Saudi Journal of Biological Sciences 28 (2021) 5310–5316

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

The potency of newly development H5N8 and H9N2 avian influenza vaccines against the isolated strains in laying hens from Egypt during 2019

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ARTICLE INFO

Article history:
Received 20 April 2021
Revised 16 May 2021
Accepted 19 May 2021
Available online 24 May 2021

Keywords:
H5N8
H9N2
Avian Influenza virus
Experimental vaccines
Viral shedding

ABSTRACT

Avian influenza (AI) is a respiratory disease complex syndrome recently recorded in vaccinated flocks causing high economic losses. This study aimed to prepare inactivated vaccine from recently isolated field strains [highly pathogenic avian influenza (HPAI) (H5N8) and low pathogenic avian influenza (LPAI) (H9N2)] and compare the efficiency of the two experimental avian influenza vaccines and some commercial avian influenza H5 and H9N2 vaccines in laying hens. The obtained results indicated that the identified experimental vaccines (H5N8 and H9N2) were protected the flocks from AI as compared to commercial H5N1, H5N3, and H9N2 vaccines, which showed a protection level of 80, 70, and 90%, respectively, indicating a high efficacy for the developed vaccines. In addition, it significantly improved the virus shedding, especially when used in booster dose. The experimental vaccines were given high antibody titer higher than commercial vaccine which was reached to 9.3 log_2, 9.7log_2 for experimental H5N8 vaccine which was significantly higher than and groups 3 and 4 especially at 2nd WPV, while at the 3rd WPV, the significant difference was with group 4 only. The HI titer was 9.3 log_2 at 2nd WPV for the experimental H9N2 vaccine that was significantly higher than group 9. In conclusion, the booster dose of the experimental vaccines could elicit strong immunity than single-dose and commercial vaccines.

1. Introduction

Poultry is a major source of meat and egg intake for animal-derived protein. In addition, the poultry industry, particularly in Egypt, is considered a major source of national income worldwide. Recently, this industry is impacted heavily by different respiratory viral diseases such as avian influenza (AI) (Hassan et al., 2019). AI is caused by the Influenza type A virus, which belongs to the family Orthomyxoviridae. It is a segmented RNA virus that is serologically categorized according to the antigenic difference of 2 surfaces glycoprotein into 18 HA (H1-H18) and 11NA (N1- N11) subtypes (Tong et al., 2013). Over the past decade, exposure to highly pathogenic avian influenza (HPAI) (H5N1, H5N8) and low pathogenic avian influenza (LPAI) (H9N2) has challenged the poultry industry in Egypt, causing high economic losses (Selim et al., 2017). The HPAI (H5N8) virus was firstly observed in Chinese live bird industry at 2010 (Lee et al., 2014). The HPAI (H5N8) viruses triggered separate outbreaks in domestic poultry and South Korean wild birds by 2014. Between 2014 and 2017, several outbreaks were subsequently recorded in many Eurasian and North American

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.05.049
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countries, either in wild or domesticated birds (OIE, 017). The surveillance of AI in Egypt revealed first introduction of HPAI (H5N8) in wild birds at the end of 2016 belongs to clade 2.3.4.4b (Selim et al., 2017) and then spread to domestic poultry, causing outbreaks in the poultry flocks and high economic losses in the poultry industry (Anis et al., 2017; Salaheldin et al., 2018; Yehia et al., 2018). The LPAI (H9N2) was first introduced in 2010 cluster to G1 lineage (El-Zoghby et al., 2012) and causing severe economic losses when co-infected with other bacterial and viral pathogens (Hassan et al., 2017). The veterinary authorities in Egypt have sought to enforce a systematic action plan to control the spread of the virus but have had little progress due to discrepancies in execution. More than 24 commercially inactivated H5 vaccines are approved to be used in poultry; the genetic incompatibility of these vaccines with the circulating viruses has led to the failure of the HPAI vaccination strategy among poultry in Egypt, causing outbreaks in vaccinated poultry flocks (Kayali et al., 2016).

The study aimed to prepare auto genus inactivated vaccine from field isolated strains (H5N8 and H9N2) and compares the efficacy of the experimental vaccine and the commercial vaccines that are already used in the field.

2. Material and methods

2.1. Antigens

Influenza virus A/ chicken/ Egypt/ AB1/ 2018 (H5N8) (Clad 2.3.4.4) and A/chicken/EGYPT/AB3/2018 (H9N2) were used as the antigen. The accession number of HA and NA genes of the H5N8 virus are MK975994 and MK975996, respectively and the accession number of HA and NA genes of the H9N2 virus are MK968881 and MK968893, respectively. They were isolated from infected layer chickens in Sharkia province, Egypt and identified at the Reference Laboratory for Veterinary Quality Control on Poultry Production (RLQP), Egypt.

2.2. Vaccine preparation

The seed virus vaccines were propagated in the allantoic of 11-day-old embryonated chicken eggs. The allantoic fluids were harvested after 72 h. The harvested material was clarified and inactivated by treatment with 0.1% formalin for 16 h at 37 °C while the fluid was continuously shaken. The absence of inactivated viruses was confirmed by inactivating tests by inoculation in susceptible embryonated eggs (Slemons et al., 1974; Stone, 1987).

Antigen was stored at −70 °C before homogenizing with oil adjuvant (Brugh et al., 1979). Water-in-oil adjuvant Montanide ISA-70 (SEPPIC, Commits/Pharmacy Division, Paris, France) was used to produce this experimental vaccine. Inactivated oil-emulsion vaccine was experimental by homogenizing three parts (v/v) of antigen with 7 parts (v/v) of Montanide ISA70. The concentration of antigen in the aqueous phase was retained at least to the equivalent of $10^{8.5}$ EID50/dose for H5N8 virus and 10^{8} EID50/dose for H9N2 (Brugh and Siegel, 1978). Details of preparation and methods used to assess emulsion viscosity and stability have been described earlier by Stone et al., (1978).

2.3. Commercial vaccine used

The commercial vaccine used in the field against H5 viruses was Merial, (H5N1) Clade 2.3.4 vaccine (Batch no. 18103173) and Zoetis H5N3 vaccine A/chicken/Vietnam/C58/2004 (H5N3), Clade 1, Zoetis USA, (Batch no. 240601). Commercial inactivated Cevac Flu H9K, CEVA (H9N2) vaccine, was used (Batch no: 0412FG1KNB). Manufacturers' recommendations were followed during the use of commercial vaccines.

2.4. Experimental challenge

In this experiment, a total of one hundred SPF chickens (100 days old) were divided into ten groups (10 birds/each), as discussed in Table 1.

Group 1 received one dose of experimental inactivated HPAI (H5N8) vaccine at 110 days, group 2 received two doses of experimental inactivated HPAI (H5N8) vaccine at 100 and 125 days, group 3 received one dose of Merial (H5N1) Clade 2.3.4 vaccine, at 110 days, group4 were received Zoetis H5N3, A/chicken/Vietnam/C58/2004 (H5N3), group 7 received one dose of experimental inactivated vaccine LPAI (H9N2) at 110 days, group 8 received two doses of experimental inactivated vaccine LPAI (H9N2) at 110 and 125 days, and group 9 were vaccinated with Cevac Flu H9K, CEVA (H9N2) vaccine. The positive controls (Groups 5 and 6) were non-vaccinated. Group 10 was a negative control (non-vaccinated and non-infected) (Table 1).

The SPF chickens vaccinated subcutaneously in the dorsal anterior of the neck with 0.5 ml/bird. The chickens were housed in separated groups and were fed with complete diets. Three serum samples were collected from each group separately at 2nd, 3rd and 4th week after vaccination. The HI titers were determined using standard method. The HI responses were measured using Influenza virus A/ chicken/Egypt/AB1/2018 (H5N8), and avian influenza (Clad 2.3.4.4) and A/chicken/EGYPT/AB3/2018 (H9N2) according to the OIE (2018).

Virus challenge was preceded four weeks post-vaccination intranasally by using 10^{7} EID_{50}/0.1 ml of the AI types H5 and H9 challenge viruses separately. The chickens that challenged were daily observed for ten days post-challenge for clinical signs, mortality and morbidity.

2.5. Determination of virus shedding

Oropharyngeal swabs were collected in 1 ml of sterile PBS at 2, 4, and 10 days post a challenge to record titers of viral shedding from all challenged birds. Swab samples were centrifuged at 2000 rpm for 10 min at 4 °C and collect the supernatants were submitted to real-time PCR for virus titration.

2.6. RNA extraction and real-time PCR

The collected oropharyngeal swabs in PBS were frozen at 70 °C. The viral RNA was extracted using QIAamp Viral RNA Mini Kit (QIAGEN, Benelux B.V., Hulsterweg 82, Venlo, The Netherlands). Quantitative real-time RT-PCR (qRT-PCR) was done by (Löndt et al., 2008; Ben Shabat et al., 2010). In short, a one-step qRT-PCR using sequence-specific probes for gene expression analysis was performed according to the instructions of the manufacturer (QIAGEN, The Netherlands) and using the ABI 7500 Real-Time PCR system (Applied Biosystems, Carlsbad, CA). Primers and probes targeting H5 and H9 influenza viruses were purchased from Metabion GmbH, Germany, as shown in (Table 2). A standard curve was established for viral quantification with viral RNA extracted from the titrated challenge virus, HPAI type H5N8 virus, and the LPAI type H9N2 virus. Results were reported as EID50/ml equivalents.

2.7. Statistical analysis

Data were reported as mean ± SD. All results were produced by SPSS version 25 (Armonk, NY: IBM Corp), and Graph Pad prism 8.0.2 (GraphPad Software, Inc) was used for graphing charts. One-way ANOVA was run to test differences among groups and

Table 1
Experimental plan.

Challenge virus (10^7 EID50/0.1 ml)	Type of vaccine	Age at vaccination (day)	No. of birds	Groups
HPAI (H5N8)	Experimental inactivated HPAI (H5N8)	110	10	Group 1
HPAI (H5N8)	Experimental inactivated vaccine HPAI (H5N8)	110 125	10	Group 2
HPAI (H5N8)	Merial (H5N1) Clade 2.3.4 vaccine	110	10	Group 3
H5N8	Zoetis H5N3, A/chicken/Vietnam/C58/2004 (H5N3)	110	10	Group 4
H5N8	Unvaccinated	110	10	Group 5 (control + ve)
H9N2	Unvaccinated	110	10	Group 6 (control + ve)
H9N2	Experimental inactivated vaccine LPAI (H9N2)	110	10	Group 7
H9N2	Experimental inactivated vaccine LPAI (H9N2)	110 125	10	Group 8
H9N2	Cevac Flu H9K, CEVA (H9N2)	110	10	Group 9
Not infected	Unvaccinated	–	10	Group 10 (control-ve)

Table 2
Primers and probes of H5 and H9 viruses.

Virus	Gene	Primer/ probe sequence 5′-3′	Ref
AIV H5 subtype	H5	H5LH1 ACATATGACTAC CCACARTATTCA G H5RH1 AGACCAGCT AYC ATGATTGC H5PRO [FAM]TCWACA GTGGCGAGT TCCCTAGCA[TAMRA]	Löndt et al. (2008)
AIV H9 subtype	H9	H9F GGAAGAATTAATTATTATTGGTCGGTAC H9R GCCACCTTTTTCAGTCTGACATT H9 Probe [FAM]AACCAGGCCAGACATTGCGAGTAAGATCC[TAMRA]	Ben Shabat et al. (2010)

significant results followed by Duncan's multiple range tests. $P < 0.05$ is statistically significant.

3. Results

The experimental inactivated vaccine and commercial avian influenza vaccines depend on the Egyptian HPAI H5N8 and LPAI H9N2 viruses used to vaccinate seven chicken groups. We assessed the different types of vaccines by the serological responses weekly until four weeks after vaccination. In group 1, which vaccinated with the experimental H5N8 vaccine (one dose at 110 day) appeared obvious increase in the antibody titers versus to the homologous virus with a mean HI titer of 5.3 $\log_2$, 9 $\log_2$ and 9.3 $\log_2$ at 2nd, 3rd and 4th week post vaccination respectively, which was significantly higher than group 3 and 4 especially in the 2nd week while at the 3rd week , the significant difference was with group 4 only as shown in Table 3 and Fig. 1. in group 2 which was injected with the same vaccine at 110 day and taken booster dose at 125 days, the mean HI titer were 6 $\log_2$, 9.3 $\log_2$ and 9.7 $\log_2$ at 2nd, 3rd and 4th week post vaccination respectively, which was significantly higher than groups 3 and 4 especially at 2nd WPV , while at the 3rd WPV , the significant difference was with group 4 only as shown in Table 3 and Fig. 1.

A/ chicken/Egypt/AB1/2018 (H5N8) virus secluded from household poultry to unvaccinated chickens was fatal. It causes 100% mortality from 4th to 8th days after challenge, with AIV infection signs, including combs and wattle cyanosis, hemorrhage in shanks, lethargy, and diarrhea. Groups 1 and 2 ere vaccinated with experimental H5N8 vaccine and remained live for ten days after challenge with no signs of infection. While groups 3and 4, the protection percent were 80% and 70%, respectively, with mild signs of AIV infection, as shown in Fig. 2.

The results of viral shedding revealed that, in group 1, the viral shedding was found only at the 2nd-day post-challenge with a mean virus titers 6.154 $\times$ 10^4 EID_{50}/mL, while in group 2, there were no viral shedding. In group three, which were vaccinated with Merial H5N1 vaccine, the viral shedding was detected at the 2nd-day post-challenge with a mean virus titer 7.206 X 10^4 EID_{50}/mL and no viral shedding was detected at 4th-day -post-challenge. In group 4 which was vaccinated with the Zoetis H5N3 vaccine, the viral shedding was detected at the 2nd day with a mean virus titer 4.843 X 105 EID50/mL at 4th day, the mean virus titer was 2.6625 X 10^5 EID_{50}/mL as shown in Fig. 3.

Table 3
Profile of HI antibody responses weekly post-challenge in chicken groups vaccinated with experimental H5N8 vaccine and some commercial H5 avian influenza vaccines.

Groups	Type of vaccine	Week 2	Week 3	Week 4	F and (P-value)
1	Experimental H5N8 vaccine (one dose)	1.61 ± 0.17^{bc}	2.71 ± 0.10^{a}	2.81 ± 0.17^{ab}	66.5 (0.0008)
2	Experimental H5N8 vaccine (2 doses)	1.81 ± 0.30^{b}	2.81 ± 0.17^{a}	2.91 ± 0.17^{ab}	22.2 (0.002)
3	Re-5 Merial	1.31 ± 0.18^{cd}	2.31 ± 0.97^{a}	2.41 ± 0.10^{b}	3.46 (0.10)
4	Zoetis H5N3	0.90 ± 0.52^{d}	1.51 ± 0.30^{b}	1.61 ± 0.17^{c}	3.34 (0.106)
5	Non vaccinated (control positive)	–	–	–	–
	F and (P-value)	10.31 (0.0002)	3.915 (0.017)	7.683 (0.001)	

Data present means ± SD of log HI antibody responses weekly post-challenge for groups vaccinated with experimental H5N8 vaccine and some commercial H5 AI vaccines. Values which carrying different small letters are statistically different according to Duncan's multiple range test ($P < 0.05$).

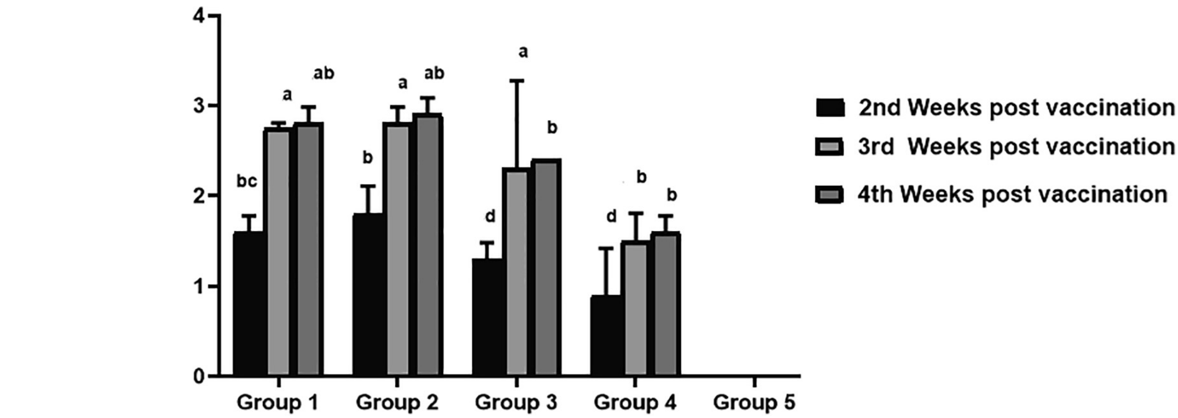

Fig. 1. Mean HI titer in groups vaccinated with experimental H5N8 vaccine and commercial H5 vaccines at 2nd, 3rd and 4th WPV. F/N. the figure showned increase in the antibody titers and significant difference in group 2 which was vaccinated with experimental H5N8 (2 doses) at the 2nd WPV and the mean HI titer was 6 log_2, 9.3 log_2 and 9.7log_2 at 2nd, 3rd and 4th wpv which was higher than the HI titer in groups 3and 4 which vaccinated with commercial vaccines.

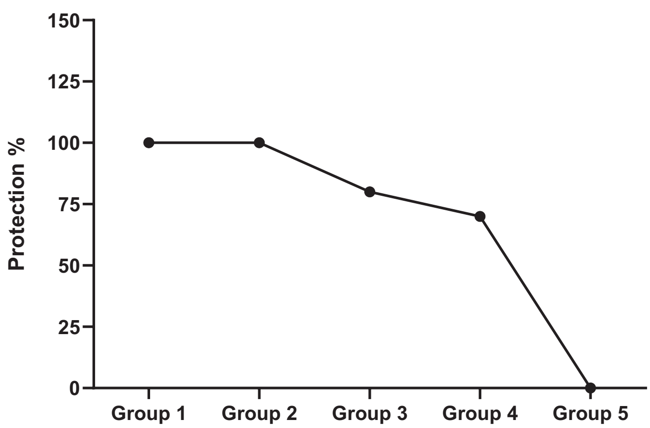

Fig. 2. Protection percent in the vaccinated and unvaccinated groups which were challenged by HPAI (H5N8), FN. The fig shown Groups 1 and 2 which vaccinated with experimental H5N8 vaccine remained live for 10 days. While, groups 3 and 4, the protection percent were 80% and 70% respectively.

Also, in group 7 which vaccinated with the experimental H9N2 vaccine (one dose) showed a high increase in the antibody titer against the homologous virus with a mean HI titer of 7.7 log2, 9 log2 and 9.7 log2 at 2nd , 3rd and 4th WPV, respectively, that was significantly higher than the commercial H9N2 vaccine especially in the 2nd and 3rd WPV. While, in group 8, the mean HI titer were 8 log2, 9.3 log2 and 10 log2 at 2nd, 3rd and 4th week post vaccination respectively which was significantly higher in 2nd WPV than group 9 as shown in Table 4 and Fig. 4.

The LPAI A/chicken/EGYPT/AB3/2018 (H9N2) virus showed 70% protection in the control group (Fig. 5), with mild clinical signs of AIV infection coughing and sneezing, depression, anorexia, respiratory distress, ruffled feather, nasal and ocular discharge. All chickens vaccinated with the experimental H9N2 vaccine still live for ten days after challenge without signs of infection. While, vaccinated group with the commercial H9N2 vaccine revealed protection percent 90% and mild signs of AIV infection compared with the control group as shown in Fig. 5.

The results of viral shedding in group 6 (control group) revealed that the viral shedding was detected at the 2nd day after the challenge with a mean virus titers 6.3565 X 10^5 EID50/mL. In contrast, at 4th day post-challenge, the viral shedding was detected with a mean virus titers 6.762 X 10^4 EID_{50}/mL. No virus shedding was detected from tracheal swabs in groups 7 and 8 which were vaccinated with inactivated H9N2 vaccine. In group 9, which was vaccinated with the commercial H9N2 vaccine, viral shedding was detected at 2nd with a virus titer of 7.924 X 10^2 EID_{50}/mL and 2.113 X 104 EID_{50}/mL at the 4th day post-challenge as shown in Fig. 6.

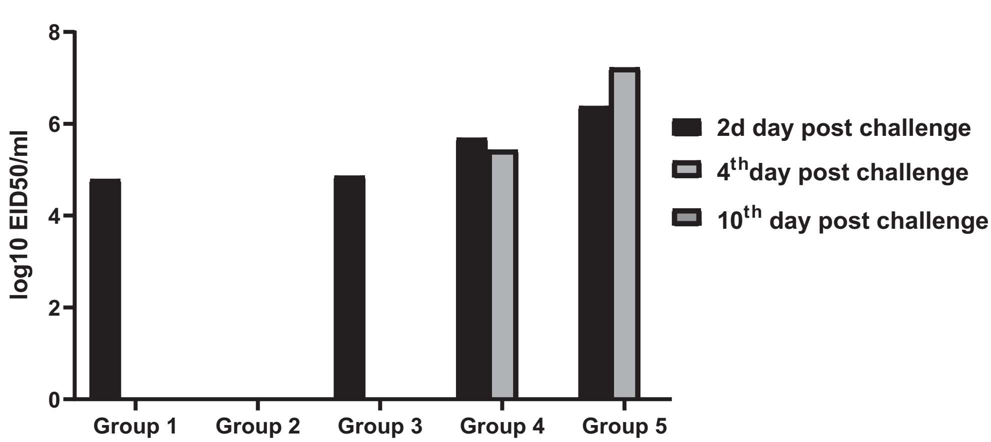

Fig. 3. Virus titter of HPAI (H5N8) in chicken vaccinated by experimental and commercial vaccine at 2, 4 and 10 days after vaccination by using RT-PCR. F/N. the figure shown there is no virus in group 2 and the virus titer is lower in chicken vaccinated by experimental vaccine in group 1 than commercial vaccine in group 3 and 4 at 2, 4.10 days.

Table 4
Profile of HI antibody responses weekly post-challenge in chicken groups vaccinated with experimental and commercial H9N2 AVIAN influenza vaccines.

Groups	Type of vaccine	Week 2	Week 3	Week 4	F and (P-value)
6	**Non vaccinated (control positive)**	–	–	–	–
7	**Experimental H9N2 vaccine (one dose)**	2.31 ± 0.17[ab]	2.41 ± 0.30[a]	2.61 ± 0.69[ab]	6.2 (0.035)
8	**Experimental H9N2 vaccine (2 doses)**	2.41 ± 0.100[a]	2.81 ± 0.17[a]	3.01 ± 0.10[a]	28.00 (0.001)
9	**Commercial H9 vaccine**	1.61 ± 0.35[c]	2.11 ± 0.10[ab]	2.51 ± 0.17[ab]	11.97 (0.008)
	F and (P-value)	10.31 (0.0002)	3.915 (0.017)	7.683 (0.001)	

Data present means ± SD of log HI antibody responses weekly post-challenge for groups vaccinated with experimental and commercial H9N2 AI vaccines. Values which carrying different small letters are statistically different according to Duncan's multiple range test ($P < 0.05$).

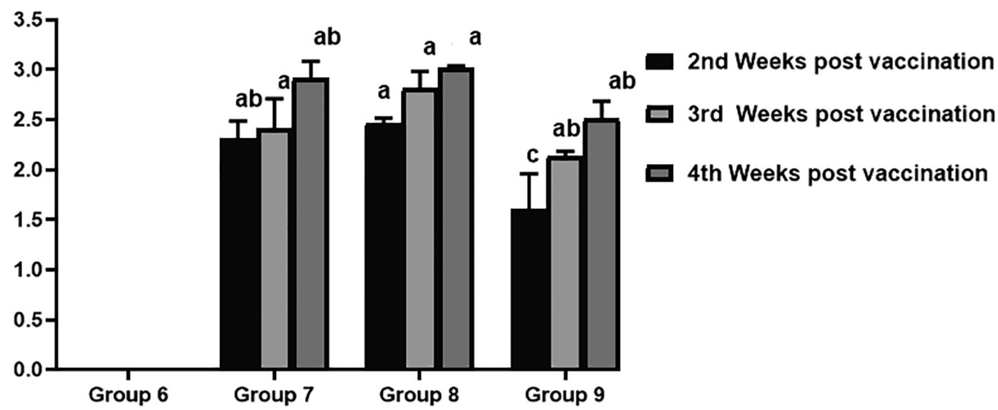

Fig. 4. Mean HI titer in groups vaccinated with experimental and commercial H9N2 vaccine in 2nd, 3rd and 4th WPV. F/N. the figure showed increase in the antibody titers and significant difference in group 8 which vaccinated with experimental H9N2 vacine (2 doses) and the mean HI titer of 8 log_2, 9.3 log_2 and 10 log_2 at 2nd, 3rd and 4th WPVwhich was higher than HI titer in groups 7 and 9.

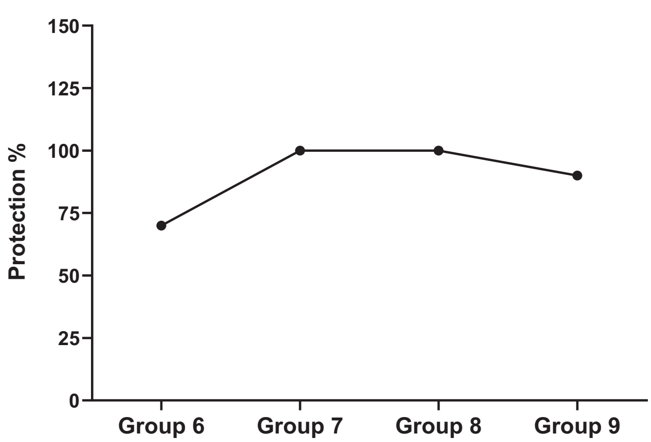

Fig. 5. Protection percent in vaccinated and unvaccinated groups which were challenged by LPAI (H9N2), FN. The fig shown group 6,7 vaccinated with experimental vaccine live for 10 days after challenge. While, group 8 vaccinated group with the commercial H9N2 vaccine revealed protection percent 90%.

4. Discussion

In Egypt, the strategy of HPAI vaccination was missed due to the genetic incompatibility of these vaccines with the revolving viruses despite the presence of more commercial AI vaccines in Egyptian poultry (El-Zoghby et al., 2012).

Various factors are influencing the effectiveness of poultry vaccines. The genetic and antigenic similarity between the field viruses and already used vaccine strains is one of these significant factors (Wong and Webby, 2013). The poultry vaccine should conserve at least 80% of vaccinated chickens from mortality. According to the (OIE) Manual for vaccine assessment, it must minimize the sheddinginimize the spread of the viruspost-infection to be efficient according to the (OIE) Manual for vaccine assessment. The H5N8 virus has recently been recorded in Egypt in wild and household birds. Vaccination in Egypt is the most prevalent technique for H5N1 control. The discovery of clade 2.3.4.4 H5N8 viruses in poultry in 2017 declared the need to reappraise the capability of the commercial H5 vaccine used in Egypt to guard the poultry from the recently appearing H5N8 virus (Kandeil et al., 2017).

This study was planned to evaluate the efficacy of the experimental and commercially applied AI vaccine regimens commonly used in Egypt. Seven groups were vaccinated with experimental and commercial H5 and H9 vaccines. The vaccine efficacy was assessed by the challenge of vaccinated layer chickens with recently field isolated H5N8 and H9N2 strains.

The antigenic similarity between the HA of the vaccine and challenge virus offering the best defence against the deaths and shedding of the virus so, in groups which vaccinated with the experimental H5N8 and H9N2 vaccines were no mortalities. While in The commercial H5N1 and H5N3 vaccines make protection rates 80% and 70%, respectively against H5N8 field strain that was similar to results obtained by (Kapczynski et al. 2017). Also, Kang et al. (2020) evaluated the protective efficacy of the clade 2.3.2.1c and 2.3.4.4c H5Nx vaccines against lethal homologous and heterologous viruses in layer and breeder chickens and founded that in the homologous challenge, all vaccinated groups exhibited 100% survival with no clinical symptoms. Kapczynski et al. 2017 stated that the virus shedding was significantly reduced following using the challenge strains as autogenous vaccines against the H5N8 challenge virus. In this study, the oropharyngeal swabs of chickens vaccinated with experimental H5N8 vaccine (2 doses) and experimentally infected with the isolated field strain chicken/Egypt/AB1/2018 (H5N8) virus showed no virus shedding. The chicken vaccinated with the experimental H9N2 vaccine (one dose and two doses) and inoculated with A/chicken/EGYPT/AB3/2018 (H9N2) showed the same results. For this reason, the booster dose

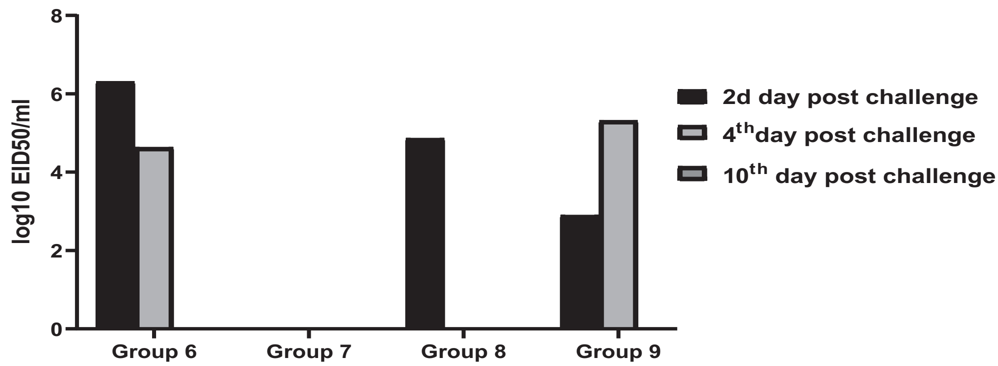

Fig. 6. Virus titter of LPAI (H9N2) in chicken vaccinated by experimental and commercial vaccine at 2, 4 and 10 days after vaccination by using RT-PCR. F/N. the figure shown there is no virus in group 7 and the virus titer is lower in chicken vaccinated by experimental vaccine in group 8 than commercial vaccine in group 9 at 2,4.10 days.

from oil-adjuvant H5 antigens give power full immunity against the homologous and heterologous HP H5 avian influenza viruses than the commercial vaccine as explained by (Jin et al., 2018), who showed that a single dose of an oil-adjuvant (1 μg) inactivated vaccination offered full protection for chickens against the infection with homologous H5N8 HP avian influenza virus (A/Waterfowl/S005/Korea/2014, clade 2.3.4.4). Still, it did not preserve them against infection by heterologous H5N6 HP avian influenza virus (A/Waterfowl/ Korea/S57/2016 (clade 2.3.4.4). Two doses of oil-adjuvant H5 antigens may give rise to strong immunity against the homologous and heterologous H5 avian influenza viruses.

The experimental vaccines were given high antibody titer higher than commercial vaccine which was reached 9.3 $\log_2$, 9.7$\log_2$ for experimental H5N8 vaccine which was significantly higher than and groups 3 and 4 especially at 2nd WPV, while at the 3rd WPV, the significant difference was with group 4 only. The HI titer was 9.3 $\log_2$ at 2nd WPV for experimental H9N2 vaccine that was significantly higher than group 9. that were similar to results of Kandeil et al. (2018) showed that chickens vaccinated with the experimental H5N8 vaccine give great result than the control group titers with a mean HI titer of 5.8 $\log_2$ at two weeks after vaccination. In chickens vaccinated with the experimental vaccine, the mean HI titer increased to 9.1 $\log_2$ at 3rd wpv and 9.5 $\log_2$ at 4th wpv. While the commercial vaccines not given any significant HI titers against the heterologous H5N8 virus until 3 up.

Also, the mean HI titers in groups 7 and 8 showed a high increase against the homologous antigen and was significantly higher than the commercial H9N2 vaccine. Our results agreed with Dharmayanti et al. (2020) who recorded the antibody titre in the vaccinated chickens with the inactivated monovalent H9N2 vaccine against the AI H9N2/2017, homolog BLi25Ut/18 and H5N1/2013 antigens and the titers were 8 log2, 8.3 log2 and 0 after three WPV. This is also coordinated with Lee et al. (2011), who stated that one dose of inactivated H9N2vaccine is very protective and immunogenic in SPF chickens.

In groups 7 and 8, no viral shedding was detected, while in group 9, viral shedding was detected at 2nd DPC with a mean titer of 7.924 × 102 EID50/mL and at the 4th DPC, the mean titer was 2.113 × 104 EID50/mL. Sultan et al. (2015) noticed a marked reduction of virus shedding in the group vaccinated with local vaccine than the other two groups.

5. Conclusions

In Egypt, various AI subtypes were recorded and make a threat to the poultry industry. More attention must be directed toward observing the circulating viruses to understand the development of the viruses. All the more likely selected viruses for immunization concentrates on limiting the widespread of the viral infection. The experimental H5N8 and H9N2 vaccines were immunogenic and provide high protection rate in SPF layer chickens against the isolated field strains HPAI (H5N8) and LPAI (H9N2). The present study also demonstrates that the booster dose of the experimental vaccines could elicit strong immunity. This strategy must simplify the vaccination programs for controlling multiple poultry viruses, especially in endemic countries.

Funding

The current work was funded by Taif University, Saudi Arabia, for financial support through its Researchers Supporting Project (TURSP-2020-105).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank the Department of Poultry and Rabbit Medicine, Faculty of Veterinary Medicine, Zagazig University, for their continuous support and technical assistance.

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