

## Impact of H5N8 duck and chicken viral isolates on the immunological profile of vaccinated specific pathogen free chickens

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Nowadays, there is a global concern about outbreaks caused by the highly pathogenic avian influenza virus H5N8 clade 2.3.4.4 which caused devastating losses in the poultry industry sector. This clade was subdivided into two waves: clade 2.3.4.4A from 2014 to 2015 and clade 2.3.4.4b from 2016 until now. In this literature we aimed to evaluate the efficacy of recently used inactivated commercial avian influenza vaccines against two new Egyptian highly pathogenic avian influenza virus H5N8 isolates of clade 2.3.4.4b, A/chicken/Egypt/1526v/2020/H5N8 (H5N8-CH) and A/Duck/Egypt/Qalubia321/2021 (H5N8-D). Three-week-old specific pathogen free chickens were vaccinated with eight types of the most recently used inactivated avian influenza vaccines containing homologous and heterologous virus to the circulating H5N8 isolates. All specific pathogen free chicken groups were bled weekly post vaccination for antibody analysis using two H5N8 isolates of chicken and duck origin as antigen in hemagglutination inhibition test. Also, all vaccinated chicken groups were challenged 4 weeks post vaccination against the H5N8 duck isolate with a dose of  $10^9$  EID<sub>50</sub>/0.1 mL per chicken to measure the protection percentage of the commercial vaccines used. The results showed that vaccines with homologous and heterologous virus showed variable degrees of accepted protection percentage ranged from 90% to 100%, thus it was concluded that not only the genetic and antigenic match of the vaccine strains with the circulating highly pathogenic avian influenza viruses influences vaccine efficiency; other factors, such as manufacturing procedures, adjuvant, antigen content, vaccine dose and administration factors could affect vaccine efficacy, therefore, further vaccine development studies are needed to improve the percentage of protection and prevention of viral shedding against local highly pathogenic avian influenza H5 viruses in Egypt.

**Keywords:** avian influenza; H5N8 virus; influenza vaccines; chickens; ducks.

### Introduction

Highly pathogenic avian influenza (HPAI) viruses are considered a resident enzootic crisis threatening global, local economy and health conditions. For almost 16 years, the HPAI viruses have gone through several genetic variations that, in turn, subjected us to different emerging strains throughout different countries.<sup>(1)</sup> In 2010, the H5N8 clade 2.3.4.4 strain was first detected in

some types of wild migratory birds in Asia and then spread worldwide.<sup>(2)</sup>

By the end of 2016, the H5N8 strain was first reported in Egypt and has become endemic, which has been enhanced by the geographical location of Egypt as an articulating region between three continents. This unique location places Egypt in the crossing ways of

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wild migratory birds from various destinations of the world.<sup>(3)</sup> The H5N8 virus of clade 2.3.4.4 was considered highly pathogenic, so the Egyptian government started to implement control plans based on biosafety and biosecurity programs; this was carried out by increasing public awareness, culling infected birds, the sanitary burial of dead carcasses, and prohibition of backyard rearing, in addition to the limitation of commercial movements of birds between governorates. Vaccination programs were the first preventive and protective measure applied to protect against infection and limit disease spreading by minimization of viral shedding.<sup>(4)</sup>

New strains of HPAI virus H5N8 are recognized almost annually, causing tragic economic losses in the Egyptian poultry industry sector. They have resulted from wide variation in the hemagglutinin segment of the virus, in association with antigenic variation in the same subtype, provoking new reassortant strains that challenge the protective ability of permitted vaccination programs.<sup>(5)</sup> Therefore, regular follow-up of the efficacy of commercially used vaccines against new isolates is very critical to avoid a crisis of high morbidity and mortality.<sup>(6)</sup>

In this study, an experiment was designed to monitor the immunological response of vaccinated specific pathogen free (SPF) chickens by hemagglutination inhibition test (HI) and challenge test; the SPF chickens were vaccinated with some widely used commercial inactivated avian influenza vaccines (AIV) formulated from different H5 strains and an inactivated recombinant baculovirus vectored vaccine expressing H5&NDv (Newcastle disease virus). The HI test was done using two Egyptian HPAI H5N8 isolates of clade 2.3.4.4b, A/chicken/Egypt/1526v/2020/H5N8 (H5N8-CH) and A/Duck/Egypt/Qalubia321/2021 (H5N8-D), while the challenge experiment was performed using only the H5N8-D strain under strict hygienic measures including isolators.

## Materials and Methods

### Specific pathogen free chicks and eggs

SPF embryonated chicken eggs (ECE) were obtained from Koum Oshiem SPF chicken farm, Fayoum, Egypt. They were used for virus titration and shedding.<sup>(7)</sup> One-

day-old SPF chicks (total number 480) were raised in HEPA-filtered isolators with controlled lighting, feed, and water-supplied adequately. They were used to determine virus lethal dose 50 (LD<sub>50</sub>) and monitoring the potency and efficacy of the tested inactivated vaccines.

### Vaccines

Different eight inactivated commercial AIV were kindly supplied by the Central Laboratory for Evaluation of Veterinary Biologics (CLEVB) Abbassia-Cairo (Table 1). Seven inactivated whole avian influenza (AI) virus vaccines were classic oil-adjuvanted inactivated vaccines with different seed viruses, the eighth vaccine was a bivalent inactivated whole virus vaccine from the inactivated recombinant baculovirus vector-H5AI, propagated in insect cells and Newcastle disease virus.

### Viruses

Two different HPAI H5N8 viruses were locally isolated and were sent to be identified and sequenced by the Reference Laboratory for Veterinary Quality Control on Poultry Production (RLQP), Animal Health Research Institute (AHRI) –DOKKI- GIZA were used:

- A/chicken/Egypt/1526v/2020/H5N8 (H5N8-CH), chicken origin, identified as clade 2.3.4.4b.

- A/Duck/Egypt/Qalubia321/2021 (H5N8-D), duck origin, identified as clade 2.3.4.4b; its percentage similarity to different vaccine strains is shown in Table 1.

The (H5N8-D) isolate was used for serology tests, challenge experiments, and shedding tracing, while (H5N8-CH) isolate was used for serology tests.

### Virus titration in specific pathogen free eggs

Serial tenfold virus dilution ( $10^{-5}$  to  $10^{-12}$ ) of the virus in sterile antibiotic saline was inoculated in five ECE via allantoic sac (0.1 mL/egg). The inoculated embryos were incubated at 37°C-38°C and candled twice, daily for 6 days. Slide hemagglutination test (HA) was applied to the allantoic fluid of inoculated chicken embryos to detect positive HA reaction. The 50% egg infective dose (EID<sub>50</sub>) was estimated using the Reed and Muench method.<sup>(8)</sup>

**Table 1.** Types of inactivated AI-H5 vaccines used in the experiment.

Name	Strain	Type	Lineage	Similarity to H5N8 challenge virus
Reassortant avian influenza virus (Re <sub>5</sub> -H5N1)	A/duck/Anhui/1/2006 (H5N1)	Imported inactivated reassortant	2.3.4	98%
Volvac B.E.S.T (rBac-H5+ND)	A/duck/china/E319-2/2003 (H5N1) and Lasota	Imported inactivated recombinant baculovirus -AI + ND	2.3.2	93.3%
Egy flu (Egy-H5N1)	RGA/chicken/Egypt/18-H/2009 (H5N1)	Imported inactivated reassortant	2.2.1.1	86.8%
Poulvac FluFendi AI (Re-H5N3)	A/chicken/Vietnam/c58/2004 (H5N3)	Imported inactivated reassortant	Clade I	91.3%
Nobilis Influenza H5N2 (Pot-H5)	A/duck/Potsdam/1402-6/1986 (H5N2)	Imported inactivated LPAIV	Eurasian	81.8%
OPTIMUNE Avian Influenza vaccine (Mex-H5)	A/chicken/Mexico/232/1994 (H5N2)	Imported inactivated LPAIV	North American	75.6%
MEFLUVAC H5 PLUS 8 (Loc1-H5N1+H5N8)	A/chicken/Egypt/RG-13CAL/2017 (H5N1)	local inactivated reassortant	2.2.1.2	88.3%
	A/chicken/Egypt/ME1010/2016 (H5N1)		2.2.1.1	86.7%
	A/chicken/Egypt/ME-2018 (H5N8)		2.3.4.4b	98.5 %
Avian Flu H5 plus (Loc2-H5N8)	A/chicken/Egypt/D10552B/2015 (H5N8) +	local inactivated reassortant	2.3.4.4b	83.3%
	A/green winged teal/Egypt/877/2016			98%

LPAIV: Low pathogenic avian influenza virus. ND: Newcastle disease.

### Virus titration in chickens

It was done according to World Organization for Animal Health (WOAH)<sup>(9)</sup> for each viral isolate. Serial tenfold dilution ( $10^{-1}$ :  $10^{-6}$ ) of each H5N8 isolate was done. Each dilution was injected into five SPF chickens, 0.1 mL/bird. Daily deaths were recorded for one week to calculate viral LD<sub>50</sub> using Reed and Muench method.<sup>(8)</sup>

### Potency test

According to WOA<sup>(9)</sup> specifications, 4 week old SPF chickens, were vaccinated subcutaneously (S/C) with the field dose recommended by the companies that produce the inactivated vaccines listed in Table 1. Blood samples were collected weekly post-vaccination and serum samples were separated, inactivated at 56°C/30 minutes, and stored at -20°C until used. Serological

analysis to determine the level of antibodies against H5 was performed by the hemagglutination inhibition (HI) test using H5N8-CH and H5N8-D isolates. At 4 weeks post-vaccination (wpv), subgroups from the vaccinated and control groups were challenged with HPAI H5N8-D virus to determine the protection percentage of the tested vaccines. The challenge dose ( $10^9$  EID<sub>50</sub>) was inoculated intranasally (0.1 mL/each bird). Chickens were observed daily for 10 days after challenge. All dead and clinically infected birds were recorded as shown in Table 4. Tracheal and fecal swabs were taken 2 days post-infection (dpi) from all groups to estimate the viral shedding reduction using SPF ECE according to WOA<sup>(9)</sup>. The neutralization index (NI) was calculated by subtracting the virus titer of vaccinated SPF chickens from the virus titer of control SPF chickens. The NI should be  $\geq 2$  according to WOA<sup>(9)</sup>.

## Experimental design

A total of 225 SPF chickens were divided into nine groups (25 chickens/group). Eight groups were numbered from 1 to 8, each group was vaccinated with one of the tested vaccines shown in Table 1. The ninth group remained unvaccinated as control group. After 4 weeks of vaccination, the nine groups were subdivided into two subgroups A and B. Subgroup A contains 10 chickens per vaccination group. Subgroup A was subjected to the challenge test to estimate the efficacy of the AIV tested against the HPAI H5N8-D virus. While subgroup B of each experimental group remained unchallenged and was monitored weekly for serological analysis of immune response of the vaccinated with the different tested AI vaccines until the end of the experiment at the 11<sup>th</sup> wpv.

## Ethical approval

All animal experiments in this study were conducted in strict compliance and adherence to the relevant policies regarding animal handling as mandated by international, national, and/or institutional guidelines for animal care, and were approved by the Research Ethical Committee at the National Research Center, Cairo, Egypt.

## Results and Discussion

Since 2006, the AI virus has been threatening the poultry industry in Egypt. Despite the great effort carried out by governmental authorities to apply strict vaccination programs to control the disease, it has not been possible to completely eliminate the virus from the poultry field.<sup>(10)</sup> Until now, novel strains have been isolated periodically due to persistent viral mutation. Recently, the 2.3.4.4 clade isolated from ducks was found to be the most predominant since 2016.<sup>(5)</sup>

This study was carried out to evaluate the ability of the most recently used licensed commercial inactivated AIV in Egypt (shown in Table 1) to protect chickens against two isolated H5N8 AI virus, as well as the influence of the percentage similarity between the challenge strain and the different vaccine strains on the immune response.

Serological analysis using HI and cross HI were carried out, in addition to challenge test,<sup>(9)</sup> to evaluate the

commercial AIVs. The HI test and cross HI antibody titers were monitored weekly post-vaccination using H5N8-CH and H5N8-D isolates (Table 2). All the imported vaccines induced low cross HI antibody titer against H5N8-D isolate at the first 3 wpv ranged from 1.4 to 6.8 log<sub>2</sub>. After that, the antibody titers induced by all imported vaccines started to show a slight increase until the 6<sup>th</sup> wpv, this low level of antibodies ranging from 4.5 to 6.8 log<sub>2</sub>. Thereafter, the antibody titer gradually decreased until the 11<sup>th</sup> wpv reaching 3.5 to 5.5 log<sub>2</sub> for the imported vaccines tested. The same results were noticed for the cross HI antibody titers against H5N8-CH as shown in Table 2.

The locally prepared vaccines (loc1-H5N1+H5N8 and loc2-H5N8) induced higher levels of HI antibody titers against H5N8-D isolate reaching 7.8 and 7.5 log<sub>2</sub> at 5<sup>th</sup> wpv; in the case of the H5N8-CH isolate, the HI antibody titer reached its peak at 6<sup>th</sup> wpv achieving 8 and 6.5 log<sub>2</sub> (Table 2). Also, the HI antibody titer against H5N8-D antigen began to decrease from the 6<sup>th</sup> wpv to reach 6.8 and 6 log<sub>2</sub> at the 11<sup>th</sup> wpv; while for H5N8-CH antigen it started to decline at 7<sup>th</sup> wpv, to reach 7 and 5.5 log<sub>2</sub> at 11<sup>th</sup> wpv for the two local vaccines, respectively.

From the previous data, it is observed that the HI antibody titer achieved by the locally prepared vaccines against H5N8-D and H5N8-CH antigens was higher than the cross HI antibody titers induced by all imported vaccines against the heterologous H5N8 antigens. These relatively low antibody titers in the cross HI test were expected due to genetic and antigenic differences in the HA gene between HPAI H5N8 antigens and the different vaccine strains of the tested imported AIV compared to the homologous HI test results in the case of local AIV.<sup>(11)</sup>

The efficacy of the tested AI inactivated vaccines was evaluated using HPAI H5N8 clade 2.3.4.4b at 4 wpv.<sup>(9)</sup>

When the viral titer and pathogenicity of the two viral isolates H5N8-D and H5N8-CH were tested, it was found that the EID<sub>50</sub> were 10<sup>9</sup> and 10<sup>10</sup> for H5N8-CH and H5N8-D, respectively, while LD<sub>50</sub> in chicken was 10<sup>5.2</sup>/mL for H5N8-D isolate, but H5N8-CH isolate was not lethal.

**Table 2.** Mean HI antibody titer of different inactivated AI-H5 vaccines using local H5N8-D and H5N8-CH antigens.

Vaccine type	Antigen type	Antibody titer (log <sub>2</sub> )										
		Weeks post-vaccination										
		1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	7 <sup>th</sup>	8 <sup>th</sup>	9 <sup>th</sup>	10 <sup>th</sup>	11 <sup>th</sup>
Re5-H5N1	H5N8-D	1.2	3.2	5.5	6.7	6.7	6.7	6.5	6	5.8	5.5	5
	H5N8-CH	1.4	3.4	5.6	6.7	6.8	6.8	6.6	6.2	5.8	5.6	5
rBac-H5+ND	H5N8-D	2	3.4	4	5.3	5.6	5.9	5.9	4.8	4.5	4.3	4
	H5N8-CH	2.3	3.6	4.2	5.5	5.5	6	6	5	4.8	4.6	4.5
Egy-H5N1	H5N8-D	1.5	3.4	5.6	6	6.2	6.3	6	5.6	5.5	5	4.7
	H5N8-CH	1.7	3.5	5.5	5.4	5.6	5.3	5.1	5	5	4.8	4.8
Re-H5N3	H5N8-D	1.6	3.8	6.5	5.8	5.9	6.1	6.1	5.6	5.6	5	4.5
	H5N8-CH	1.5	4	6.4	6.6	6.2	6	6	5.8	5.5	5.3	5
Pot-H5	H5N8-D	1.5	3.6	4	4.2	5	5	4.8	4.5	4.3	4	3.5
	H5N8-CH	1.4	3.8	4	4.5	4.8	4.8	4.5	4.3	4	3.8	3.6
Mex-H5	H5N8-D	1.3	3	3.5	4	4.5	4.5	4.2	4	3.6	3.4	3
	H5N8-CH	1.5	4.2	5.6	6.2	6.4	6.4	6	5.8	5.6	5.5	5.5
Loc1-H5N1+ H5N8	H5N8-D	1.6	4.4	6.7	7.8	7.8	7.6	7.5	7.5	7.3	7.1	6.8
	H5N8-CH	1.6	4.8	6.8	7.8	7.8	8	7.8	7.8	7.6	7.4	7
Loc2-H5N8	H5N8-D	1.2	3.4	6.1	7	7.5	6.9	6.4	6.3	6.3	6.4	6
	H5N8-CH	1.4	3.4	6.6	6.5	7	6.5	6	5.8	5.8	5.6	5.5
Control	H5N8-D	0	0	0	0	0	0	0	0	0	0	0
	H5N8-CH	0	0	0	0	0	0	0	0	0	0	0

**Table 3.** Pathogenicity test of HPAI-H5N8 strains in SPF chickens.

Tested viruses	Viral dilution	Chicken No./ group	Days post inoculation (DPI)							Total Deaths/ total No.	Mortality % at DPI
			1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	7 <sup>th</sup>		
<b>H5N8-D</b>	dil 1	5	-	-	3	2	-	-	-	5/5	60% at 3 <sup>rd</sup> DPI 100% at 4 <sup>th</sup> DPI
	dil 2	5	-	-	-	3	2	-	-	5/5	60% at 4 <sup>th</sup> DPI 100% at 5 <sup>th</sup> DPI
	dil 3	5	-	-	-	3	-	1	-	4/5	60.7% at 4 <sup>th</sup> DPI 80% at 6 <sup>th</sup> DPI
	dil 4	5	-	-	-	-	2	1	-	3/5	40% at 5 <sup>th</sup> DPI 60% at 6 <sup>th</sup> DPI
	dil 5	5	-	-	-	-	-	1	-	1/5	20% at 6 <sup>th</sup> DPI
	dil 6	5	-	-	-	-	-	-	-	0/5	0%
<b>H5N8-CH</b>	dil 1	5	-	-	-	-	-	-	-	0/5	0%

(-): no deaths were recorded.

**Table 4.** Efficacy of different inactivated AI-H5 vaccines in chicken challenged with HPAI H5N8-D virus.

Vaccines	Chicken/ group	Daily deaths										Total mor- tality	Pro- tec- tion %	Viral shedding			
		1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	7 <sup>th</sup>	8 <sup>th</sup>	9 <sup>th</sup>	10 <sup>th</sup>			VT	TS	NI	FS
Re5-H5N1	10	-	-	-	-	-	-	-	-	-	-	0	100%	4	5	5	-ve
rBac-H5+ND	10	-	-	-	-	-	-	-	-	-	-	0	100%	4	5	5	-ve
Egy-H5N1	10	-	-	-	-	-	1	-	-	-	-	1	90%	2	7	7	-ve
Re-H5N3	10	-	-	-	-	-	-	-	-	-	-	0	100%	3	6	6	-ve
Pot-H5	10	-	-	-	-	-	1	-	-	-	-	1	90%	5	4	4	-ve
Mex-H5	10	-	-	-	-	1	-	-	-	-	-	1	90%	5	4	4	-ve
Loc1-H5N1+ H5N8	10	-	-	-	-	-	-	-	-	-	-	0	100%	3	6	6	-ve
Loc2-H5N8	10	-	-	-	-	-	1	-	-	-	-	1	90%	4	5	5	-ve
Control	10	-	-	4	6	-	-	-	-	-	-	10	0%	10	0	0	+ve

\*TS: tracheal swap; FS: fecal swap; VT: viral titer (log EID<sub>50</sub>); NI: neutralization index; -ve: absence of virus particles; +ve: presence of virus particles; (-): no deaths were recorded.

The HA titer was  $2^8$  and  $2^7$  for H5N8-CH and H5N8-D, respectively. As the H5N8-CH isolate was not lethal, the challenge doses were determined depending on pathogenicity of H5N8-D in chickens (Table 3) that could infect 100% of susceptible chickens within 3 to 4 dpi and it was  $10^{4.2}$  LD<sub>50</sub> or  $10^9$  EID<sub>50</sub>. This agrees with<sup>(12)</sup> who stated that LD<sub>50</sub> of the circulating AI virus was very low in comparison to the previously isolated AI strains.

Table 4 shows the protection percentage of all AI vaccines tested against the H5N8-D virus. It was observed that 100% of chickens in groups vaccinated with Re-H5N1, rBac-H5+ND, Re-H5N3 and Loc1-H5N1+ H5N8 vaccines survived the challenge with HPAI H5N8-D, while the Egy-H5N1, Pot-H5, Mex-H5 and loc2-H5N8 vaccines protected 90% of chickens against the same challenge virus. All the non-vaccinated chickens showed severe clinical signs with 100% mortality at 4-day post challenge (dpc) against the challenge dose  $10^9$  EID<sub>50</sub> (0.1mL/bird) of HPAI H5N8-D virus.

Although few reports indicated that HPAI H5N8-D viruses induced asymptomatic disease in ducks with prolonged virus shedding,<sup>(13)</sup> an increased viral adaptation to chicken was observed within the HPAI of 2.3.4.4.b clade viruses.<sup>(14)</sup> This was supported by the findings that the HPAI H5N8 challenge group showed typical AI signs and 100% mortality for the H5N8-D isolate and not for H5N8-CH isolate.

Also, there was a reduction in the viral shedding from the challenged vaccinated chicken groups. The NI 5, 5, 7, 6, 4, 4, 6 and 5 for the AI vaccines are listed in Table 4, respectively. The NI should be  $\geq 2$  according to WOAHA.<sup>(9)</sup>

From the previous results it was observed that despite the relatively low HI antibody titers against H5N8-D virus achieved by the imported commercial AI vaccines, formulated from different H5 seed virus strains of clade 2.3.4 (Re5-H5N1), clade 2.3.2 (rBac-H5+ND) and clade I (Re-H5N3), there was a high protection percentage reaching 100% in the vaccines and a reduction of viral shedding titers against the same virus with a range of 5:6 log<sub>10</sub> EID<sub>50</sub>.

More over other imported vaccines as Pot-H5 and Mex-H5 showed lower protection levels against the H5N8-D challenge virus reaching 90% with low viral shedding reduction of  $10^4$  EID<sub>50</sub>. This might be due to the lower similarity between the vaccinal strain and the challenge HPAI H5N8-D virus strain (shown in Table 1). This in agreement with Swayne et al.,<sup>(11)</sup> who stated that the more similarity between the AI strains the more reduction in viral shedding.

Yuk et al.,<sup>(15)</sup> showed that while commercial clade 2.3.2 H5 vaccines protected chickens against the HPAI H5N8-D virus challenge, they failed to prevent shedding. Also, Kandeil et al.<sup>(5)</sup> found that although the protection percentage of some commercial H5 vaccines was greater than 90% against the H5N8 strain belonging to clade 2.3.4.4b, with viral reductions in shedding greater than  $10^3$  EID<sub>50</sub> considered acceptable for any good quality vaccine, other factors can reduce the efficacy of a good quality vaccine, such as uncontrolled field conditions or inadequate biosecurity measures.<sup>(16)</sup>

On the other side, it was noticed that despite being the locally prepared inactivated AIV as loc1-H5N1+H5N8 and loc2-H5N8 formulated from clade 2.3.4.4b viral strains, the same as the circulating challenge viruses, provided protection percent of 100% and 90%, respectively. This is in agreement with Swayne et al.,<sup>(17)</sup> who explained that not only the genetic and antigenic match of vaccine strains with circulating HPAI viruses influences vaccine efficacy; other factors, such as manufacturing procedures, adjuvant, antigen content, vaccine dose and administration factors, affect vaccine efficacy, therefore, it is essential to conduct vaccine development studies to improve the percentage of protection and prevent viral shedding against local HPAI H5 viruses in Egypt.<sup>(14)</sup>

## Conclusions

Vaccines with homologous and heterologous seed virus showed variable degrees of accepted protection percentage ranged from 90% to 100%, thus it was concluded that not only the genetic and antigenic match of the vaccinal strains with the circulating HPAI viruses influences vaccinal efficiency; other factors such as manufacturing procedures, adjuvant, antigen content, vaccine dose and administration factors could affect

vaccine efficacy, allowing greater chances of being more immunogenic and effective against different HPAI viruses in endemic regions, therefore, it is essential to conduct further vaccine development studies aimed at improving the protection and prevention of viral shedding against local HPAI H5 viruses in Egypt.

### Conflict of interest

The authors declare that there is no conflict of interest.

### Author's contribution

Hala Nourhanne Mohamed: conducted the experiment and drafted the manuscript, designed and followed up the experiment and critically reviewed the manuscript.

Lamiaa M Omar: designed and followed up the experiment and critically reviewed the manuscript.

Ayman H El-Deeb: designed and followed up the experiment and critically reviewed the manuscript, participated in designing and followed up the practical work.

Mohamed AM Atia: designed and followed up the experiment and critically reviewed the manuscript, participated in designing and followed up the practical work.

Ahmed A El-Sanousi: conducted the experiments and drafted the manuscript, designed and followed up the experiment and critically reviewed the manuscript.

All authors read and approved the final manuscript.

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## **Impacto de virus H5N8 aislados de pato y pollo en el perfil inmunológico de pollos libres de patógenos específicos vacunados**

### **Resumen**

En la actualidad, existe una preocupación mundial por los brotes causados por el virus de la gripe aviar altamente patógena H5N8 clado 2.3.4.4 que causó pérdidas devastadoras en el sector de la industria avícola. Este clado se subdividió en dos oleadas: clado 2.3.4.4A de 2014 a 2015 y clado 2.3.4.4b de 2016 hasta ahora. En el presente trabajo, dos aislamientos egipcios de la gripe aviar altamente patógena H5N8 del clado 2.3.4.4b, A/chicken/Egypt/1526v/2020/H5N8 (H5N8\_CH) y A/Duck/Egypt/Qalubia321/2021 (H5N8\_D), se utilizaron para evaluar la eficacia de vacunas comerciales inactivadas contra la gripe aviar de reciente utilización. Pollos libres de patógenos específicos de tres semanas de edad fueron vacunados con ocho vacunas inactivadas contra la influenza aviar, de uso reciente, que contenían virus homólogos y heterólogos a los aislamientos circulantes de H5N8. Todos los grupos de pollos libres de patógenos específicos fueron sangrados semanalmente tras la vacunación para el análisis de anticuerpos; dos virus H5N8 aislados de pollo y pato se utilizaron como antígeno en la prueba de inhibición de la hemaglutinación. Además, todos los grupos de pollos vacunados fueron retados 4 semanas después de la vacunación con el virus H5N8 aislado de pato, con una dosis de  $10^9$  EID<sub>50</sub>/0,1 mL por pollo, para medir el porcentaje de protección de las vacunas comerciales utilizadas. Los resultados mostraron que las vacunas con virus homólogos y heterólogos presentaron grados variables de aceptada protección, la que osciló entre el 90% y el 100%, por lo que se concluyó que no sólo la coincidencia genética y antigénica de las cepas vacunales con los virus circulantes de la influenza aviar altamente patógena influye en la eficacia de la vacuna; otros factores, como los procedimientos de fabricación, el adyuvante, el contenido en antígenos, la dosis de la vacuna y los factores de administración podrían afectar a la eficacia de la vacuna, por lo que es necesario seguir estudiando el desarrollo de vacunas para mejorar la protección y la prevención de la excreción viral contra los virus H5 de la influenza aviar altamente patógena locales en Egipto.

**Palabras clave:** virus influenza aviar; virus H5N8; vacunas contra la influenza; pollos; patos.