control group (D) in the antibody values at 14th days of age, while at the 7th day post

vaccination group D was showed zero maternal antibody titer (MAb), with respect to the results of group B it was higher than other vaccinated groups with significant difference from day 21 to the end of the experiment (Table 4).

Table (3) represents the morbidity and mortality rates.

Groups	No. of dead birds	Days post challenge	Morbidity rate	Mortality rate
A (3 doses)	2	4th day p.c.	50% b	20% b
	1	5th day p.c.		
B (2 doses)	Zero	Zero	30% c	0% c
C (1 doses)	1	4th day p.c.	30% c	10% b
D Control group (unvaccinated group)	1	2th day p.c.	100% a	100% a
	4	3th day p.c.		
	3	4th day p.c.		
	2	5th day p.c.		

Different small letters vertically refer to significant differences at level ($P < 0.05$) among groups.

Table (4): ELISA antibody titers; the MAb, and Ab after vaccination.

Groups	Maternal Antibody	8days/pre-vaccination	14 days	21 days	28 days	35 days	42 days
C one dose	4849.8 ±1161.8	69.9 ±35.92	545.50 ±17.00 A	959.5 ±169.00 A	2014.00 ±22.00 C	3364.00 ±92.00 C	4055.00 ±166.00 A
B two doses			736.60 ±94.00 A	1856.00 ±142.00 B	2980.00 ±16500 B	4500.50 ±130.0 B	6550.00 ±145.00 B
A three doses			255.30 ±15.00 A	820.20 ±105.00 A	1565.00 ±138.0 A	2543.60 ±253.0 A	3223.00 ±222.00 A
D (control)			0±0	0±0	0±0		

Different capital letters vertically refer to significant differences at level ($P \leq 0.05$) among groups.

Discussion

ELISA antibody titers are seen increased gradually from the 1st week post vaccination till record the highest level at 21 days in group (B). This in agreement with (12) who referred to those ELISA antibody titers increased gradually from 1st week till record the highest level in 3rd week for group which received bivalent CR88 and H120. (13) mentioned that the EID₅₀ at 10^3 was not sufficient to provide protection against virulent virus challenge and according to international standards. Live vaccine should contain no less than $10^{3.5}$ EID₅₀ per dose per bird. The EID₅₀ at $10^{3.2}$ (the titer of the product vaccine) was sufficient to provide protection against virulent virus challenge when used two doses was gave infallibility in group B against challenge test, this study demonstrated that the vaccine dose is an important factor to give protective effect against avian IBV in chicks. The titer values of vaccinated groups (A, B & C) after 7 days

post vaccination there were no statistically significant difference ($P < 0.05$) although there were a numerically differences in titer values among the three groups which may due to the interfering between the maternal antibody and the homogeneous immune response to the vaccine which promoting the neutralization. These findings were in harmony with (14) who referred to that according to high or low maternal antibodies, the response to the first and second vaccine maternal antibody titers can interfere with the neutralization of the first vaccination and a different response to the second one, while there were statistically significant difference ($P < 0.05$) among vaccinated groups with group of control (D) which showed zero maternal antibody (MAb) titer at the 6th day post vaccination. At the day 14 post vaccination also there were no significant difference ($P < 0.05$) between A and C although group A was recorded the highest

titer, but when compared group B with each of A & C showed that there were significant difference ($P < 0.05$). Pre-challenge at the day 28, group B was recorded the highest titer which was 2980.0 ± 165.0 then group C which was reached 2014.0 ± 22.0 , and after 7 days of challenge test the titer values increased in all groups but group B also obtained the highest titer among them and there is a statistically significant difference ($P < 0.05$) between the vaccinated groups A&B, A&C and B&C respectively while in 14 post challenge also all groups revealed increase in titer values with significant difference ($P < 0.05$) between A & B and also between B&C although group B was recorded the highest titer values. The combination of these three strains were gave higher levels of cross-protection against variant-2 heterologous strains and this in agreement with (15) who referred that the combination of some strains such as Mass and Conn or Mass and JMK produce higher levels of cross-protection to some heterologous strain. Also (16) reported that Ma5 can be used included with IB 4/91 vaccine and inactivated vaccine for broad protection against different IB serotypes. (17) reported that to give 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 the results of this study were in agreement with (18) who referred to the use of heterologous vaccine strains, broadened the protection spectrum. Also in this study there were statistically significant difference ($P < 0.05$) in antibody titers among vaccinated groups at 21 days of age. Pre-challenge at the day 28, group B was recorded the highest titer which was 2980.0 ± 165.0 then group C which was reached 2014.0 ± 22.0 , and group A which was recorded the lowest titer 1565.00 ± 138.00 this results mentioned that

there were statistically significant difference ($P < 0.05$) among an immune response of the vaccinated groups. Post 7 days of the challenge test the titer values were increased in all groups but group B obtained the highest titer among them and in attendance were an important statistical differences $P < 0.05$ among groups A&C, A&B, as well as B&C respectively while the 14th day post challenge also all groups were recorded significant statistical differences $P < 0.05$ among A with B as well as B with C but group B was recorded the highest titer values, this is in agreement with (19) who referred to that ELISA antibody titers increased gradually from 1st week till record the highest level in 3rd week, also they reported that the group which received bivalent CR88 and H120 vaccine shows gradually elevation in titer, and this in agreement and explain the results which show gradually elevated in titer values in all groups when was used live attenuated multivalent IB vaccine, also (19), was reported that there was evidence that local antibody prevents re-infection when used the same vaccination route to produce local antibody secretion. Also this results were in agreement with (13), who suggested that EID_{50} at 10^3 was not sufficient to provide protection against virulent virus challenge and according to international standards, live vaccine should contain no less than $10^{3.5}$ EID_{50} dose/ bird, so, it could be concluded that the multivalent vaccine was safe, potent and induce high levels of antibody titers, in addition it was gave a high protection in vaccinated chickens. The titer $10^{3.2}$ EID_{50} was sufficient to provide protection against virulent virus challenge when was used two doses to give infallibility in group B against challenge test. This study was demonstrated that the dose is an important to give protective effect against avian IBV in chicks.

References

- 1-Abro SH, Renstrom LH, Ullman K, Isaksson M, Zohari S, Jansson DS, Belak S, Baule C (2012) Emergence of novel strains of avian infectious bronchitis virus in Sweden. *Veterinary Microbiology*, 155(2-4): 237-46.
- 2-Ababneh M, Dalab AE, Alsaad S, Al-Zghoul M (2012) Presence of Infectious Bronchitis Virus Strain CK/CH/LDL/97I in the Middle East. *Faculty of Vet. Med. Jordan University of Sci. and Technol.*, P.O. Box 3030, Irbid 22110, Jordan.
- 3-Worthington KJ, Currie RJ, Jones RC (2008) A reverse transcriptase-polymerase chain reaction survey of infectious bronchitis virus genotypes in

- Western Europe from 2002 to 2006. *Avian Pathol* 37(3), 247-57.
- 4-Seifi S, Asasi K, Mohammadi A (2009) A study of natural co-infection caused by avian influenza (H9 subtype) and infection bronchitis viruses in broiler chicken farms showing respiratory signs. *Online J.Vet. Res*, 13: 53-62.
 - 5-Abbass HA (1987) A study of infectious bronchitis in chicken M.Sc. Thesis-College of Vet. Medicine Baghdad University – Iraq.
 - 6-AL-Husairaji FF (2005) Isolation, identification and pathogenesis of infectious bronchitis virus in broilers in Northern area of Iraq. M.Sc. Thesis College of Veterinary Medicine, Mosul University
 - 7-Khammas EJ (2008) Avian Influenza (H9N2) outbreak in Iraq. *Iraqi J. Vet. Med.*, 32:(1)
 - 8-Hussein MA (2012) Isolation and identification of infectious bronchitis virus and experimental infection in broilers. M.Sc. Thesis, Collage of Veterinary Medicine, Baghdad University – Iraq.
 - 9-Zana H, Mahmood R, Sleman R, Aumaid UU (2010) Isolation and molecular characterization of Sul /01 /09 avian infectious bronchitis virus, indicate the emergence of anew genotype in the middle east. *J. Vet. Mic.* 10: 1016.
 - 10-Office International des Epizooties (2008) Avian infectious bronchitis. In: "Manual of Standards for Diagnostic Tests and Vaccines" 5th Ed., Paris,: 1-18.
 - 11-Allam S (1975) "Breeding and Management of Poultry" 2nd Ed. Cairo-Egypt. Pp: 32-38.
 - 12-Toro H (1994) and Fernandez I: Avian infectious bronchitis: specific lachrymal IgA level and resistance against challenge. *Zentralbl Veterinary med. (B)*, 41:467–472.
 - 13-Rosner B (1995) Hypothesis testing: categorical data. In: *Fundamentals of biostatistics*. 4th ed. Belmont, CA: Duxbury Press, Wadsworth Publishing; 345-442.
 - 14-Zahid AH, Kadhum LI, AK Al-Safy (2011) Interfering 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)*, Vol.16: No.2.
 - 15-Terregino C, Toffan A, Beato MS, Nardi RD, Vascellari M, Meini A, Ortali G, Mancin M, Capua I (2008) Pathogenicity of a QX strain of infectious bronchitis virus in specific pathogen free and commercial broiler chickens and evaluation of protection induced by a vaccination program based on the Ma5 and 4191 serotypes. *Avian Pathol.*, 37 (5): 487-493.
 - 16-De Wit jj, H Van de Sande (2009) Efficay of combined vaccines at day of hatch against a D338 challenge in SPF and commercial chickens. *Proceeding of the 6th international Symposium on corona and Pneumoviruses and Complicating Pathogens*, June 14-17, 2009, Rauischholzhausen, Germany, PP177-182.
 - 17-Mase M, T Inoue, S Yamaguchi, T Imada, (2008) Existence of Avian IBV with a European-prevalent 4/91 genotype in Japan. *Journal Vet. Med. Sci.*, 70: 1341-1344.
 - 18-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.
 - 19-Toro H, Fernandez I (1994) Avian infectious bronchitis: specific lachrymal IgA level and resistance against challenge. *Zentralbl Veterinary med. (B)*, 41:467–472.