American Journal of Microbiology and Biotechnology
2016; 3(3): 18-22
http://www.aascit.org/journal/ajmb
ISSN: 2375-3005

AASCIT American Association for Science and Technology

Keywords
HPAIV,
Challenge Dose,
Immune Response,
Haemagglutination Test,
H5N1 and H5N2 Vaccines,
Efficacy and Potency

Received: March 8, 2016
Accepted: March 21, 2016
Published: May 10, 2016

The Influence of Different Challenge Doses of Highly Pathogenic Avian Influenza on the Efficacy of Different Avian Influenza Vaccine

Nourhanne M. Said[1, *], Lamiaa M. Omar[1], Ahmed A. El-Sanousi[2]

[1]The Central Laboratory for Evaluation of Veterinary Biologics, Cairo, Egypt
[2]The Virology Department, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt

Email address
nourhannemohamed@yahoo.com (N. M. Said)
[*]Corresponding author

Citation
Nourhanne M. Said, Lamiaa M. Omar, Ahmed A. El-Sanousi. The Influence of Different Challenge Doses of Highly Pathogenic Avian Influenza on the Efficacy of Different Avian Influenza Vaccine. *American Journal of Microbiology and Biotechnology.* Vol. 3, No. 3, 2016, pp. 18-22.

Abstract
This study aim to investigate the influence of different challenge doses of HPAIV on the efficacy of different AI vaccine was studied by use of two different types of inactivated AI vaccines H_5N_1, and H_5N_2 and their potency were evaluated by using different doses of HPAI challenge virus. Groups of specific pathogen free chicken (SPF) were vaccinated with the recommended dose of the two vaccines according to the manufacturer, and another group was kept as control. Four weeks post vaccination (DPV) both vaccinated and control chicken groups were bled for the detection of Ab titer in response to vaccination using HI (haemagglutination inhibition test). The vaccinated chicken with the two vaccines were subdivided into 4 subgroups to be challenged by the doses (10^4, 10^5, 10^6, 10^7) EID_{50} of HPAI challenge virus correspondingly. The results revealed that the Ab titer produced with H5N1 vaccine was 8 log^{-2} and 7 log^2 for H5N2 vaccine. The protection percent of the vaccinated chickens was calculated for each challenge dose for each vaccine. The protection percent of the chicken vaccinated with H5N1 vaccine was 100% in case of all AIv challenge doses while it was 90% for the chicken groups vaccinated with H5N2 in cases of (10^4, 10^5, 10^6) challenge doses and 85% in case of (10^7) challenge doses. These results demonstrate that chicken vaccinated with good quality inactivated AI vaccines under good condition were protected from clinical signs (morbidity) and deaths (mortality) caused by AIv infection even with high doses.

1. Introduction

Avian influenza (AI) is a viral respiratory disease of many species of domestic and wild bird. AI viruses have been circulating previously among domestic poultry over the past 100 year [1]. AI viruses were classified on the basis of the severity of clinical signs in susceptible species. Highly pathogenic avian influenza (HPAI) is a devastating disease of poultry caused by some viruses of the H5 and H7 subtypes [2], but all subtypes of AI can cause low pathogenicity avian influenza (LPAI) form. Virus shedding and the development of clinical signs occur by a variety of avian influenza viruses in chickens. In experimentally infected birds, some HPAI and LPAI viruses can occur in faeces and respiratory secretions as early as 1 to 2 days after inoculation. Some HPAI viruses have also been found in meat 1 day after inoculation and in eggs 3 days after inoculation. LPAI viruses can be shed in

asymptomatically infected or minimally affected flocks [3, 4, 5, 6]. Vaccination protects against disease and mortality, but does not always prevent infection and virus spread. Inactivated whole virus vaccines were considered the main type that are licensed widely by several countries and proved efficacy. There is also live virus vaccines which were developed for AI using alternative recombinant live vectored constructs and can provide some of immunological advantages of live vaccines but without the reassortant risk of live AI virus [7]. Moreover, the disadvantages of some live recombinant vaccines include the risk of generating revertants and allow spread of genetically modified organisms in the environment [8]. The evaluation of inactivated AI vaccines depend on testing their potency and efficacy achiving protection in chicken against AI viruses [4, 5].

However, vaccinated birds shed far less field virus after infection than unvaccinated birds [9, 10].

This study aims to investigate the effect of challenge dose of local field HPAI virus on the efficacy of different AI H5 vaccines in protecting chicken against morbidity and mortality.

2. Materials and Methods

2.1. Chicken

Group of 220 SPF chicken of four weeks-old were obtained from a farm at Kom Oshem, El-Fayoum, Egypt. They were kept in positive pressure stainless steel isolation cabinets till used.

2.2. Vaccine

2.2.1. Inactivated H5N2 AI Vaccine

Inactivated oil emulsion LPAI H5N2 vaccine was produced by Boehringer Ingelheim vetmedica S. A. De. C. V., Mexico. The vaccinal strain is A/Chicken/ Mexico/232/94/CPA. It was administrated subcutaneously at the lower third of the neck at a dose of 0.5 ml /bird.

2.2.2. Inactivated Egyptian H5N1 AI Vaccine

Inactivated oil emulsion reassortant Avian Influenza H_5N_1 vaccine and was produced by Harbin Veterinary Research Institute (HVRI), China. The vaccinal strain is A /chicken / Egypt /A-18-H / 09. It was administrated subcutaneously at the lower third of the neck in a dose 0.3 ml /bird.

2.3. Virus

2.3.1. Antigens

(1) For potency and identity test we used three antigens

Inactivated Mexican H5N2 Antigen (A/Chicken/ Mexico/232/94/CPA), Inactivated H5N1 (H5N1 subtype, Egy/PR8-1 strain) Antigen (A/chicken/Egypt/A-18-H/09). Standard positive AI antisera were obtained with each homologous antigen.

(2) For purity test

Standard antigens against ND (lasota) and EDS'76 HI antigens and IB (M41), IBD (52/70), REO (S1133) AGP antigens were obtained from Central Veterinary laboratory (CVL), Weibrige, England. These antigens were used for detection of any extraneous agent in the purity test. Standard positive antisera were obtained with each homologous above antigens.

2.3.2. Challenge Virus

The highly pathogenic avian influenza virus A / Chicken / Egypt / 1709-6 / 2008 (H5N1) was isolated locally. Its titer was 10^{10} EID50/ml in embryonated chicken egg (ECE). It was submitted by National Laboratory for Quality control of Poultry (NLQP), Dokky, Egypt.

2.4. Sterility Test

To determine if the vaccine samples were free of bacterial and fungal contaminants and acceptable for release, all vaccine bottles were tested individually. One ml each of vaccine samples was inoculated into bacterial and fungal media plates. The inoculated media were incubated aerobically and anaerobically at 37°C for 21 days. Inoculated media were inspected for possible growth. It was done according to OIE manual for 2014.

2.5. Safety and Extraneous Test: (OIE, 2014)

About 20 healthy, 4 weeks old, SPF chickens were inoculated S/C and I/O with twice or 10X the normal recommended dosage for each vaccine. The birds were observed for any possible local or systemic adverse reaction due to each vaccine for 21 days. After 3 weeks, in case of inactivated vaccines, each bird was inoculated S/C with one field dose from the tested vaccines. Serum samples were collected two weeks later and tested for antibodies to extraneous agents were performed. It was done according to OIE manual for 2014.

2.6. Serological Tests

Haemagglutination inhibition (HI) test, Potency and efficacy test were carried out due to OIE manual for 2014

2.6.1. Haemagglutination Inhibition (HI) Test

The HI titer was the highest dilution of serum causing complete inhibition of 4 HAU of antigen. The agglutination was assessed by tilting the plates. Only those wells in which the RBCs stream at the same rate as the control wells (containing 0.025 ml RBCs and 0.05 ml PBS only) should be considered to show inhibition.

2.6.2. Potency and Efficacy Test

To demonstrate the antigenic capacity of the tested AI vaccines; SPF chickens, four weeks old, were vaccinated S/C and I/O with field dose recommended by the productive companies for inactivated and recombinant vaccines, respectively. Blood samples were taken weekly and the serum samples were separated, inactivated at 56°C /30 mint and kept at -20°C till used. The serological analysis of AI antibody level against H5 was determined by HI test using different type of

AI antigens corresponding to each vaccine type provided by the manufacturing companies. The efficacy of the vaccines was carried out by challenge test by using ascending challenge doses (10^4, 10^5, 10^6, 10^7) EID_{50} /ml of HPAI challenge virus. The protection percent was calculated according to daily deaths recorded during the challenge period.

Experimental design:

Population of 200 SPF chickens were divided into three groups A, B, C as shown in table 1

Table 1. *Vaccinal treatment and challenge dose for each subgroup.*

Group No.	Subgroup	Chicken No.	Vacc. Treatment	Challenge dose
A	A1	20 for each subgroup	H5N1	10^4 EID_{50}/ml
	A2			10^5 EID_{50}/ml
	A3			10^6 EID_{50}/ml
	A4			10^7 EID_{50}/ml
B	B1	20 for each subgroup	H5N2	10^4 EID_{50}/ml
	B2			10^5 EID_{50}/ml
	B3			10^6 EID_{50}/ml
	B4			10^7 EID_{50}/ml
C	C1	10 for each subgroup	Unvaccinated	10^4 EID_{50}/ml
	C2			10^5 EID_{50}/ml
	C3			10^6 EID_{50}/ml
	C4			10^7 EID_{50}/ml

Blood was collected from all birds before inoculation to ensure that the birds were serologically naive to influenza viral antigens, chicken were divided into control group contain 40 chicken and 160 vaccine inoculated chicken all are reared for 28 days, Booster dose was given at the day 21 post Vaccination, Blood sampling after 28 days of vaccination to detect antibody titer formed in response of vaccination, Oculo-nasal challenge was carried out. The control group received the same volume of normal uninfected allantoic fluid and the daily deaths and clinical signs were monitored.

3. Results

3.1. Identity and Sterility Tests

When the vaccinated chicken serum was examined by HI test using standard homologus H5 AI antigens, gave positive results. This results revealed that the produced HA antibodies were identical to the used antigen and the tested vaccines contain AI virus. The vaccines were found to be sterile and free from any contaminants as shown in table 2.

3.2. Serological Test

When the serum samples where tested after four weeks of vaccination the haemagglunation inhibition test shows that the antibody titer in chicken vaccinated with H5N1 was 7log2 and in chicken vaccinated with H5N2 was 8log2 as shown in table 2.

3.3. Safety Test

It was found to be safe for chicken vaccination when it was given as double doses as shown in table 2.

Table 2. *Characterization of two types of the tested AI Vaccines.*

Type of vaccine	Identity	Sterility	serology	Safety
H5N1	+ve	Sterile	8 log 2	Safe
H5N2	+ve	Sterile	7 log 2	Safe

3.4. Effecacy Results

The protection percent of chicken vaccinated with inactivated Egyptian H5N1 AI vaccine was 100% for all the four doses and all the chicken were a life till the end of the experiment. This confirm the complete protection against the virus without any clinical signs of infection as shown in table 3, while the protection percent of chicken vaccinated with the inactivated H5N2 AI vaccine was 85% for 10^7 EID_{50}/ml and 90% for 10^4, 10^5, 10^6 EID_{50}/ml as shown in table 4, respectively. The control chicken were dead. The results revealed that at 1st day, 2 chickens were died for dose 10^5 EID_{50}/ml, 14 chickens for dose 10^6 EID_{50}/ml and 20 chickens for dose10^7 EID_{50}/ml. At the 3rd day, 4 chickens died for dose 10^4 EID_{50}/ml, 18 chickens for the dose 10^5 EID_{50}/ml and 6 chickens for dose 10^6 EID_{50}/ml. At the 4 day, 4 chickens died for the dose10^4 EID_{50}/ml, one chicken died, while, at the 7th day, the dose 10^6 EID_{50}/ml as shown in table 3 and table 4 for the two vaccines.

Table 3. *Protection percent of H5N1 vaccine due to the use of different challenge dose.*

Titer/EID50	Type	No of chickens	Days post challenge										Mortality/total	Protection%
			1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th		
10^4	Vaccinated	20											0/20	100%
	Control	10			2	2							4/10	40%
10^5	Vaccinated	20											0/20	100%
	Control	10		1	9								10/10	0%
10^6	Vaccinated	20											0/20	100%
	Control	10		7	3								10/10	0%
10^7	Vaccinated	20											0/20	100%
	Control	10		10									10/10	0%

Table 4. *Protection percent of H5N2 vaccine due to the use of different challenge dose.*

Titer/EID50	Type	No of chickens	Days post challenge										Mortality/total	Protection%
			1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th		
10^4	Vaccinated	20								1	1		2/20	90%
	Control	10			2	2							4/10	40%
10^5	Vaccinated	20						1	1				2/20	90%
	Control	10		1	9								10/10	0%
10^6	Vaccinated	20						2	1				3/20	90%
	Control	10		7	3				1				10/10	0%
10^7	Vaccinated	20					1	1	1				3/20	85%
	Control	10		10									10/10	0%

4. Discussion

Continuous replication of HPAI H5N1 virus in vaccinated birds in Egypt during the last five years represent a challenge to study the influence of immune pressure on vaccine efficacy against the newly emerging viruses in different host species especially chicken. Avian influenza viruses is an important veterinary and human health pathogens around the world which can cause wide range of pathological effect in poultry industry. The AI can causes not only subclinical infection or high virulence but also high mortality rate can reaches 100% [11]. To date, vaccination is the most commonly used to prevent or reduce losses due to AI infection.

A variety of vaccines are therefore used to control the disease and limit its spread. We used two types of vaccines Volvac inactivated H5N2 AI vaccine and Egy-flu inactivated Egyptian H5N1 AI vaccine given with the recommended dose by the manufacturer at the age of four weeks old SPF chicken. Four weeks post vaccination, all the vaccinated chickens and the control group were challenged with HPAIV (H5N1) as a challenge virus. It was given with ascending challenge doses (10^4, 10^5, 10^6, 10^7) and deaths were monitored during the observation period.

The protection percent of chicken vaccinated with inactivated Egyptian H5N1 AI vaccine was 100% for all the four doses and all the chicken were a life till the end of the experiment. This confirm the complete protection against the virus without any clinical signs of infection, The protection percent of chicken vaccinated with inactivated Egyptian H5N2 AI vaccine was 85% on the broad aspect and the control chickens were dead

Our findings demonstrate the potential benefit of using H5N1 AI vaccine in vaccination as it gave higher rate of protection more than the protection accomplished by H5N2 vaccine. In this study as we fixed the other parameters as chicken age, the uniformity of the immune level as we used SPF chicken, the same ration and living condition with minimization of stress factors. The use of challenge test can predict flock response to AI exposure. This method can be considered as effective way to test the vaccine immunological efficacy. These findings substantiates the previous studies [12, 13]. Regular evaluation of the current vaccines in H5N1 endemic countries is a paramount challenge to mitigate the socio-economic impact of the virus in birds and human [14, 15]. It was also observed that despite the increase of the challenge dose both vaccines were capable of producing satisfactory immunological level that can provide protection to the chicken as the prevention of respiratory and general clinical signs (morbidity) and death (mortality) has been the most frequent used criteria to assess protection [16, 17].

The use of the vaccines can be effective tool under the use of strict control programs that includes biosecurity, education, surveillance, sufficient database, isolation and diagnosis, hygienic elimination and culling of infected birds, applying the environmentally sound disposal of carcasses and applying quarantine measures on the commercial movement of humans and birds that can provide convenient regime for the control of viral spread [12].

The protection percent accomplished by the two vaccines was capable on facing the ascending challenge dose of the HPAV. We also found that the good manufacturing procedures, proper adjuvant system, route and site of immunization are important for the insurance of protection against the infection with H5 viruses.

This study could be considered a fundamental platform for further investigations on the efficacy of H5 vaccines to protect chickens against H5N1, H5N2 virus infections [18].

5. Conclusion

As a result of the carried out work the used vaccines were capable of protecting the chicken against the ascending challenge doses even with the dose of 10^7 EID_{50} /ml. These findings reveals the importance of the good manufactured vaccine in the protection of chicken flocks against HPAIV. So, it is important to carry out a strict vaccination program with good quality manufactured vaccine to ensure satisfactory protection and immunization of the chicken flocks.

References

[1] Tumpey T. M., Alvarez, R., Swayne, D. E. and Suarez, D. L. (2005): Diagnostic Approach for Differentiating Infected from Vaccinated Poultry on the Basis of Antibodies to NS1, the Nonstructural Protein of Influenza A Virus, J. Clin. Microbiol., 43 (2): 676–683.

[2] Capua I. and Mutinelli F. (2001): A colour atlas and text on avian influenza, - Influenza Aviaria, Testo e atlante, - Atlas sobre la Influenza Aviar. A book, edited by Ilaria Capua and Franco Mutinelli, pp. 1-236, Published by: PAPI EDITORE, Edizioni.

[3] Anna R. S., Darrell W. T. and James A. R. (2008): The onset of virus shedding and clinical signs in chickens infected with high-pathogenicity and low-pathogenicity avian influenza viruses Avian Pathology Volume 37, Issue 6, 2008 pages 555-577.

[4] Capua I. and Alexander D. J. (2004): Avian influenza: recent developments. Avian Pathol., 33, 393–404.

[5] Van der Goot J. A., Koch G., de Jong M. C., van B. M. (2005): Quantification of the effect of vaccination on transmission of avian influenza (H7N7) in chickens. Proc. Natl. Acad. Sci. U.S.A.

[6] Ellis T. M., Leung C. Y., Chow M. K., Bissett L. A., Wong W., Guan Y., Malik Peiris J. S., (2004): vaccination of chickens against H5N1 avian influenza in the face of an outbreak interrupts virus transmission. Avian Pathol. 33: 405-412.

[7] Swayne D. E. (2008): Vaccines, vaccination and immunology for avian influenza virus in poultry. In: Avian influenza. Blackwell publeshers, Ames, Iowa. In press. Blackwell publishing. p. 407-451.

[8] Toro H, Tang D. G, Suarez, D. L, Zhang, J. and Shi Z. (2008): Protection of chickens against avian influenza with replicating adenovirus-vectored vaccine. Vaccine, 26: 2640-2646.

[9] Brugh M., Beard C. W., Stone H. D. (1979): Immunization of chickens and turkeys against avian influenza with monovalent and polyvalent oil emulsion vaccines. Am. J. Vet. Res.40: 165-169.

[10] Karunakaran D., Newman J. A., Halvorson D. A., Abraham A. (1987): Evaluation of inactivated influenza vaccines in market turkeys. Avian Dis. 31: 498-503.

[11] WHO (2006) World Health Organization, weekly epidemiological record, (5), 81, 41-48.

[12] Wood J. M, Kawaoka Y, Newberry L. A, Bordwell E and Webster R. W. (1985): Standardization of inactivated H5N2 avian influenza vaccine and efficacy against lethal A/chicken/Pennsylvania/1370/83 infection. Avian diseases: 29.867-872.

[13] Stone H. D. (1988): Optimization of hydrophile- lipophile balance for improved efficacy of New-castle disease and avian influenza oil emulsion vaccines. Avian diseases 32: 68-73.

[14] Tian G., Zeng X., Li Y., Shi J., Chen H. (2010): Protective efficacy of the H5 I nactivated vaccine against different highly pathogenic H5N1 avian influenza viruses isolated in China and Vietnam. Avian Dis. 54, 287–289.

[15] Pfeiffer J., Suarez D. L., Sarmento L., To T. L., Nguyen T., Pantin-Jackwood M. J., (2010): Efficacy of commercial vaccines in protecting chickens and ducks against H5N1 highly pathogenic avian influenza viruses from Vietnam. Avian Dis. 54, 262–271.

[16] Stone H. D. (1987): Efficacy of avian influenza oil emulsion vaccines in chickens of various ages. Avian diseases 31: 483-490.

[17] Xie Z. X and Stone H. D (1990): immune response to oil-emulsion vaccines with a single or mixed antigens of Newcastle, Avian influenza, and infectious bronchitis. Avian diseases 34: 154-162.

[18] Kapczynski D. R, Esaki M., Dorsey K. M, Jiang, H, Jackwood M, Moraes M, Gardin Y. (2015): Vaccine protection of chickens against antigenically diverse H5 highly pathogenic avian influenza isolates with a live HVT vector vaccine expressing the influenza hemagglutinin gene derived from a clade 2.2 avian influenza virus.2015 Feb 25; 33 (9): 1197-205. doi: 10.1016/j.vaccine.2014.12.028. Epub 2015 Jan 20.