Serological Evaluation and Challenge Tests of the Recombinant and Inactivated AI Vaccines to Egyptian HPAIV H5N1 under Field Condition and Experimental Trails

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Abstract: Four different commercial vaccines of AI were evaluated in this study (VolVac® inactivated vaccine H5N2, VolVac® recombinant vaccine, Merial® inactivated vaccine H5N and Merial® recombinant vaccine) by serological and challenge tests under field condition and experimental trails. Egyptian HPAIV H5N1 (A_chicken_Egypt_11VIR4453-266_2010) used in challenge tests. Five broiler farms in Alexandria governorates used in field trail evaluation and the results indicated that the GM titers of the HI against AIV vaccine in the flocks (1, 2 & 3) vaccinated with recombinant and oil inactivated vaccine of (Merial®) were 7, 6 and 7.8 log 2, respectively, while in Flocks (4 & 5) vaccinated with recombinant and oil inactivated vaccine (VolVac®) were 5 & 5.1 log 2, respectively. Flocks (1, 2, & 3) revealed 70, 20, and 40 % protection respectively and flock (4 and 5) have 30 and 90 %, respectively. 270 one day old commercial broiler chicks (obtained from local hatchery were reared on a floor pens and divided into 9 groups) used in experimental trail evaluation were vaccinated S/C route and infected by HPAIV H5N1 (A_chicken_Egypt_11VIR4453-266_2010) and the results showed that the highest HI titers either at 28 days or 35 days of age was recorded in group (1) vaccinated with recombinant Merial® 7.7 log 2 at 28 days and 7.8 log 2 at 35 days followed by group (7) vaccinated with inactivated vaccine Merial® 7.4 log 2 at 28 days and 7.5 log 2 at 35 days then group (2) vaccinated with recombinant and inactivated Merial® 6.8 log 2 at 28 days and 7.1 log 2 at 35 days. While, other groups vaccinated with recombinant and inactivated vaccine Volvac® or inactivated vaccine of Volvac® gave the lowest titers ranged from 4.2 - 2.7 log 2. The highest percent of protection after challenge was recorded in group (3) vaccinated with recombinant vaccine Volvac® (90%) followed by group (1) vaccinate with recombinant vaccine Merial® (70%), while, group (2) vaccinated with recombinant vaccine and inactivated Merial® and group (5) vaccinated with inactivated Volvac® gave the same protection percent (50%) and the lowest protection percent was reported in the groups (4, 6 & 7) it was 40, 30 and 14.2%, respectively.

Keyword: Avian Influenza, Serological, Vaccine, GM, Recombinant, Inactivated

INTRODUCTION

The Avian influenza viruses (AIVs) subtype H5N1, belonged to the family Orthomyxoviridae, genus Influenza A, are negative single stranded, enveloped RNA viruses. A few members of H5 and H7 subtypes cause major and frequently
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fatal disease in experimental birds\(^1\). At the beginning of 2004, the outbreaks of avian influenza virus subtype H5N1 were reported in several Asian countries including Vietnam, South Korea, Japan, Taiwan, Laos, Cambodia, China, Pakistan, Indonesia and Thailand caused severe losses to the poultry industry\(^2\). Additionally, the H5N1 virus also poses a significant threat to human health\(^3\). Highly pathogenic avian influenza virus (HPAIV) subtype H5N1 which originated in southeast Asia in 1996, 1997 has spread across Eurasia since 2003 and entered Africa in 2005\(^4\). In Egypt, H5N1 HPAI outbreaks were reported in February 2006.

In March 2006, Egyptian official reported more than 900 H5N1 outbreaks among poultry in governorates spanning the length of the Nile land in an effort to control the outbreaks, authorities culled more than 34 million birds and implemented a poultry vaccination programme, their efforts largely controlled outbreaks in the second half of 2007\(^5\).

When poultry are infected, they may have no disease, mild disease or very severe disease. Chickens, quail and turkeys are especially susceptible, while ducks more commonly show no disease but act as a reservoir for the virus. Other poultry species including guinea fowl and pheasants, and also ostriches, can be came affected. While wild birds are generally not affected by the AI viruses that they carry, and can occasionally suffer disease. This has been observed as a result of infection with the H5NI virus in Asia and parts of Europe and may be a result of the virus's first becoming highly virulent in domestic birds\(^6\).

Vaccination efficacy in individual birds can be quantified using the following three parameters: (1) degree of protection from infection when exposed to a given amount of infectious virus, (2) the degree of reduction of morbidity and mortality given infection occurred, and (3) the level of reduction of virus excretion by infected poultry\(^7\). The efficacy of most commercially available vaccines has been determined in studies with chickens or turkeys, since globally they represent the economically most important poultry species. However, in many developing countries, particularly in Southeast Asia, other species such as ducks, Muscovy ducks, and quails also represent significant parts of the poultry sector. Thus, vaccines use in poultry populations with significant shares of species other than chickens and turkeys require efficacy testing in these species to contain viral transmission. Although, almost all commercially available vaccines provide some level of protection from infection with virulent virus and significantly reduce mortality in infected chickens no HPAI vaccine has so far proved to satisfactory perform on all three of the above parameters\(^8,9\).

The time lag between vaccination and protective immunity and the respective duration of protection depends on the vaccine used, timing of vaccination, number of doses given, species, immunologic condition of the birds, and the challenge virus. The literature on vaccine efficacy is dominated by studies with specific pathogen free (SPF) birds reared under laboratory conditions. The results of these studies cannot safely be extrapolated to field conditions, and in these protection is often assessed by serology, the assumption being that birds with a specified antibody level (e.g., a hemagglutination inhibition [HI] titer $\geq 16$)
are protected. However, under conditions of antigenic variability and diversity of the HPAI viruses circulating in the field, a given titer found against a laboratory strain cannot unequivocally be interpreted as protective against field virus challenge. Published literature on vaccine efficacy and the onset of protection under field and laboratory conditions indicated that protection in chickens is in no circumstances achieved prior to 11–13 days post vaccination\textsuperscript{10, 11, 12}. About 20.5 weeks after successful vaccination of layer chickens with two doses of killed LPAI H6N2 vaccine, all birds tested sero-negative again\textsuperscript{13}. 

Avian influenza viruses vary antigenically and evolve rapidly, which poses a major challenge for the sustained use of vaccines as HPAI control measure. Although, several studies demonstrated cross-protection for HPAI viruses, a correlation between virus shedding and antigenic differences of vaccine and field strains was shown\textsuperscript{14} for the Mexican lineage H5N2 virus and\textsuperscript{15} for a range of H5 HPAI viruses. During and after the extensive use of about two billion doses of H5N2 vaccine in commercial poultry farms in Mexico, molecular drifts with a yearly trend have been shown\textsuperscript{16}. Antigenic drift of avian influenza viruses was also observed in the USA after vaccination programmes for LPAI in commercial poultry\textsuperscript{17}. Variant field strains that escaped the protection by the commonly used vaccines emerged in Shanxi China during 2006, in Egypt in late 2006, and in Indonesia early 2007\textsuperscript{18}. Using a deterministic patch-structured model,\textsuperscript{19} found that an avian influenza vaccination campaign can lead to the prevalence of a vaccine-resistant strain, which could result in the replacement of viral strains in areas without vaccination via migration of asymptomatic birds.

The aim of this study is to evaluate different commercial vaccines (recombinant and inactivated) by serological and challenge tests under field condition and experimental trails.

**MATERIAL AND METHODS**

1. **AI virus:** Egyptian HPAIV H5N1 was isolated in embryonated chicken eggs at the Faculty of Veterinary Medicine Alexandria Univ. Egypt, obtained during routine surveillance of poultry holdings from a commercial broiler farm in Alexandria governorate during late 2010 and 2011. These flocks suffering from high mortality and respiratory distress. After RT-PCR test and gene sequencing was done at to OIE, FAO National Reference Laboratory for Newcastle Disease and AI, Istituto Zooprofilattico Sperimentale delle Venezie, Viale della universita (Padova)-Italy, was used for the challenge test.

2. **Specific pathogen free eggs (SPF):** Fertile, Specific pathogen free egg for H5N1 propagation and titration were obtained from Specific pathogen free governmental farm.

3. **Washed chicken RBCs:** Blood was collected from wing vein with sterile syringe containing anticoagulant (3.8% sodium citrate) at the rate of 4:1. Following collection, blood sample was mixed with equal amount of PBS (PH 7.2) and centrifuged at 3000 rpm for 15 min. The supernatant was discarded and the cell sediment was resuspended with PBS and centrifuged at 3000 rpm for 15 min
for three times and finally the supernatant was discarded. Finally, 1% and 10% washed RBCs suspensions in PBS were prepared and used for HI and slide HA test, respectively.

4. Determination of median embryo infective dose (EID50) of Egyptian HPAIV: H5N1 (A_chicken_Egypt_11VIR4453-266_2010) virus for challenge tests:

This was carried out according to. Allantoic fluid of the Egyptian HPAIV H5N1 was tenfold diluted in PBS and inoculated in 10-day-old SPF embryonated eggs via allantoic sac (5 eggs/dilution and 0.1ml/egg). The eggs were incubated at 37°C and candled daily for mortality up to 3 days post inoculation (PI). The dead embryo within 1st 24 hrs were discarded as nonspecific mortality. Embryos which die later and survivors were opened 3 days PI and harvest allantoic fluid from each egg and test for hemagglutination to determine the presence or absence of Avian Influenza virus.

The EID50 was calculated after. The end point of the titration is used to calculate the infectivity titer of the original suspension of virus. The Reed and Muench equation is used to calculate this end point from the results of the hemagglutination (HA) test on each of the inoculated eggs. A formula is used to calculate an index (proportionate distance) that is then applied to the appropriate dilution. The infectivity titer is expressed as EID50 per ml.

5. Slide hemagglutination test: This test was done by placing one drop of 10% washed chicken RBCs suspension in sterile saline (0.8% sodium chloride) onto a clean microscopic slide and thoroughly mixed with one drop of the harvested allantoic fluid. The result was recorded within one minute. If the haemagglutinating agent is present the RBCs will clump.

6. Mono specific antiserum: Mono specific antiserum for AI H5N1 kindly supplied by Boehringer Ingelheim ® Company.

Mono specific antiserum for AI H5N2 kindly supplied by Merial® company.

7. Hemagglutination inhibition (HI) test: HI test was carried out according to [23] to detect the H5-specific antibodies titer for each vaccine using mono specific antisera of each vaccine individually.

8. Origin of vaccines:

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>VolVac® inactivated vaccine H5N2</td>
<td>A/chicken/Mexico/323/94</td>
</tr>
<tr>
<td>VolVac® recombinant vaccine</td>
<td>A/chicken/Scotland/1959</td>
</tr>
<tr>
<td>Merial® inactivated vaccine H5N1</td>
<td>A/duck/Anhui/1/2006</td>
</tr>
<tr>
<td>Merial® recombinant vaccine</td>
<td>A/turkey/Ireland/1378/83</td>
</tr>
</tbody>
</table>

9. Field experiments:

Five broiler farms in Alexandria governorates were vaccinated S/C route and evaluate as follows:

<table>
<thead>
<tr>
<th>Flock No.</th>
<th>Vaccination regime</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>One day old recombinant vaccine (Merial®)</td>
</tr>
<tr>
<td></td>
<td>Ten days old inactivated reassortant vaccine (Merial®)</td>
</tr>
</tbody>
</table>
After 21-22 days post last vaccination 10 blood samples were collected from each flock and antibody titer was measured using HI test then 10 birds from each flock were transferred to Department of Poultry and Fish Diseases, Fac. Vet. Med., Alexandria Univ., for the challenge test. Each bird was inoculated intranasal with 0.5 ml of fluid contain 106/EID50 viral particles of Egyptian HPAIV H5N1 (A_chicken_Egypt_11VIR4453-266_2010). The birds kept under daily observation for 10 days. The signs, morbidity and mortality were recorded during the observation period. Also, tracheal and lung samples from dead birds were collected for reisolation of AI virus.

10. Experimental Trail:
* A total number of 270 one day old commercial broiler chicks obtained from local hatchery were reared on a floor pens with straw letter and divided into 9 groups. Each group contain 30 chicks. The chicks were vaccinated against Newcastle disease at 8 and 20 days of age using a clone 30 by eye drop vaccine and against IBD using IBD vacc in drinking water.

* Groups (1-7) were vaccinated against AI using recombinant and/or inactivated vaccines according to the following regime:

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rec. vacc. Merial® (1st day)*</td>
</tr>
<tr>
<td>2</td>
<td>Rec. vacc. Merial® (1st day) ++ inactivated Merial® (10th day)</td>
</tr>
<tr>
<td>3</td>
<td>Rec. vacc. Volvac® (1st day)*</td>
</tr>
<tr>
<td>4</td>
<td>Rec. vacc. Volvac® (1st day) ++ inactivated Volvac® (10th day)</td>
</tr>
<tr>
<td>5</td>
<td>Inactivated vacc. Volvac® (10th day)</td>
</tr>
<tr>
<td>6</td>
<td>Inactivated 0.3ml Volvac® (1st day) ++ 0.5 ml Volvac® (10th day)</td>
</tr>
<tr>
<td>7</td>
<td>Inactivated vacc. Merial® (10th day)</td>
</tr>
<tr>
<td>8</td>
<td>Control positive challenged with AI virus</td>
</tr>
<tr>
<td>9</td>
<td>Control negative non Challenged with AI virus</td>
</tr>
</tbody>
</table>

* 0.3 ml was inoculated at 1 day of age

* The chicks were reared for 6 weeks the temperature adjusted according to the age of birds. Water and feed were available addlibtum. Anticoccidial drugs were added two times at 18 and 28 days of age for 3 successive days each.

* Blood samples were collected at one day old, 8th, 21st, 28th, 35th days of age for determining the antibodies against AI virus using HI test with homologous antigen for each vaccine.
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*At 29 days of age 10 birds from each group transferred to the lab for challenge test using HPAIV H5N1 (A_chicken_Egypt_11VIR4453-266_2010) virus. Each bird inoculated through naso-ocular route with a dose 10^6/ EID 50 and kept under daily observation for 10 days. The signs, morbidity and mortality were recorded during the observation period. Also, tracheal and lung samples from dead birds were collected for reisolation of AI virus.

RESULTS
Results of field trail evaluation:

1. Serological evaluation

Data of the GM titers against AI virus vaccines in ten serum samples were collected at 30 days of age for each flock were presented in (Table 1). The GM titers of the HI against AIV vaccine in the flocks (1, 2 and 3) vaccinated with recombinant and oil inactivated vaccine of (Merial®) were 7, 6 and 7.8 log 2, respectively. While in Flocks (4 and 5) vaccinated with recombinant and oil inactivated vaccine (VolVac®) the GM titers were 5 and 5.1 log 2, respectively.

2. Challenge test: Data of mortality and protection percentages are presented in (Table 5). It was clear that flocks 1, 2, and 3 revealed 70, 20, and 40 % protection respectively and flock 4 and 5 have 30 and 90 %, respectively.

<table>
<thead>
<tr>
<th>Farm No.</th>
<th>GM titers H1</th>
<th>Mortality</th>
<th>Mortality %</th>
<th>Protection %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2^7</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>2^6</td>
<td>2</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>2^2.8</td>
<td>2</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>2^7</td>
<td>3</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>2^3.1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 5. Results of mortality and protection percent against AI virus for field trail

No= numbers Gm = Geometric titers (log 2) at 30 days of age.

Results of experimental trail evaluation:

1. Serological evaluation

Data of the GM titers against AI virus vaccines in serum samples collected weekly during the experimental period (35 days of age) were presented in (Table 2). The highest HI titers either at 28 days or 35 days of age was recorded in group 1 vaccinated with recombinant (Merial®) 7.7 log 2 at 28 days and 7.8 log 2 at 35 days followed by group 7 vaccinated with inactivated vaccine (Merail®) 7.4 log 2 at 28 days and 7.5 log 2 at 35 days then group 2 vaccinated with recombinant and inactivated (Merial®) 6.8 log 2 at 28 days and 7.1 log 2 at 35 days. While, other groups vaccinated with recombinant and inactivated vaccine (Volvac®) or inactivated vaccine of (Volvac®) gave the lowest titers ranged from 4.2 - 2.7 log 2.
### Table 6. Geometric means titers of H5 AI virus vaccines post vaccination
(Experimental trail 30 chicks/group)

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Vaccination program</th>
<th>Geometric means titer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 day old</td>
</tr>
<tr>
<td>1</td>
<td>Rec. vacc. Merial® (1st day)*</td>
<td>4.4</td>
</tr>
<tr>
<td>2</td>
<td>Rec. vacc. Merial® (1st day) *+ inactivated Merial® (10th day)</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>Rec. vacc. Volvac® (1st day)*</td>
<td>4.5</td>
</tr>
<tr>
<td>4</td>
<td>Rec. vacc. Volvac® (1st day) *+ inactivated Volvac® (10th day)</td>
<td>4.5</td>
</tr>
<tr>
<td>5</td>
<td>Inactivated vacc. Volvac® (10th day)</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>Inactivated 0.3ml Volvac®(1st day)* + 0.5 ml Volvac®(10th day)</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>Inactivated vacc. Merial® (10th day)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Control positive challenged with AI virus</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Control negative non Challenged with AI virus</td>
<td></td>
</tr>
</tbody>
</table>

0.3 *ml was inoculated at 1 day of age

2. Challenge test:

Data of mortality and protection percentages are presented in (Table 3). It was clear that the highest percent of protection after challenge was recorded in group 3 vaccinated with recombinant vaccine Volvac® (90%) followed by group 1 vaccinated with recombinant vaccine Merial® (70%). While, group 2 vaccinated with recombinant vaccine and inactivated Merial® and group 5 vaccinated with inactivated Volvac® gave the same protection percent (50%). On the other hand, the lowest protection percent was reported in the groups 4, 6 and 7 it was 40, 30 and 14.2%, respectively.

### Table 7. Results of mortality and protection percent against H5N1 AI virus of experimental trail

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Mortality</th>
<th>Total**</th>
<th>MDT/Day*</th>
<th>Mortality %</th>
<th>Protection %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st day</td>
<td>2nd day</td>
<td>3rd day</td>
<td>4th day</td>
<td>5th day</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
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<tr>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td></td>
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<td>1</td>
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<tr>
<td>4</td>
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<td>1</td>
<td>1</td>
<td></td>
<td>6</td>
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<tr>
<td>5</td>
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<tr>
<td>6</td>
<td>1</td>
<td>2</td>
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<td>7</td>
<td>1</td>
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<tr>
<td>8</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>

- Challenge with HPAI H5N1 at 28 days of age.
- Birds kept under daily observation for 10 days.
- MDT: Mean death time.
- ** Total: Total mortality after 10 days post-challenge.

DISCUSSION
Avian influenza (AI) is a widely highly contagious disease of poultry. In Egypt, in February 2006, sever outbreaks of HPAI, H5 NI; have emerged in several governorates and were associated with drastic mortality up to 100 % infected chickens 24.

Egypt has been most severely affected by continuous outbreaks, resulting in sever losses in the poultry industry. Efforts to control highly pathogenic H5NI avian influenza virus in poultry have failed despite increased biosecurity, quarantine and doses of oil emulsion H5N1 and H5N2 vaccines and the disease became endemic in Egypt 23. The ongoing circulation of HP H5NI AI in Egypt has caused >168 human infections and remains an unresolved threat to veterinary and public health 25.

Broiler production is the most important branch in the Egyptian poultry industry. Annually, more than 600-700 million broilers are fattened within approximately 5–6 weeks when more than 80% of them are traded as live bird markets (LBMs) throughout the country 26. During trade to LBMs the risk of exposure to endemically circulating HPAIV H5N1 is grossly increased. Marketing and processing of HPAIV-infected broilers in LBMs would pose a high risk interface of virus transmission to humans 27. Therefore, protection of broilers when ready to trade is a highly desirable goal in the combat against HPAI in Egypt. However, due to the short time life span of only 35–42 days of these birds it is difficult to induce resilient specific immunity by (repeated) vaccination. Furthermore, interference of vaccine efficacy in chicks hatching from H5-vaccinated breeders by maternal derived antibodies has been claimed to further jeopardize efforts to achieve protection of broiler chicks 28, 29, 30. Moreover, another possible cause for the increased percent of infection in broiler chickens is the presence of 21 commercial vaccines in Egypt during 2010 and 2011 leads to subsequent selection of mutants escaping vaccine pressure. Interestingly, a previous study estimated the emergence of antigenically distinct viruses in Egypt as also occurring 18 months after the beginning of vaccination in poultry 31. In China the emergence of vaccine escape variant viruses was reported one year after the implantation of vaccination in poultry 32.

Regarding the vaccine evaluation, using homologous H5N2 antigens half-life of maternally derived H5-specific antibodies in broiler chicks was approximately 4.4 log 2 at 1st day based on an initial mean HI titer of H5. This value was in agreement with results obtained by 33 4.2 log 2 at 1st day and lower than that (5.2 log 2) reported by 28.

Evaluation of the efficacy of the AI vaccines and vaccination program applied in 5 broiler flocks under field condition was carried out. Serological monitoring of immune response after 21 days post vaccination ( 30 days of age) indicated that the GM titers (log 2) ranged from 5- 7.8 34,35. Mentioned that the protected titers ranged from 4-6 log 2, this means that all the examined broiler flocks have a protected titer. Simultaneously at 30 days of age 10 birds from each flock was challenged using HPAI H5N1 virus. After 10 days of observation period the protection percent ranged from 20 - 90% (Table 5).
Amazingly, the high protection percent (90%) was in a flock (No. 5) which had a GM titers of 25. On the other hand flock No. 3 gave 40% protection while GM titers was high 27.8 (Table 5).

Under experimental condition evaluation of different AI vaccines included recombinant and oil emulsion vaccine (H5N1 and H5N2) was studied. GM titers of AI virus vaccines were determined weekly from 3rd to 5th weeks of age (2-3 weeks post vaccination). The data clearly indicated that after 3 weeks post vaccination the recombinant vaccine of Merial® or recombinant vaccine plus oil emulsion inactivated vaccine of Merial® gave the highest titers (27.8 - 27.1), respectively (Table 6).

While, recombinant vaccine Volvac® or recombinant vaccine plus oil emulsion inactivated vaccine gave low titers (23.2 - 24.2), respectively. On the other hand, oil emulsion inactivated vaccine of Merial® administered alone gave high titer 27.2 at the end of the 3rd week post vaccination. But, oil emulsion inactivated vaccine in both groups gave a low titer 23.2, 22.8 (Table 6).

At 29 days of age (3 weeks post vaccination) the different experimental groups were challenged with HPAIV H5N1 and kept under daily observation for 10 days. The high protection percent was recorded in the groups vaccinated with recombinant vaccine of Volvac® and Merial® (90% and 70%), respectively. While, the lowest protection percent was recorded in the group vaccinated with oil emulsion inactivated vaccine of Merial® (14.3%) (Table 7).

In fact, the previous mentioned data clarify that the chicken groups (1, 2 and 7) had the highest GM titers for AIV vaccines 27.2, 27.1 and 27.5 respectively gave only a protection of 70%, 50% and 14.3% respectively. While group 3 which had only GM titer of 23.2 gave a protection of 90%. These data lead us to state that there was no clear correlation between the GM titers and the protection rate. This could be attributed to, under field conditions antigenic variability and diversity of HPAI viruses circulating in the field, a given titer found against a laboratory strain cannot unequivocally be interpreted as a protective against field virus challenge. Also, serological monitoring of H5 vaccinated flocks by the HI test using the homologous vaccine antigen is a routine laboratory procedure to evaluate vaccination efficacy of poultry in Egypt and elsewhere [36]. A correlation between the level of HI antibody titer and protection against disease and virus excretion was predicted by 34,35 to be at least 4.0 _ HI _ 6.0. However, results obtained in this study showed that such relations are only valid if there is a close antigenic match between the vaccine and the challenge viruses 37. Relying on the homologous HI titer measured against the commercial vaccine H5N2 antigen may result in an erroneous prediction of protection against currently circulating H5N1 viruses in Egypt. In countries endemically infected with divergent HPAI H5N1 viruses, antigens from the circulating field viruses must be used for HI assay-based predictions. Using different H5N1 viruses circulating in China and Vietnam38 found that the levels of cross reaction of HI antibody titer differed 8–32 fold between different strains of H5N1 even within the same clade.
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Also, Variation in the protection percent of challenged birds either with low or high GM titer could be explained as follow, vaccination of MDA chicks with the same vaccine that has been used for breeder immunization was useless in terms of inducing protection, a vaccine that was based on the antigenically highly distinct HPAIV EGYvar/H5N1 strain (clade 2.2.1) did not suffer from interference with MDA. HI antibodies of similar titers were induced in chicks independent of their MDA status and their age. These chicks were clinically protected when challenged with EGYvar/ H5N1.

Our findings support studies by 29, 38, 39, who mentioned that the antigenic match between the vaccine strain and the circulating field virus is one of the most decisive factors in determining the H5 vaccine efficacy to prevent replication and transmission of H5N1 virus. However, previous studies stated that homology between H5 vaccine strains and challenge viruses did not influence virus excretion and/or protection 1,40,41. This means that the strain selection should be based not only on genotype but also protectotype.

In fact, in the field and experimental trails the broiler chicks with MDA were delivered from broiler breeder flocks vaccinated several times with inactivated oil emulsion vaccine of AI virus. Vaccination of MDA-positive chickens at a later age (10 days) seems to be a valuable, although MDAs may still interfere with vaccination to a lesser extent because they are present up to 3 weeks post hatching. Therefore, in areas with high infection pressure, when possible, two vaccinations are recommended for optimal protection 28. The induction of antibody titers by vaccination was severely inhibited by maternal antibodies, with only a few chickens showing responses similar to the control chickens. It is concluded that high maternal antibody titers are required for clinical protection and reduction of virus titers after infection of chickens, whereas low antibody titers already interfere with vaccine efficacy 30. Also, this idea in accordance with field observations from Egypt in late 2006 and early 2007 where 295 out of 355 (83%) examined broiler flocks possessing MDA did not seroconvert upon vaccination with vaccines similar to those received by the breeders [41]. This may lead us to a state that it might be advisable to take into account day-old AI MDA titers when one is determining the optimal age of vaccination, as the MDA make clearance of the viral antigen within the vaccine thus the vaccine efficacy reduced.

Generally, recombinant vaccines of Merial® and Volvac® gave a protection of 70% and 90% in group 1 and 3 respectively either with low or high GM titers 27.8, 23.2.

The chickens with high levels of maternal antibodies against NDV and AIV may be well and fully protected when vaccinated with 106.8 and 107.8 CEID50 of a live recombinant LaSota Newcastle disease virus (NDV) - Avian Influenza H5 vaccine (rNDV-LS/AI-H5) respectively. Lower doses (105.8 or less) of the same vaccine conferred poor protection. There was no correlation of protection induced by rNDV-LS/AI-H5 with NDV and AIV HI antibody levels 21 days after vaccination. Even those groups that were fully protected by 107.8 CEID50 had HI antibody titers as low as 20.7 against NDV and 22.4 against AIV. This may indicate...
that the induced protection is from enhanced innate immunity or cell mediated immunity\textsuperscript{32}.

Therefore, annual or biannual evaluation of the vaccine efficacy in the face of antigenic drift due to the immune pressure exerted by the vaccine has been suggested\textsuperscript{9,27}. For the foreseeable future routine update of vaccines and diagnostics will remain the major challenge of all attempts to restrict circulation of HPAIV H5N1 in poultry populations of endemically infected regions and to decrease risks of human exposure.

In addition to, high tittered vaccines, more aggressive vaccine strains, multiple immunization especially if high MDA in chicks, or a combination of all three approaches all these are required for successive vaccination as supported by idea of\textsuperscript{33}.

Finally, we can concluded that continuous active and passive surveillance is very necessary for monitoring virus evolution, manager of infected flock appropriately, improve vaccination protocols and identify or exclude the presence of other strain. Also, AI vaccines should be evaluated regularly to ensure that they are still protective against the circulating virus strain.

REFERENCES


Serological Evaluation and Challenge Tests ...


