Comparative Studies On The Immune Response Of Chicken Vaccinated With Recombinant Live Newcastle And Avian Influenza Vaccine With Inactivated Oil Emulsion Newcastle And Avian Influenza

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ABSTRACT

Live recombinant Newcastle virus containing the avian influenza H5 haemagglutinin (HA) gene (rND-AI-H5) and combined inactivated oil emulsion ND-AI vaccines were evaluated for their ability to protect chickens against infection with local isolate of avian influenza and ND. Susceptible SPF chickens were vaccinated with the previous vaccines according to specific vaccination schedules to study the immune response against them through monitoring the haemagglutination inhibition (HI) titres and protection % against local circulating highly pathogenic avian influenza (HPAI) H5N1 and velogenic viscerotropic Newcastle disease (VVND) strains. It was found that two separate doses of inactivated ND-AI induced the highest HI titres (9.0) with protection % (93.3%) at 5th week post booster vaccination. Chicken groups were vaccinated with rND-AI-H5 as two separate doses produced HI titers reached a peak of 5.5 with protection 40%, while those vaccinated with rND-AI-H5 then boosted by inactivated combined ND-AI vaccine produced peak HI titre (8.0) and 80% protection which nearly similar with that results obtained by the combined inactivated ND-AI vaccine used as one dose (peak HI titre 7.7 log2 with 80 % protection). While it was observed that chicken group vaccinated with rND-AI-H5 as one dose produced a weak and non protective immune response where peak HI titre reached 5.0 log2 with 30.3 % protection. The viral shedding was detected two days post challenge with 10^5 EID_{50}/0.1 ml of local HPAI virus by using oropharyngeal swabs from the live infected control group as well as the vaccinated chicken. it was observed that the reduction in the challenge viral shedding through oropharyngeal swabs treatment were 0.5, 1.1, 2.4, 3.9 and 3.2 corresponding for chicken vaccinated with one and two doses of rND-AI-H5, one and two doses of inactivated ND-AI and rND-AI-H5 boosted with inactivated ND-AI, respectively. Finally, it was noticed that the vaccinated chicken group with two homologous doses from inact.ND-AI vaccine and that vaccinated with the rND-AI-H5+inact. ND-AI showed the best and the highest level of reduction in the viral shedding after challenge with local HPAI virus.

INTRODUCTION

Avian influenza (AI) is one of the dangerous and pandemic diseases caused by influenza type "A" virus. It belongs to Ortho-myxoviridae familly. In addition, the avian influenza viruses are further subtyped based on the antigenic properties of their surface glycoproteins into 16 HA subtypes (H1-H16) and 9 NA subtypes (N1-N9) *(1)*. The HA glycoprotein plays a major role in pathogenicity and immunogenicity of AI virus infections and is the critical component of AI vaccines. So, AI viruses are further classified as either high pathogenicity avian influenza (HPAI) or low pathogenicity avian influenza (LPAI) *(2-4)*. Only viruses of the H5 and H7 subtypes have been known to be classified as HPAI virus that threatens the poultry industry and humans health *(5)*. In December 2005, the World Organization for Animal Health and the Food and Agriculture Organization of the United Nations recommended that the using of vaccination of poultry for the control of AI viruses *(6)*.

The control of AI depends on eradication strategies in some countries but this policy had led to very high cost and economical losses. So, other countries depend on the vaccination

strategies especially in areas with high animal densities leading to increased risk of disease spread *(7)*. Vaccination against AI has proven to be a successful additional control measure implemented along side controlled culling *(8)*.

Inactivated, whole virus vaccines were considered the main type that are licensed widely by several countries and have proven efficacy. Also, in recent years, multiple experimental recombinant vaccines have been developed, some of which have been reported to be efficiently protect chickens against HPAI *(1)*.

The live virus vaccines have been developed for AI using alternative virus vectored constructs which can provide some of the immunological advantages of the live virus vaccine but without the re-assortment risk of using of live AI virus *(9)*. Moreover, the disadvantages of some live recombinant vaccines include the risk of generating revertants and allow spread of genetically modified organisms in the environment *(10)*.

In Egypt, there are two conventional types of AI vaccines, the Chinese Re-assortant AI H5N1 inactivated vaccine and inactivated LPAI H5N2 vaccine either monovalent or combined with NDV. Recombinant live Newcastle disease vectored vaccine express AI-HA genes (rND-AI-H5) is still unlicensed till now in Egypt.

So, this study aimed to compare the immune response of rND-AI-H5 vaccine and combined inactivated oil adjuvant vaccine of ND-AI. The comparison was based of results on HI test and protection percentage.

MATERIAL AND METHODS

1. Experimental birds

A total of 300 SPF chickens were obtained from Kom Oshiem, El-Fayoum Farm as one day old. They were maintained at Central Laboratory for Evaluation of Veterinary biologics, Abbasia, Cairo (CLEVB) and housed in positive pressure stainless steel isolation cabinets with continuous light exposure till used.

2. Vaccines

a) Live Recombinant ND-AI vaccine(rND-AI-H5)

Lyophilized vaccine contains a suspension of a live recombinant Newcastle disease virus (LaSota strain) used as vector containing an insert of the heamagglutinin subtype H5 gene of avian influenza virus. It was produced by laboratories of Avimex S.A. de C.V. The H5 gene of AI is derived from the vaccinal strain A\Chicken\Mexico\232\94. The vaccine was administrated by ocular route in a dose 0.2 m\l per bird.

b) The combined inactivated ND-AI vaccine (inac-ND-AI)

The inactivated oil emulsion combined ND-AI (LPAI H5N2) vaccine was produced by laboratories of Avimex S.A. de C.V. The vaccinal Strain is (A/Chicken Mexico/232/94/CPA) .It was administrated subcutaneously at the lower third of the neck in a dose 0.5 ml /bird.

3. Antigens

The homologous AI and ND antigens were obtained from the vaccines manufacture corresponding the vaccine type. While and these antigens were used in serological tests (HA test – HI test) they are:

Inactivated Mexican H5N2 Antigen (A\Chicken\ Mexico\232\94\CPA)

Standard ND antigen (lasota strain)

4. Antisera

Avian influenza antisera were obtained from the vaccine manufacture against each standard vaccinal antigen.

Newcastle antisera were obtainced with the standard antigen.

5. Challenge viruses

a. AI challenge virus

Local HPAI field isolate was used as challenge virus. It was isolated and identified by National Laboratory for Quality control of Poultry (NLQP) as A / Chicken / Egypt / 1709-6 / 2008 (H5N1). Its titer was 10^{10} EID_{50}

/ ml. The challenge dose was adjusted to be 10^5 EID_{50} / 0.1 ml per bird and administered intra-nasal.

b. ND challenge virus

Local velogenic viscerotropic ND virus (VVNDV) was obtained from Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo and used in challenge test against ND vaccine. Its titer was 10^9 EID_{50} / ml. The challenge dose was adjusted to be 10^6 EID_{50} / 0.5 ml per bird and injected intra-muscular.

6. The serological tests

Hemagglutination (HA) and Hemagglutination inhibition (HI) tests were performed *(11)*.

7. Challenge

Fifteen birds out of each vaccinated and non-vaccinated groups were challenged with both virulent strains of AIV (HPAIV) 4 weeks post vaccination (WPV) and NDV (VVNDV) 3 WPV. The challenged birds were observed for 10 days post challenge and all the diseased and dead birds were recorded and examined for signs and lesions of AI and ND.

The assessment of viral shedding due to replication of HPAI challenge virus were performed *(11)* through collection of oropharyngeal swabs on 2nd days post challenge from the live vaccinated and control groups.

8. Experimental Design

The birds were divided into 6 experimental groups (50 birds /each), each group was divided into 2 subgroups, one had 20 birds for serological assessment of the immune response and the second had 30 birds for challenge test against ND and AI viruses as follows:

Group (1)

The chickens were vaccinated intra-ocularly at 10 days with one dose of $10^{6.5}$ EID_{50} in 0.03 ml of rND-AI-H5 vaccine.

Group (2)

The chickens were immunized intra-ocularly with 2 doses of $10^{6.5}$ EID_{50} in 0.03 ml of rNDV-AI-H5 vaccine. The first dose was inoculated on 10th day old while the booster dose was on 25th day old.

Group (3)

The chickens were immunized intra-ocularly on 10th day of age with rND-AI-H5 then boosted subcutaneously on 25th day of age by combined inactivated oil adjuvant ND-AI vaccine.

Group (4)

The chickens were immunized subcutaneously with single dose of inac-ND-AI vaccine on 21st day of age.

Group (5)

The chickens were immunized subcutaneously with two doses of inac-ND-AI vaccine on 21st and 35th day of age.

Group (6)

The chickens were kept as non-vaccinated control group.

Serum samples were collected from each groups weekly post vaccination till 14 weeks.

RESULTS

The results of HI to avian influenza vaccines are illustrated in Tables 1, 2. At 2nd week post vaccination sero-conversion to AI H5 was began to be detected in vaccinated chickens with one dose of rND-AI-H5 vaccine. The mean HI titre was 3.4 log2 against homologous strain H5N2 antigen. The antibodies titers remain increased to reach to the peak at 4th WPV to be 5.2 log2.

While, it was found that mean HI titre of the tested sera collected from chicken vaccinated with inactivated ND-AI vaccine reach the peak level (7.7 log2) against homologous antigen at 5th weeks post vaccination. Then, It was began decrease gradually to become 5log2 at 14th WPV.

In case of two doses of rND-AI-H5 vaccine, the HI titer was to somewhat higher than one

dose where reach the maximum HI level 5.6 at 3rd Weeks Post booster (WPB). But, the results of HI titers were much higher and reach to the maximum level 8 log2 at 5th WPB in the chicken vaccinated with rND-AI-H5 then boosted by inac-ND-AI vaccines.

On the other hand, the vaccination of a chicken group with two doses of inactivated ND-AI vaccine evoked a high level of HI titer reach peak (9log2) at 6th WPB and persists till 7th WPB then decrease gradually to become 7.7 log2 at the 12th WPB.

From the available data in Tables 3, 4, it was shown that one dose of imported inact.-ND-AI vaccine gave a reliable and protective level of HI titre at 4th week post vaccination according to Egyptian Authorities (Protective level $\geq$ 6 log2). Then, the HI titre was increased slightly and remained approximately at constant level till 7th WPV, then decreased gradually. But, the HI titre levels of ND remain protective till end of 14th WPV. In contrary, the rND-AI-H5 did not reach the authorized protective level at any WPV.

Meanwhile, antibody level measured by ND antigen in case of vaccinated chicken with two doses from inact.ND-AI also, increased from 6.7log2 at 1st WPB and reach to peak 7.4 log2 at 4th WPB which persist then began to decrease to reach 6.5 log2 at 12th WPB. Also, in case of chicken group vaccinated with rND-AI-H5 as primary dose followed by inact-ND-AI vaccine, the antibody titer increased gradually till became 7.1log2 at 6th WPB. But, the vaccination with two doses from rND-AI-H5 fall to produce a protective titer (6log2) at any WPB.

It was observed that, the Protection % of the chicken group vaccinated with one field dose of rND-AI-H5 vaccine was 33.3 % during 10 days post challenge against the local HPAI H5N1virus as shown in Table 5. Mean while, 40 % of the chicken that received 2 homologous doses from imported rND-AI-H5 vaccine were protected during the observation period. But, on challenging the immunity of chickens vaccinated by the rND-AI-H5 vaccine as primary vaccine then boosted by the imported H5N2 vaccine, 80 % of them were protected. On the other hand, the protection rates of chickens groups that vaccinated with the inact-ND-AI vaccine as one or two doses were 80 and 93.3 % during 10 days observation post challenge respectively. However, the control group that received the same challenge dose, 100%of chickens showed death with sever symptoms during 3 day post challenge.

On day 2 post challenge (pc), in all control and vaccinated chickens, some titers of virus were shed from the oropharynx depending on the vaccine type. The using of inact-ND-AI as two doses was considered the best vaccine reduced the number of chickens infected and shedding the challenge virus (3.9) followed by group vaccinated with rND-AI-H5 + inact-ND-AI(3.2) then that vaccinated with one dose of inact-ND-AI. While, one and two doses from rND-AI-H5 vaccine did not able to reduce the viral shedding from oropharyngeal tract.

The data presented in table (6) indicated, that in chickens group vaccinated with one field dose of rND-AI-H5 and inact-ND-AI vaccine, 60 and 93.3% and from chicken were protected during 10 days against the local VVND isolate. While, in case of two doses from rND-AI-H5, inact-ND-AI and rND-AI-H5+inact-ND-AI, the protection % were 78.6, 100 and 100 % respectivelly.

Table 1. Results of AI HI antibody titres of chicken vaccinated with one dose of recombinant and inactivated ND-AI vaccines

Vaccine types	No. of serum samples	Mean HI titres at weeks post vaccination														
		Pre	1	2	3	4	5	6	7	8	9	10	11	12	13	14
r-ND-AI-H5 one dose	15	0	0	3.4	4.2	5.2	5.0	5.0	4.8	4.5	4.0	3.6	3.0	2.7	2.0	2.0
Inac-ND-AI one dose	15	0	5.5	6.3	7.4	7.6	7.7	7.5	7.0	6.5	6.1	5.9	5.5	5.0	5.0	5.0
Control	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 2. Results of AI HI antibody titres of chicken vaccinated with two homologous and heterologous doses of recombinant and inactivated ND-AI vaccines

Vaccine types	No. of serum samples	Mean HI titer at weeks post 1st dose			Mean HI titres at weeks post second dose											
		Pre	1	2	1	2	3	4	5	6	7	8	9	10	11	12
r-ND-AI two dose	15	0	0	3.4	4.8	5.5	5.6	5.6	5.5	5.5	5.0	4.5	4.2	4.0	3.7	3.0
r-ND-AI + inac-ND-AI	15	0	0	3.4	4.9	6.0	6.9	7.7	8	7.8	7.7	7.3	7.0	7.0	6.6	6.0
Inac-ND-AI two doses	15	0	5.5	6.3	7.6	7.9	8.0	8.5	9.0	9.0	8.9	8.9	8.5	8.1	8.0	7.7
Control	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 3. Results of ND HI antibody titres of chicken vaccinated with one dose of recombinant and inactivated ND-AI vaccines

Vaccine types	No. of serum samples	Mean HI titres at weeks post vaccination														
		Pre	1	2	3	4	5	6	7	8	9	10	11	12	13	14
r-ND-AI one dose	15	0	0	1.8	2.8	4.7	5.7	5.1	5.1	4.9	3.1	2.1	2.1	2.0	1.8	1.8
Inact-ND-AI one dose	15	0	0	3.4	6.5	6.5	6.8	6.8	6.7	6.5	6.4	6.3	6.1	6.1	6.0	6.0
Control	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 4. Results of ND HI antibody titres of chicken vaccinated with two doses of recombinant and inactivated ND-AI vaccines

Vaccine types	No. of serum samples	Mean HI titer at weeks post 1st dose			Mean HI titres at weeks post second dose											
		Pre	1	2	1	2	3	4	5	6	7	8	9	10	11	12
r-ND-AI two dose	15	0	0	1.8	3.0	5.1	5.5	5.7	5.7	5.5	5.4	5.1	5.0	4.9	3.5	2.7
r-ND-AI + inact-ND-AI	15	0	0	1.8	4.5	5.7	6.0	6.3	6.5	7.1	7.1	7.0	6.7	6.5	6.0	6.0
Inact-ND-AI two doses	15	0	0	3.4	6.7	6.9	7.1	7.4	7.4	7.3	7.3	7.3	7.0	6.9	6.5	6.5
Control	10		0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 5. Results of the efficacy of different ND-AI vaccines against the challenge with local strain of HPAI virus at 4 weeks post vaccination

Vaccine type	No. of birds	No. of dead birds / days post challenge										Total deaths	Protection % *	Titre of viral shedding ** (EID_{50} /ml)	Reduction in viral shedding*** (log_{10}/ml)
		1	2	3	4	5	6	7	8	9	10				
rND-AI-H5 one dose	15			5	3	1	1					10/15	30.3	4.9	0.5
rND-AI-H5 two doses	15			4	3	2						9/15	40	4.3	1.1
rND-AI-H5 + inac.ND-AI	15			1	1	1						3/15	80	2.2	3.2
Inac.ND-AI one dose	15				2	1						3/15	80	3.0	2.4
Inac.ND-AI two doses	15								1			1/15	93.3	1.5	3.9
Control non-vaccinated	10			9	1							10/10	0	5.4	-

* protection is considered when challenge results are 80% and above, while values lower 80% mean no protection

** Viral shedding using tracheal swabs 2 days post challenge

*** Reduction in viral shedding must be ≥ 2 log_{10}

Table 6. Results of the efficacy of different ND-AI vaccines against the challenge with local strain of VVNDV virus at 3 weeks post vaccination

Vaccine type	No. of birds	No. of dead birds / days post challenge										Total deaths	Protection %
		1	2	3	4	5	6	7	8	9	10		
rND-AI-H5 one dose	15					3	2	1				6/15	60
rND-AI-H5 two doses	15	1*				1	2					3/14	78.6
rND-AI-H5 + inac. ND-AI	15											0/15	100
Inac. ND-AI one dose	15								1			1/15	93.3
Inac. ND-AI two doses	15											0/15	100
Control non-vaccinated	10				6	4						10/10	0

* Non specific death

DISCUSSION

Avian Influenza (AI) is an economically-important disease of poultry and human health around the world. It is unusual in that it can cause a range of disease symptoms in poultry from a subclinical infection to being highly virulent with 100% mortality ***(12)***. Traditionally, HPAIV is controlled by elimination of infected flocks. Due to economical reasons, culling of infected flocks is no longer a pratical method for control of AI in either developed or developing countries. So, effective vaccination is a critical tool that supports public health efforts to reduce influenza virus morbidity and mortality. Although vaccination has been recommended by the world organization ***(6)*** to control AI, few effective AI vaccines are available ***(13)***. The most currently used vaccines against *AI* consists of 1) inactivated whole virus, 2) In vivo expressed HA protein (like live vector vaccines) as adenovirus ***(14, 15)***, fowl pox virus ***(16, 17)***, baculovirus ***(18-20)***, or Newcastle disease virus ***(21-23)***. Recently, the recombinant vaccines using NDV as a vector has been used and gave promising results ***(21-23)*** since this rND-AI-H5 can be administrated to large numbers of birds with ease via spray or drinking water which would allow a coast-effective and rapid immunization.

The Haemagglutination Inhibition test is the most convenient, rapid and economical serological method for evaluating the immunity of chicken to AI vaccines. Although, the HI test generally does not detect low levels of circulating antibodies, it has proved to be an indicator of immune status of a flock when individual sera are tested after vaccination ***(24-26)***.

Challenge under strictly controlled conditions with virulent HPAI virus may also be used to predict flock response to exposure. This method can add considerable significance to the HI values obtained with sera from the same chicken. These findings substantiate the previous cited results of ***(27, 28)***.

The results of HI titers of the chicken sera vaccinated with one dose of combined inactivated ND-AI vaccine was 7.7 $\log_2$ at 5th weeks post vaccination (WPV) with protection 80 % while in case of vaccination with rND-AI-H5 vaccine it reached 5.2 $\log_2$ at 4th WPV and protection 30.3 % (Tables 1 and 5). The present results are consistent with the studies carried out by several auther ***(29-34)***.

As shown in Tables 2 and 5, it is noticed that in case of vaccination of chicken groups with two homologous doses from each AI vaccine type, the peak of HI titres reached 9 $\log_2$ at 5th week post boostering (WPB) with protection percentage 93.2 % in chickens vaccinated with combined inactivated ND-AI vaccines, while reached up to 5.6 $\log_2$ at 3rd WPB and protection percentage 40 % in vaccinated chickens with rND-AI-H5 vaccine. The low level of immunity produced by the rND-AI-H5 confirmed earlier work ***(35-37)*** which showed that rND-AI-H5 and some adenovirus vectors could be applied by misapplication via drinking water, intraocular or intranasal route of administration and replicate in mucus membranes where some vectors require injection to produce an effective immune response. However, inactivated vaccines could be administered in local or imported form via SC or IM without loss of potency inducing a satisfactory immune response to AI virus. Inactivated AI vaccines are used extensively in the most parts of the world. The major advantage of inactivated vaccines is that they do not produce the undesirable side effects sometimes associated with live virus vaccines and inclusion of adjuvant greatly enhances immunogenicity ***(38, 39)***.

The present work also describes the serological response to ND vaccines. The results of HI titers produced by combined inactivated ND-AI vaccine reached to 6.8 $\log_2$ at 5th WPV when used as one dose and of 7.4 $\log_2$ at 4th WPB when used as 2 doses with a protection 93.3 % and 100 %, respectively. While in case of rND-AI-H5 vaccine used either as one or 2 doses, the maximum HI titers were 5.7 $\log_2$ at 5th WPV and the protection were 60 % and 67%, respectively (Tables 3 and 6). The recombinant NDV

expressing AIVH5 which had been derived from HB1 vaccine strain provided only 70% of the vaccinated chickens against NDV infection because of excessive attenuation *(22)*. They added that insertion of an addition gene exits a further attenuation effect.

The HI antibody levels of chicken group vaccinated with rND-AI-H5 as primer dose and boosted with combined inactivated ND-AI vaccine reached the peak up to 8 log_2 after 5 WPB against AI and to 7.1 log_2 at 6^{th} WPB against ND, while the protection was 80 % and 100% respectively (Tables 2-6). As the replication of live vector vaccine in upper respiratory and digestive tract cells resulted in mucosal and systemic response against both NDV and AIV. The rND-AI-H5 vaccine provides a rapid local and systemic protection when using the inactivated combined vaccine as a booster after it (80%) *(22)*.

In case of virus shedding experiment, it was found from Table 5 that the titer of virus shedding after challenge with HPAI strain (Egyptian local field isolate) were different, where it was reduced, in the group of chickens vaccinated with the inactivated combined ND-AI vaccine as booster dose after rND-AI-H5 vaccine, with 3.2 log_{10}. While, the reduction in viral shedding were 2.4log_{10} and 0.5log_{10} in the chicken vaccinated with one dose of combined inactivated ND-AI and rND-AI-H5, respectively. But, in case of chicken vaccinated with two doses of inactivated ND-AI and rND-AI-H5 vaccines, the levels of the virus shedding were reduced with 3.9log10 and 1.1log_{10}. The concerns of vaccination against AI is that single dose of current vaccines do not produce sufficient immunity to completely prevent infection and subsequent virus transmission, although recent experiments demonstrated that vaccination with inactivated vaccines may be able to reduce the spread of AIV within flock *(19,22)*. It has been illustrated that inactivated whole influenza virus vaccine produce uniform protection of chickens from clinical signs and death following challenge by HPAI viruses *(17, 29)*.

The obtained results illustrated that the using of combined inactivated ND-AI as 2 doses induced higher HI titers, protection percentage and reduction in viral shedding, more than any of the other vaccines. Also, the usage of the rND-AI-H5 as prime-boost schedule vaccine with inactivated one in order to protect chicken flocks from avian Influenza infections is more effective *(29, 40)*. So, it suggested further studies on many new batches of recombinant and inactivated avian influenza vaccines.

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الملخص العربى

دراسات مقارنة بين رد الفعل المناعى للدجاج المحصن لكل من لقاح الأنفلونزا المحمل على فيروس النيوكاسل ولقاح الأنفلونزا والنيوكاسل المثبط

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تم معايرة لقاح حى من النيوكاسل المحمل عليه جين الهيمأجليوتينين لفيروس الإنفلونزا H5 ومقارنته باللقاح المثبط المزدوج من النيوكاسل والإنفلونزا فى قدرتهم على حماية الطيور ضد الإصابة بكلا من الفيروسات المحلية لمرض النيوكاسل والإنفلونزا. تم تحصين مجاميع من الدجاج الخالى من المسببات المرضية طبقاً لنظام معين من التحصين تم وضعه لدراسة مدى إستجابة الجهاز المناعى لهذه الطيور لتلك اللقاحات عن طريق قياس نسبة الأجسام المناعية فى اختبار التلازن الدموى المثبط ونسبة الصد فى إختبار التحدى. وقد وجد أن جرعتين منفصلتين من اللقاح المثبط للنيوكاسل والأنفلونزا قد أعطى أعلى نسبة للأجسام المناعية حيث وصلت إلى أعلى قيمة لها وكانت (9.00) فى الإسبوع الخامس بعد التحصين بالجرعة الإضافية بالإضافة إلى نسبة صد (93,3%). الدجاج المحصن بلقاح النيوكاسل الحى المحمل بالإنفلونزا بجرعتين منفصلتين قد أعطى نسبة أجسام مناعية وصلت إلى (5.5) ونسبة صد (40%) بينما الدجاج المحصن باللقاح الحى المحمل تليه جرعة إضافية من اللقاح المثبط قد أعطت نسبة أجسام مناعية (8) ونسبة صد (80%) وهذه النتائج وجد أنها متماثلة مع النتائج التى حصلنا عليها فى المجموعة المحصنة باللقاح المثبط كجرعة واحدة والتى أعطت نسبة أجسام مناعية وصلت إلى (7.7) و (80%) نسبة صد. وقد لوحظ أن المجموعة المحصنة باللقاح الحى المحمل كجرعة واحدة قد أعطت استجابة مناعية ضعيفة حيث كانت نسبة الأجسام المناعية (5) ونسبة الصد (30.3%). وبقياس مدى قدرة اللقاحات السابقة على تقليل نسبة القوى العيارية لفيروس الإنفلونزا الضارى المعزول والمفرز بعد اختبار التحدى بيومين من خلال أخذ مسحات من القصبة الهوائية وجد أنها أعطت (0،5 ، 1،1 ، 4،2 ، 3،9 ، 3،2) لو$_{10}$ فى المجموعات المحصنة بكلا من اللقاح المحمل كجرعة واحدة أو جرعتين - اللقاح المثبط كجرعة واحدة أو جرعتين – اللقاح الحى المحمل يليه جرعة اضافية من اللقاح المثبط – اللقاح الحى المحمل يليه جرعة أضافية من اللقاح المثبط) بالترتيب. وفى النهاية وجد أن اللقاح المثبط المركب عند استخدامه كجرعتين وكذلك المجموعة المحصنة باللقاح الحى للنيوكاسل والمحمل عليه جين الإنفلونزا ثم التحصين باللقاح المثبط أستطاعوا التقليل من إفرازات الفيروس الضارى بنسبة كبيرة بالمقارنة بباقى المجاميع.