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EFFECTIVENESS OF THE PIGEON POX VACCINE ON THE CHICKEN VACCINATED WITH AVIAN INFLUENZA VACCINE

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ABSTRACT

Evaluation of the effectiveness of pigeon pox (PP) vaccine in improvement the required immunity to avian influenza (AI) virus vaccine in birds and reducing its shedding after challenge was the object of this study.Specific pathogen free chicks were vaccinated with PP and AI in 10 groups at 5 and/or 8 days of age and/or boosted after 35 days then challenged after 28 days with virulent highly pathogenic AI virus local Egyptian field isolate. The development of immune responses to AI haemagglutinine was recorded and also AI virus shedding after challenge.Vaccinated 10 groups induced protective immune responses; especially in the groups which were boosterly vaccinated with PP vaccine. All birds vaccinated and experimentally challenged 28 days later were protected against virulent AI (H5N1); mild clinical signs of infection developed in few number of vaccinated birds. In contrast, all unvaccinated birds died within 72 hours of challenge. Vaccination of chicks with PP and AI vaccines provided good effectiveness of the PP vaccine on the immune response of vaccinated birds with AI vaccine and showed decreasing in shedding after challenge; especially in the groups which take a booster vaccination of PP and AI vaccine. Although eradication still remain the 1st of choice for controlling the AI in the circumstances of a continuing and wide spread outbreak, but also the availability of new designing future vaccination regime by using avipox virus vaccine should be applied.

Keywords: Avian influenza, Chickens, prim-boost , pigeon pox vaccines, shedding.

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INTRODUCTION

The threat that highly pathogenic avian influenza (HPAI) virus, a virus subtype H5N1 threaten to poultry and public health has intensified (**Chuang–Ling *et al.*, 2003**). As the virus become established in poultry in developing countries, the number of human cases increases (**Qiao *et al.*, 2003** and **OIE 2019**). Vaccination is a useful tool to control avian influenza (AI) especially when biosecurity and stamping out strategies alone are not successfully implemented **(Qiao *et al.*, 2003** and **WHO 2008).**

In late 2016, the epidemiology of avian influenza (AI) in Egypt was exhibited a substantial change due to the emergence of HP H5N8 in wild birds. **(Selim*et al.*, 2017).** An immunization strategy depending on using bivalent and multivalent vaccines containing whole inactivated viruses has been advocated before to control several avian pathogens (**Lee *et al.*, 2013**). **Tripathy and Reed (2003)and Weli and Tryland(2011)**reported that prophylaxis can be achieved by vaccination, live fowlpox virus or pigeon pox virus for vaccination against avipox viruses. These vaccines are very effective and have undoubtedly contributed immensely to the prevention of the disease in commercial poultry farming.

Pigeon pox (PP) virus produces mild infection in chicken and turkey but it is more pathogenic for pigeons ***(*Bossinger *et al.*, 1982*).*** For this reasons, PP vaccine is used not only for vaccination of pigeons but also against pox infection in chickens and turkeys ***(*Gottstein *et al.*, 2004** and **Wang *et al.*, 2006*).***

Vaccines of fowl pox or pigeon pox virus origin have been routinely used for more than half a century to prevent fowl pox in commercial poultry in endemic areas **(Siddique et *al.*, 2011*),*** or has been diagnosed in previous flocks **(OIE 2019).**

Pigeon pox vaccine appears to provide better cross protection to some wet pox field strains. The combination of fowl pox and pigeon pox stimulates a broader spectrum immune response needed for optimum protection (**Hy-Line, 2019*).*** Poxviruses, encode multiple classes of immunomodulatory proteins that have evolved specifically to inhibit such diverse processes as apoptosis, the production of interferons, chemokines, and inflammatory cytokines, and the activity of cytotoxic T lymphocytes (CTLs), natural killer (NK) cells, complement, and antibodies. Often, the evolutionary origins of these virus-encoded immunomodulatory proteins are difficult to trace (**Johnston and Grant, 2003 and Meseko *et al*., 2012).**

Attenuated avipox viruses (APVs) have been employed as vaccines for chickensor as nominate vectors for the delivery of recombinant proteinsin both mammalian and avian species (**Taylor *et al.* 1988 and Paoletti, *1990***). Elicit T helper 1 (Th1) and CD_8 T-cell and resulted in the induction of antibodyand cell-mediated responses (**Radaelli, *et al.* 2003**), led to the induction of gamma interferon (IFN) in spleen shown promising results.For instance, **Webster*et al. (*2005*)*** reported that a prime-boost vaccination regimen, which included FPV could confer protection against challenge.

An in vivo study also it was conducted to further examine host responses to Avipox viruses. Cellular responses were characterizedby a slight, although statistically significant, increase in thepercentage of CD_4 cells and a decrease in the percentage ofCD_8 cells among spleen cells at 1 day post inoculation and also utilize IL-10 as a mechanism to evade the host immune system (**Diener *et al.*, 2008**).

Avipox viruses induced immunity in vaccinated host which, with the most immunogenic antigens, was protective when use as recorded in **Beard, *et al. (*1991*)*, Taylor, *et al. (*1991 and 1992*)*, Webster, *et al. (*1996*)* and Giotis and Skinner, (2019)** who used it as a recombinant vaccine vector.The current study aimed to evaluate the effect of pigeon pox virus vaccine efficacy to improve the avian influenza virus vaccine immune response in chicken and reduce its shedding after experimental infection.

MATERIALS AND METHODS

1. Experimental chicks

Six hundred (600) one day old, specific pathogen free (SPF) chicks were obtained from the SPF Egg Production Farm, Koum Oshein, El-Fayoum, Egypt. All birds were housed in separated negative pressure filtered air isolators and were provided with autoclaved commercial water and feed. The chicks were used for evaluation of the tested vaccination programs.

2.Vaccines

1. **Pigeon pox virus vaccine:** The Hungarian strain of pigeon pox virus in freeze dried form was used for vaccination of the chicks. Its EID_{50} was found to be 10^7/ml. Itwas obtained from the Veterinary Serum and Vaccine Research Institute (VSVRI**)**, Cairo.
2. **Avian influenza virus vaccine:** The vaccine viruses used in this study include the reassortant AI-H5N1 viruses, 2016 (H5N1) from the Veterinary Serum and Vaccine Research Institute, Cairo.

3.Viral strains

1. **Fowl pox virus strain:** The Beaudette strain of fowl pox virus was used for SNT. Its EID_{50} was found to be 10^9/ml . It was kindly obtained from the Veterinary Serum and Vaccine Research Institute, Cairo.
2. **Avian influenza virus**

It was used as antigens to detect antibodies against H5N1 viruses for vaccinated birds with 10^6 EID_{50}/100 µl of highly pathogenic avian influenza (HPAI).Virus.H5N1A/chicken/Egypt/D10552B/2015 (H5N1) viruses were used

4.Challenge virus

1. **Local Egyptian HPAI (2015) field isolate** Obtained from Newcastle Disease department, VSVRI; and used for challenge. Its titer was 10^{10} EID_{50}/ml. It was submitted by National Laboratory for Quality control of Poultry (NLQP), Dokky, Egypt. Challenge test was done according to **OIE** (**2017**).
2. **Fowl pox virus:** Having a titer of 10^6EID_{50}/ ml from the VSVRI, Cairo.

5.Serum Samples

Serum samples were collected weekly from vaccinated chicks on different intervals before and after vaccination on weekly intervals for 5 months, and stored at -20°C till application of Heamagglutination test (HI) and Serum Neutralization Test (SNT).

6.Swapping for avian influenza shedding detection

Twenty birds from each group were randomly removed from all sites, placed in BSL-3 certified isolators and infected with challenge virus. Challenge was carried out at 28 days post vaccination (DPV) using HPAI virus at a dose 10^6 EID_{50}/0.1 via the natural route Birds were then monitored daily for morbidity and mortality. Oral swabs were obtained from each bird at days 2, 5, and 7 post infections for virus titration in eggs. The challenge dose was according to the standard dose being used in Egypt to evaluate all HPAI-H5 submitted to the Central Laboratory for Evaluation of Veterinary Biologics, Egypt.

Oropharengial swabs were collected from all challenged birds in 1 ml of sterile phosphate buffer saline at 2, 5, and 7days post AI challenge to monitor virus shedding titers. Swab samples were vortexed and centrifuged at 2000 rpm for 10 min at 4°C. Supernatants were used for virus titration in 10 days old SPF embryonated chicken eggs, according to **Tumpey *et al.*, (2004)** and EID50/ml was calculated according to **Reed and Munech (1938).** Then each swab was examined using individually HA plate was carried out according to **(OIE 2018).**

7.Serological tests

Serum Neutralization Test (SNT):

All collected sera were screened against avipox according to the method described by **OIE (2012).** The neutralization index (NI) was calculated according to **Reed and Munech (1938).**

- **Heamagglutination test:** All collected sera were screened against avian influenza according to the method described by **Anon (1971)** and **OIE (2018).**
- **Heamagglutination Inhibition test (HI):** All collected sera were screened against avian influenza antibody titer according to the method described by **Allan *et al.*, (1978).**

8. **Experimental design**

Six Hundred chicks were divided into 10 groups (Each group contain 50 chicks, beside 100 chicks as control groups) as shown in table (1).

Table 1: Vaccination programs

Group	Age	Vaccines	Method of use
1	5 days	Pigeon pox	Pigeon Pox (PP) vaccine was administered to chickens by the wing-web (WW) stab method with single needle. Avian influenza (AI) vaccine was administrated by intra-muscular injection
2	5 days	Pigeon pox	
	8 days	Avian influenza	
	Challenged with HPAI after 28 days		
3	5 days	Pigeon pox	
	8 days	Avian influenza	
	Boostered after 35 days with PP and AI		
	Challenged with HPAI after 28 days		
4	5 days	Pigeon pox	
	8 days	PP and AI	
	Challenged with HPAI after 28 days		
5	5 days	Pigeon pox	
	8 days	PP and AI	
	Boostered after 35 days with PP and AI		
	Challenged with HPAI after 28 days		
6	8 days	Avian influenza	
	Challenged with HPAI after 28 days		
7	8 days	Avian influenza	
	Boostered after 35 days with AI		
	Challenged with HPAI after 28 days		
8	8 days	Avian influenza	
	Boostered after 35 days with PP and AI		
	Challenged with HPAI after 28 days		
9	8 days	PP and AI	
	Challenged with HPAI after 28 days		
10	8 days	PP and AI	
	Boostered after 35 days with PP and AI		
	Challenged with HPAI after 28 days		

HPAI: highly pathogenic Avian influenza.

Ten non-vaccinated control birds from the original groups were challenged with a fowl pox virus. The challenge virus was administered by the wingweb stab method in the opposite wing from that used for vaccination. Reactions were observed ten days post challenge.

RESULTS

The vaccine has the ability to give good takes and immunity in chicks vaccinated with pigeon pox vaccine applied by the wing web stab method. Potency tests of pigeon pox virus vaccine were tested for potency in 5 day old and 35 day old birds.

Table 2: Average Body Weight (gm) in different vaccinated bird groups:

	1 days	2 weeks	4 weeks	6 weeks	8 weeks	10 weeks	12 weeks	14 weeks	16 weeks	18 weeks	20 weeks	24 weeks
Control	**35.8 - 41.4**	**129**	**273**	**546**	**690**	**917**	**1048**	**1193**	**1379**	**1587**	**1787**	**1860**
G1		**131.6**	**305.2**	**533.1**	**706.8**	**925.9**	**1058.6**	**1189.0**	**1386.5**	**1616.9**	**1798.0**	**1880.5**
G2		**131.3**	**311.0**	**527.2**	**711.2**	**922.9**	**1054.5**	**1180.9**	**1389.3**	**1622.3**	**1807.5**	**1877.3**
G3		**134.8**	**308.8**	**551.8**	**769.9**	**951.3**	**1080.6**	**1274.1**	**1411.0**	**1689.3**	**1866.8**	**1917.6**
G4		**135.4**	**319.2**	**577.3****	**746.1**	**936.0**	**1068.9**	**1199.6**	**1400.5**	**1655.9**	**1858.2**	**1902.1**
G5		**136.9****	**321.7****	**570.1**	**788.8****	**989.5****	**1123.5****	**1290.9****	**1457.9****	**1693.4****	**1903.1****	**1934.5****
G6		**122.6**	**288.0**	**517.7***	**698.1***	**906.6***	**1059.6**	**1192.6**	**1378.2***	**1611.1**	**1780.6**	**1853.9***
G7		**127.1**	**278.9***	**521.3**	**700.8**	**912.0**	**1054.8***	**1188.0***	**1385.4**	**1610.9***	**1779.0***	**1868.3**
G8		**119.6***	**288.0**	**547.7**	**749.2**	**942.4**	**1077.7**	**1245.8**	**1437.2**	**1684.4**	**1896.7**	**1911.2**
G9		**138.0**	**320.1**	**551.0**	**739.8**	**930.9**	**1061.6**	**1239.5**	**1386.8**	**1627.3**	**1860.0**	**1918.7**
G10		**33.7**	**318.9**	**557.7**	**771.5**	**972.9**	**1098.7**	**1205.8**	**1453.3**	**1666.4**	**1896.4**	**1925.6**

* : Lowest body weight within the same week.

**: Highest body weight within the same week.

Table 3: Immune response for PPV:Neutralizing index of different vaccinated bird groups: (SNT)

Age	5 days	8 days	15 Days	21 days	28 days	35 days	42 days	48 days	55 days	8 weeks	10 weeks	12 weeks	14 weeks	16 weeks	18 weeks	20 weeks	24weeks
G1	0.5	0.5	1.25	2.5	3.0	3.0	3.0	3.0	3.0	3.0	2.5	2.75	2.5	2.5	2.5	2.0	2.0
G2	0.5	0.5	1.0	2.5	3.0	2.75	2.75	3.0	3.0	3.0	3.0	2.5	2.5	2.5	2.5	2.25	2.25
G3	0.25	0.5	0.75	2.25	2.75	3.25	3.0	3.75	3.5	3.5	3.5	3.5	3.5	3.25	3.5	3.25	3.00
G4	0.5	0.5	1.75	2.75	3.0	3.0	3.75	3.75	3.75	3.75	3.75	3.75	3.25	3.75	3. 5	3. 5	3.25
G5	0.5	0.5	1.5	2.5	3.25	3.25	3.5	4.0	3.75	4.0	4.0	4.0	4.0	4.0	4.0	3.75	3.75
G6	0.25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G7	0.25	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-
G8	0.5	-	-	-	-	-	-	0.5	0.75	1.0	2.5	3.0	3.0	2.75	3.0	3.0	3.0
G9	0.25	0.25	1.5	2.75	3.0	3.0	3. 5	3.75	3.75	3.75	3.75	3.75	3.25	3.55	3. 5	3. 5	3.25
G10	0.5	0.25	0.75	2.25	2.75	3.0	3.5	3.5	3.75	3.75	3.75	3.75	3.75	3.75	3. 5	3.25	3.25

G6 & G7: Not none, they were not vaccinated against pox.

Neutralizing Index (NI) ≥1.5 is considered protective against pox viruses Cotral (1978).

Table 4: Mean HI antibody titers of avian influenza vaccine (HI) titer log_2 in different vaccinated bird groups:

	15 days	21 Days	28 days	35 days	42 days	48 days	55 Days	8 weeks	10 weeks	12 weeks	14 weeks	16 weeks	18 weeks	20 weeks	24 weeks
G1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G2	8.5	9.8	10.8	10.3	9.8	9.5	9.5	9.5	9.5	9.0	9.0	9.0	9.0	9.0	9.0
G3	8.1	10.0	10.5	10.1	11.0	10.6	10.5	10.3	10.1	10.0	10.0	9.9	9.8	9.6	9.3
G4	8.9	10.0	11.0	11.0	11.0	11.0	11.0	11.0	10.0	10.0	10.0	9.9	9.8	9.6	9.0
G5	9.9	10.8	10.6	11.0	1.01	11.0	11.0	10.4	10.2	10.2	9.8	9.6	9.7	9.7	9.6
G6	8.3	9.8	10.6	10.0	10.0	9.8	9.5	9.2	9.3	9.0	9.0	9.0	9.0	9.0	9.0
G7	8.5	9.5	10.2	10.0	11.0	11.0	10.5	10.0	9.7	9.7	9.5	9.0	9.0	9.0	9.0
G8	8.4	10.0	10.5	10.0	11.0	11.0	10.8	10.5	10.3	9.7	9.3	9.3	9.0	9.0	9.0
G9	9.8	11.0	11.0	11.0	10.6	10.1	10	10	9.9	9.9	9.5	9,5	9.5	9.5	9.3
G10	9.6	11.0	11.0	11.0	10.3	10.8	10.8	10.6	10.0	10.0	10.0	10.0	10.0	9.5	9.5

G1: Not none, it was not vaccinated against influenza.
HI titer considered protective if it is 2^4 or more according to Allan *et al.* (1978).

Avian Influenza shedding detection

Table 5: Challenge virus shedding titers in vaccinated and non-vaccinated challenged control groups.

Group	Days post challenge		
	2 dpc	5dpc	7dpc
Control	3.6(4/5)	Not detected	Not detected
2	0.6	0.2	0
3	0.6	0	0
4	0.4	0	0
5	0.2	0	0
6	0.8	0.2	0
7	0.8	0.2	0
8	0.6	0	0
9	0.4	0	0
10	0.2	0	0

-No reisolation virus was recovered from the oral swabs collected from chickens vaccinated with the experimental vaccine at 2,5and 7 post infection.

- All the titration of virus shedding by HA was less than 1.

Protection percent:

Table 6: Result of Challenge test and protection percent

Group	Animal protection	Protection %
Control	0/20	0 %
2	17/20	85%
3	18/20	90%
4	19/20	95%
5	19/20	95%
6	17/20	85%
7	18/20	90%
8	18/20	90%
9	18/20	90%
10	19/20	95%

Protection percent is 80% according to Egyption standard protocol (2017)

DISCUSSION

Countries such as Egypt in which H5N1 HPAI infection became endemic have not eradicated the infection by vaccination. This failure has many explanations, including the suboptimal use of vaccines that seems to have led to the emergence of new antigenic variants against which the classical vaccines are not fully protective. Maternal dependant

antibodies(MDA) interference on vaccine efficacy likely contributes also to the lack of control by vaccination. The high MDA interference on inactivated vaccines and suggest that the use of a prime-boost strategy using avipox vector to prime may be able to overcome at least partially MDA interference(**Richard-Mazet *et al.*, 2014).** In young chicks, their efficacy is optimal only at 2–3 weeks of age, and they cannot induce optimal protection in one-day-old birds ***(*Michel *et al.*,2006).**

Due to the bad effect of early investigation of fowlpox vaccine like retarded growth (**Goldhaft, 1956),** high mortality rate **(Brandly and Dunlop 1939)** and increased susceptibility to pullorum infection and infestation with coccidia**,** and according to these findings, vaccination of chicks of less than 30 days old was not recommended **(Graham and Brandly 1940).** Also **Dalling (1933)** recommended the use of pigeon pox virus vaccine instead of fowl pox virus to avoid the complications arising after using the fowl pox virus vaccine; and what founded by **Taslima *et al.*, (2008)** which founded that maternally derived antibody from fowl pox vaccine until day 4 of age PPV vaccine is recommended for the prevention of fowl pox in chicks aged at day 1 to day 5. And also that recorded by **Michael *et al.*, (1986)** that up till now fowlpox virus vaccine cannot be used for vaccination of baby chicks but only for chicken of not less than 2 months old.

So this study is a trial to use pigeon pox vaccine in young chicks according to that applied with **BYC (2012)** that said that the chicks may be vaccinated as young as one day of age by using the wing web method and using a one needle applicator. **Tripathy and Reed (2008)** recorded that live attenuated vaccines, its strains come from fowlpox of chicken or pigeons has been used to prevent fowlpox disease in susceptible birds.

Paramunity inducers (PIND) from attenuated pox virus directly activate and regulate the paraspecific (innate) immune system. Pox viruses are good paramunity inducers. In passaging they very early loose the specific immunizing epitopes whereas the phylogentic older epitopes are conserved and hence the paraspecific cytokine regulating function is retained **(Brames and Mayr 2006**).

Table (2), showed good body weight in different vaccinated groups; which show high body weight in the groups vaccinated with pigeon pox vaccine especially in the groups which were taken a booster dose of PP as group 5 even after vaccination in the contrast the groups which were vaccinated with Avian influenza alone as group 4or the control, that agree with **Okwor *et al.*, (2012),** who founded that the performance of the bird as measured by average weight gain was 775 $\pm$ 2.89gm in vaccinated group with avipox vaccine which were significantly higher ($P<0.05$) than unvaccinated group which it's average weight gain was 570 $\pm$11.54 gm.

The representing results in table (3) showed that the SNT against FPV was protective from 15 to 21 days of age in all PPV vaccinated groups and still to the end of experiment and it agreed with **Michael *et al.*, (1986)** who recorded that the pigeon pox vaccinated chicks at one and three week old given 80-90% protection when challenged 4 week after vaccination. **Hala, *et al.*, (2008)** recorded that the PPVV act as immunostimulant to counter act the immunesuppression effect of the intermediate strains of IBDV vaccines. This agreed with the results in table (4) which show increasing of the immune response against AIV not less than HI10 log2 titer in all groups that vaccinated with PPV previously 3rd week post vaccination especially in groups 3,5,9 and 10 which were boosted after 35 days.

This high antibody titer was continuously high till the end of this study at 24 week post vaccination in all groups that was not less than 9 log2 titer at the end of the study.While in groups 3,5,9 and 10 that prime-boost with the two vaccines together the HI antibody titer were high till 8 weeks post vaccination not less than 10 log titer in groups 9 and 10 and this agreed with **Steensels *et al.*, (2008)** who reported that the prime-boost vaccination scheme was shown to be immunogenic in 1 day old.

Previous studies showed a correlation between serologic response and protection against mortality and viral shedding (**Kumar *et al.*, 2007).** Also, the OIE terrestrial manual suggests that the protection from mortality and protection from virus shedding depend on serological potency **(OIE, 2012). Kumar *et. al.*, (2007)** showed that low antibody titers of 10 to 40 prevent mortality with no viral shedding; while titers greater than 40 prevent mortality and reduce shedding .Previous evidence showed that serological titers are associated with protection when the challenge and vaccine viruses are genetically and antigenically closely related. The presence of HI antibodies predicted protection in the field as well **(Swayne *et al.*, 2015).**

The results were also confirmed by **Kemal Karaca *et al.*, (2005)** who reported that poxvirus vectored influenza vaccines should be considered as an alternative in the development of specific influenza vaccines. The antibody responses were detected as early as 1 week after the first vaccination, mounted a booster response to the second vaccination that cross-

reacted with a recent highly pathogenic Asian H5N1 isolate.

Full protection against clinical signs and shedding was induced by the different vaccination schemes. However, the broadest antibody response and the lowest antibody increase after challenge were observed in thegroup whose immune system was primed with the fowlpox vectoredvaccine and boosted with the inactivated vaccine, suggesting that this prime-booststrategy induced optimal immunity against H5N1 and minimal viral replication afterchallenge. In addition, this prime-boost vaccination scheme was shown to be immunogenic in 1dayold **(Steensels *et al.*, 2008*).***

The use of a prime-boost vaccination on a routine basis provides a sufficient level of protection to minimize highly pathogenic avian influenza (HPAI) virus shedding and decrease the viral load, leading to a successful and effective HPAI virus control strategy.Prime–boost vaccination with a fowlpox vector and an inactivated avian influenza vaccine is highly immunogenic when challenged with Asian H5N1 HPAI (**Boehringer, 2017**).

Following challenge with HPAIV, all chickens vaccinated with AI inactivated vaccine with PPV were protected, while the chickens vaccinated with either the unaltered parent pox vaccine virus or unvaccinated controls experienced 100% mortality following challenge. This protection was accompanied by the high levels of specific antibody to the respective components showed that pigeon pox and avian influenza could be a potential vaccine to replace current inactivated vaccines for preventing AI and this agreed with **Qiao et al., (2003).**

The results in table (5) agreed with **Ali *et al.***, (**2019**) Challenged SPF chickens showed significant decreases in the virus shedding titers up to <3log10 compared to challenge control chickens. detected No virus shedding was 6 “days post-challenge” in all vaccinated challenged groups, and with **Niqueux *et al.*, (2013),** who reported that the reduction of oropharyngeal shedding levels was also constantly observed from the onset of the followup at 2.5 or three days post-infection in vaccinated ducks compared to unvaccinated controls, and was significantly more important for vFP89+vNDV-H5 vaccination**.** Also confirmed with **Ellis *et al.*, (2006)** who said that vaccination with commercially available inactivated vaccines based on avian influenza virus subtype H5 can confer clinical protection and reduce virus shedding after infection**.**

Table (6) showed that Pigeon pox virus and AIV was evaluated for its ability to protect chickens against intramuscular challenge with a lethal dose of highly pathogenic (HP) AIV; Following challenge 28 days later with HPAIV, all chickens vaccinated were protected, especially in groups 4, 5 and 10were with protection percent 95%, while unvaccinated controls experienced 100% mortality following challenge. This protection was accompanied by the high levels of specific antibody to the respective components showed that pigeon pox and avian influenza could be a potential vaccine to replace current inactivated vaccines for preventing AI. This is in agreement with **Qiao *et al.*, (2003*).***

CONCLUSION

In conclusion, full protection against clinical signs and shedding of avian influenza virus was induced by the different vaccination schemes; However, the present work is demonstrating the high efficacy of the successive administration pigeon pox vaccine , the broadest antibody response and the lowest antibody increase after challenge were observed in the groups 5 and 10 whose immune system was primed with the PPV vaccine and boosted with the PPV and inactivated AI vaccine also the group that primed with both vaccines and boosted with them together gave high immune response that continued to the end of this study; while the group 10 is the preferable one because in which we applied both PP and AI at the same time in the primary and booster vaccinations, suggesting that this prime-boost strategy induced optimal immunity against H5N1 and minimal viral replication after challenge.

Declaration of Competing interest

On behalf of all authors, I hereby declare that no conflict of interest may interfere with the publication of the manuscript.

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