

## Comparative study between the efficacy of the recombinant HVT-H<sub>5</sub> avian influenza vaccine and the reassorted inactivated H<sub>5</sub>N<sub>1</sub> vaccine in broiler chickens

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**Abstract:** In a trial to control the wide spread of highly pathogenic avian influenza (HPAI) H5N1 virus outbreaks among poultry flocks in Egypt, many inactivated oil adjuvant avian influenza (AI) virus vaccines were used. The objectives of this trial were to evaluate the efficacy of different vaccination programs including rHVT-H5 and inactivated H5N1 vaccines (Re-5 vaccine (Merial), Re-5 vaccine (Qyh Biotech) and Egy-flu 1 vaccine) applied alone or in combination in broiler chickens by using haemagglutination inhibition (HI) test against either homologous or heterologous AI antigens. It be concluded that the combination between the live rHVT-H5 vaccine and the inactivated Egy-flu 1 vaccine in a vaccination program was the best vaccination program compared to the other vaccination programs including the reassorted inactivated H5N1 vaccines alone and the rHVT-H5 vaccine alone.

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**Keywords:** vaccines, homologous or heterologous AI antigens, haemagglutination inhibition.

### Introduction

Highly pathogenic avian influenza A virus (HPAIV) of subtype H5N1 caused outbreaks in poultry in many Asian, European and African countries including Egypt. In attempts to control the disease, millions of birds have been destroyed. Despite these efforts, HPAI H5N1 virus has become endemic in several regions in domestic and wild birds (*Taha et al., 2006; Liks Guan et al., 2004 and Smith et al., 2006*). This situation represents a constant threat to poultry and wild birds in Egypt and worldwide. The imminent danger of introduction of HPAIV into domestic poultry led to implementation of vaccination in an increasing number of countries. However, vaccination as a tool to combat HPAIV is a contentious issue. The most convincing argument against vaccination coverage within poultry flocks in Egypt resulting in endemicity rather than in eradication. Continuous circulation of AI virus in vaccinated birds may then result in antigenic drift as has been reported by (*Taha et al., 2007 and Lee et al. 2004*).

However, vaccination may also serve as a tool for reduction of viral load in the environment, thus decreasing the risk of transmission within poultry

The current work was planned to study the efficacy of the recombinant HVT-H5 avian influenza vaccine and the reassorted inactivated H5N1 vaccines in broiler chicken in a different vaccination programs.

### 2. Material and Methods

**Birds:** three hundred and forty, one day old cubb broiler chicks were obtained from El-Ahram Poultry Company. Chicks were fed on commercial ration and water ad libitum

**Embryonated Chicken Eggs:** Specific pathogen free SPF Embryonated Chicken Eggs (ECEs), 9-11 day old, were used for performing completion of inactivation test. These eggs were obtained from Kom Oshim farm for SPF-eggs, El-Fayoum, Egypt.

**Vaccines: Egy-flu 1 (H5N1 subtype, Egy/PR8-1 strain):** The inactivated oil adjuvant reassorted avian influenza vaccine (H5N1 subtype, Egy/PR8-1 strain) was produced by Harbin Veterinary Research Institute (HVRI).

**Reassortant Avian influenza virus vaccine, inactivated (H5N1 subtype, Re-5 strain):** The inactivated oil adjuvant reassorted avian influenza vaccine (H5N1 subtype, Re-5 strain) was produced by Merial Nanjing Animal Health Co., Ltd.

**Reassortant Avian influenza virus vaccine, inactivated (H5N1 subtype, Re-5 strain):** The inactivated oil adjuvant reassorted avian influenza vaccine (H5N1 subtype, Re-5 strain) was produced by QYH Biotech Company Limited.

**VECTORMUNE® HVT AIV vaccine (Recombinant Avian influenza Marek's disease vaccine):** VECTORMUNE® HVT AIV vaccine contains a genetically engineered Marek's disease virus of serotype 3 (turkey herpes virus or HVT) expressing an avian influenza H5 type key protective

antigen. This vaccine is recommended for use in chickens as an aid in the prevention of Avian Influenza, H5 type and Marek's disease.

**Antigens: Homologous Antigens:** Inactivated H5N1 Antigen (A/ chicken/ Egypt/18-H/2009) and inactivated H5N1 Antigen (A/duck /Anhui/ 1/2006)

**Heterologous antigens:** A/chicken/ Egypt/5612NAMRU3-S/2006 (H5N1) antigen and A/chicken/ Egypt/ 9403 NAMRU3-CLEVB214 /2007 (H5N1) antigen

**Identity test (OIE 2008):** The identity of AIV type incorporated in the vaccine under test is carried out through RT-PCR and PCR tests

**Reverse Transcriptase Polymer-ase chain reaction (RT- PCR):** RT-PCR was conducted on the viral RNA of the reassorted inactivated H5N1 vaccines using primer pair specific for H5 gene of AI virus (Table 1) according to *Munch et al., 200*. The RT-PCR reaction mixture of 50 µl was 10 µl of 5x QIAGEN One Step RT-PCR Buffer, 2 µl of dNTP mix, 1 µl of Mgcl, 3 µl of the forward primer, 3 µl of the reverse primer, 2 µl of QIAGEN One Step RT-PCR Enzyme Mix, 15 µl of the viral DNA and 14 µl of water. The RT-PCR reaction condition was reverse transcription at 50°C for 30 min followed by initial denaturation at 95°C for 5 min, then followed by 30 cycles of denature at 94°C for 1 min, annealing at 55°C for 2 min and extension at 72°C for 1 min, with final extension at 72°C for 10 min. T-Gradient thermal cyclers of Biometra was used.

**Polymerase Chain Reaction (PCR):** PCR was conducted on the viral DNA of the recombinant HVT-H5 avian influenza vaccine using primer pair specific for H5 gene of AI virus (Table 1) according to *Munch et al., 200*. The PCR reaction mixture of 50 µl was 25 µl of Taq PCR Master Mix, 3 µl of the forward primer, 3 µl of the reverse primer, 10 µl of the viral DNA and 9 µl of water. The PCR reaction condition was initial denaturation at 95°C for 5 min, then followed by 40 cycles of denature at 95°C for 30 sec, annealing at 55°C for 1 min and extension at 72°C for 1 min, with final extension at 72°C for 5 min. T-Gradient thermal cyclers of Biometra was used.

**Safety Test:** According to *(OIE 2012)*, twenty susceptible chickens were inoculated S/C with twice the normal recommended dosage for each batch of the candidate inactivated AI vaccines and another 20 chickens were kept as an isolated control group. The birds were observed for any possible local or systemic adverse reaction due to each vaccine for 21 days. Their clinical signs and immune response to AI were evaluated by HI test.

**Experimental Design:** three hundred, one day old cubb broiler chicks were used in this experiment. They were divided into 6 groups:

**Group (I):** vaccinated with 0.5 ml of H5N1 subtype, Re-5 vaccine produced by Merial Nanjing Animal

Table 1: Sequence of primer pair species for H5 gene of AI virus

Primer Name	Sequence	Target	Expected Product Size
MMU2	(5'-ATA CCA TCC ATC TAC CAT TCC-3')	H5	290 bp
MMU9	(5'-TAT GCC TAT AAA ATT GTC AAG-3')		

Health Co., ltd S/C at 2 weeks of age and used them to follow the immune response for 3 weeks after vaccination

**Group (II):** vaccinated with 0.5 ml of H5N1 subtype, Re-5 vaccine produced by Qyh Biotech Company Limited S/C at 2 weeks of age and used them to follow the immune response for 3 weeks after vaccination

**Group (III):** vaccinated with 0.5 ml of Egy-flu 1 (H5N1 subtype, Egy/PR8-1 strain) S/C at 2 weeks of age and used them to follow the immune response for 3 weeks after vaccination

**Group (IV):** vaccinated with 0.2 ml of VECTORMUNE® HVT AIV vaccine S/C at one day old and used them to follow the immune response for 5 weeks after vaccination

**Group (V):** vaccinated with 0.2 ml of VECTORMUNE® HVT AIV vaccine S/C at one day old and then after two weeks with 0.5 ml of Egy-flu 1 vaccine (H5N1 subtype, Egy /PR8-1 strain) S/C as

booster dose and follow their immune response for 3 weeks after vaccination

**Group (VI) Non-Vaccinated Control Group:** for collecting blood weekly to be used in serological test as a negative serum

#### Serological Tests

**Haemagglutination and Haema- gglutination Inhibition Tests:** They were used for evaluation of the humoral immune response of the vaccinated chicken groups against AI virus vaccines (*OIE., 2012*).

### 3. Results

Concerning the rHVT-H5 avian influenza vaccine and the reassorted inactivated H5N1 vaccines evaluation results, the most important points were: firstly the rHVT-H5AI vaccine proved to be containing H5 gene, as detected by PCR and the reassorted inactivated H5N1 vaccines proved to be containing H5 gene, as detected by RT-PCR

On comparing the potency of the three reassorted inactivated H5N1 vaccines (Re-5 vaccine (Meril), Re-5 vaccine (Qyh Biotech) and Egy-flu 1 vaccine), in broiler chickens; with each others by using HI test. the results were 243, 367 and 356.4 mean HI unites against the homologous AI antigens; 8.4, 8.8 and 142.6 mean HI unites against the heterologous AI antigen prepared from the A/chicken/Egypt/9403 NAMRU3-CLEVB214/2007(H5N1) and 146, 267 and 320 mean HI unites against the heterologous AI antigen prepared from the A/chicken/ Egypt/ 5612NAMRU3-S /2006 (H5N1) respectively. (table 2)

Regarding to the evaluation of the rHVT-H5 vaccine, the results of humoral immune response by HI test were 104, 89.6, 32.6 and 260 mean HI titers

against the heterologous AI antigens prepared from A/duck/ Anhui/1/06; A/chicken/ Egypt/18-H/ 2009 (H5N1); A/chicken/ Egypt/9403 NAMRU3-CLEVB214/07 (H5N1) and A/chicken/Egypt/5612NAMRU3-S/06 respectively (table 3).

Regarding to the evaluation of rHVT-H5 vaccine in combination with Egy-flu 1 vaccine, the results of humoral immune response by HI test were 326.4, 1186, 566 and 1060 mean HI titers against the heterologous AI antigens prepared from A/duck/Anhui/1/06; A/chicken/Egypt/18-H/ 2009 (H5N1); A/chicken/Egypt/ 9403 NAMRU3-CLEVB214/07 (H5N1) and A/chicken/Egypt/5612NAMRU3-S/06 respectively (table 3).

**Table 2: Results of haemagglutination inhibition mean titre of AIV using different antigens 3 weeks post vaccination**

Type of vaccine	Homologous antigens	HA antigens	
		A/chicken/Egypt/9403NAMRU3-CLEVB 214/07	A/chicken/Egypt/5612NAMRU3-S/06
Re-5 vaccine (Meril)	243	8.4	146
Re-5 vaccine (QYH)	367	8.8	267
Egy-flu 1 vaccine	356.4	142.6	320

**Table 3: Results of haemagglutination inhibition mean titre of AIV using different antigens 3 weeks post vaccination**

Type of vaccine	HA antigens			
	A/duck/Anhui/1/06	A/chicken/Egypt/18-H/09	A/chicken/Egypt/9403NAMRU3-CLEVB 214/07	A/chicken/Egypt/5612NAMRU3-S/06
rHVT-H5 vaccine	104	89.6	32.6	260
rHVT-H5/ Egy-flu 1	326.4	1186	566	1060

### 3. Discussion

Inactivated oil adjuvant AI vaccines could be used with restricted biosecurity measures in a comprehensive strategy to control HPAI virus outbreaks in many countries including Egypt. Concerns have been raised about inconsistencies in field protection with quality of some vaccines (Swayne, 2004 and Swayne, 2008). The recombinant vaccines contained the H5 gene of AI were used to control AI in Mexico, USA and other countries (Swayne et al., 1997; Swayne et al., 2000 and Bublot et al., 2007).

The current study was planned to evaluate the efficacy of different vaccination programs, including the live rHVT-H5 vaccine and three inactivated vaccines, applied alone or in combination in broiler chickens, and then determine the best vaccination program which give the highest HI Ab titers against different homologous and heterologous antigens.

For all the three reassorted inactivated H5N1 vaccines (Re-5 vaccine (Meril), Re-5 vaccine (Qyh Biotech) and Egy-flu 1 vaccine), it could be concluded that the best inactivated vaccine which give the highest HI titers against either homologous or heterologous antigens was Egy-flu 1 vaccine.

About the results of the potency of rHVT-H5 vaccine by HI test, it showed that the mean HI titer at the 5<sup>th</sup> WPV was vary from 32.6 to 260 against different heterologous antigens. And these results may be due to the different identity percentages (95-99 %) when the sequence of the vaccinal strain is aligned with that of the different heterologous strains. These results disagree with that of (Rauw et al., 2012), who reported that the HI titers of chickens vaccinated at 1 day old with the rHVT-H5 vaccine against H5N1 Egypt 2008 and H5N1 Hungary 2006 antigens were < 3 and 3.8 log<sub>2</sub> HI unit respectively. Although they have identity percentages of 97- 99 %

when the sequence of the vaccinal strain was aligned with that of the Egypt and Hungary strains.

From the above results, the combination between the live rHVT-H5 vaccine and the inactivated Egy-flu 1 vaccine in a vaccination program was suggested to be done and its results indicated that it was the best vaccination program compared to the other vaccination programs including the reassorted inactivated H5N1 vaccines alone and the rHVT-H5 vaccine alone. These results were in agreement with that of (*Rauw et al., 2012*).

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