Efficacy of fowl pox vaccines against Egyptian isolated strain during 2012

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Abstract

Aim: This study was designed to evaluate the protection induced by different commercial fowl pox (FP) vaccines against local FP virus isolated from Giza governorate in Egypt during 2012.

Materials and Methods: Efficacy of FP vaccines against local field isolates was determined in seven groups of (20 birds/group) 6 weeks old specific-pathogen free chicks. Each group was vaccinated via wing web with one of different FP living vaccines and (20) chicks in group (8) did not receive any FP vaccine and served as controls. At the 3rd, 5th, 7th and 10th-day post-vaccination (DPV), all birds were examined for the presence of takes at the site of vaccination. Three weeks post vaccination, serum samples were collected, and the levels of induced antibodies were detected by passive hemagglutination (PH) test. All birds in Groups (1-8A) were challenged individually via the wing web with EID_{50} $10^{3.0}$ of the Egyptian isolated strain during 2012.

Results: Takes detected at the site of vaccination at the 3rd day were ranging from 45 to 70%. This percent increased to 80-95% at the 5th DPV with a maximum elevation of takes at the 7th DPV (90-100%). Geometric mean titer of PH assay antibody titer, 3 weeks post vaccination, was ranging between 5.60 and 9.60 according to the type of vaccine used and with protection 90-100%.

Conclusion: Efficacy of some commercial FP vaccines used in the poultry field against the Egyptian isolated strain during 2012 ranged between 90% and 100% according to the type of used vaccine.

Keywords: chickens, fowl pox vaccines, local Egyptian isolated strain.

Introduction

Avipoxviruses (APVs) are large-complex DNA viruses that belong to the subfamily chordopoxvirinae of the family pox viridae [1]. APV infection is a highly contagious disease of birds and has been reported in more than 200 bird species affecting both domesticated and free-ranging birds around the world [2]. They have been shown to naturally infect more than 278 of the approximately 9000 species of wild and domestic birds [3]. Despite the large number of host species according to the International Committee on Taxonomy of Viruses, there are currently only 10 defined APV species, with species names originally assigned according to the bird species that they infect or from which they were isolated [1]. APVs are often not host-specific and differ with respect to their virulence [4]. Pox viruses are classified into two categories entomopox (insect infecting) and chordopox (vertebrate infecting). The chordopox viruses are further divided into eight categories, including orthopox, which includes variola major and vaccinia virus and avipox, which include fowlpox virus (FPV) [5]. The shape of pox viruses resembles a brick [6]. FP is a disease of poultry that can affect egg production. The disease has two recognized forms; the cutaneous form, which is characterized by nodular lesions of skin, particularly the eyelids, combs and thighs with low mortality, and the diphtheritic form, which is characterized by respiratory signs such as sneezing, gasping, head-shaking and relatively high mortality [7]. The general signs of the diseases include weight loss, loss of feathers and scaly skin on the leg, neck and back [8]. Infected birds display various clinical signs of pox virus infection, depending on the route of transmission, viral virulence and host susceptibility to the infecting strain [9]. Thus, all avian species are susceptible to avian pox because natural pox infections have been reported in several species of wild birds of different families, as well as in domestic birds [10].

Vaccination remains a cornerstone for the control and prevention of infectious diseases in both humans and animals [11]. For a long period of time FP did not play an affecting role in commercial poultry farms in Egypt. Beginning in 2011 an increasing number of new infections were identified from different domesticated birds reared under backyard management systems [12]. Also canary pox-like virus was isolated and identified from a canary suffering from pox-like lesion in 2012 [13]. A layer poultry flock of 30 day age in Giza governorate in Egypt at the end of 2012 had nodular lesions on the comb; these lesions were collected and identified using polymerase chain reaction (PCR) as FPV [14].

The objective of the present study was to evaluate the protection afforded by some living FP commercial

vaccines against such recent Egyptian FPV isolates.

Materials and Methods

Ethical approval

Institutional Animal Ethics Committee has accorded permission for conducting this trial.

Living FP vaccine

Seven FP commercial live attenuated vaccines were used; one tissue culture (TC) attenuated FPV; DIFTOSEC; Batch No. (L393996) and another six egg attenuated FP vaccines: Hiprapox; Batch No. (41YB-9); Izovac fow-Pox; Bach No (OOSSH); Avipox; Batch No (0101A3DKA) Vaiol-Vac; Batch No (303092); Poxine; Batch No. (1200760) and FP vaccine Batch No (2/2013).

Challenge FPV strain

Field isolated FPV (FPV isolated Ch-08 TK gene submitted in Gen Bank at Accession No: (KF 314718) was kindly supplied by [14] Central Lab for Evaluation of Veterinary Biologics (CLEVB); it was isolated and identified by using reverse transcription-PCR from poultry flock (chicken) at 2012. Virus titration was done using specific pathogen free-embryonated chicken eggs (SPF-ECE) as described by Villages [15] showing a titer of $10^{4.5}$ embryo infected dose (EID_{50}/mL) as calculated according to the method described earlier [16].

Experimental Hosts

SPF-ECE

These eggs were obtained from the SPF production farm, Koum Osheim, El-Fayoum, Egypt. The eggs were kept in egg incubator at 37°C with humidity of 40-60%. SPF eggs used for titration of egg adapted FP vaccines according to CFR [17] and the EID was estimated according to the method described earlier [16].

TC and cell culture media

Primary chicken embryo fibroblast cells were prepared as described by Schat and Purchase [18] and obtained from (CLEVB). Trypsin-version solution was prepared according to hanks balanced salt solution. Minimum essential medium was prepared according to the manufacturer's instructions and bovine serum was mycoplasma free and virus screened "Gibco Limited, Scotland and UK." The method used for inoculation in the microtitre plates was done as described earlier [15]. TC used for titration of TC FP strains vaccine as described earlier [17]. TC infective dose (ID_{50}) was calculated according to the procedure described earlier [16].

Experimental chicks

One day old SPF chicks were obtained from SPF production farm, Koum Osheim, El-Fayoum, Egypt. This farm is part from Ministry of Agriculture. The birds were housed in separate negative pressure filtered isolators and provided with autoclaved commercial water and feed. Chicks were used for evaluation of the efficacy of some commercial FP vaccines against local field isolated strain, which was isolated from Giza governorate of Egypt during 2012.

PCR

PCR was used for detection of the identity of commercial vaccines under test according to the protocol described earlier OIE [19] using DNA extraction kit "Genomic DNA purification kit."

Passive hemagglutination (PH)

PH test was carried out according to the procedure described earlier Shahzad et al. [20] where soluble FP antigen was prepared from the chorioallantoic membrane harvested from FPV inoculated embryonating SPF eggs and concentrated 100-field by ultracentrifugation. The concentrated FPV strains were treated and used as hemagglutination assay (HA) antigen as described earlier [21].

Experimental design

160 six weeks old SPF chicks were used in this study. Birds from Groups (1-7 of 20 birds/group) were vaccinated via the wing web at the right wing with the recommended dose of different examined commercial vaccines as shown in Table-1. While birds in Groups 8A and B (10 birds/subgroup) were kept as control. The chicken immune response was determined in vitro by two parameters; one of them by chicken examination in Groups (1-7) at 3; 5; 7 and 10 days after vaccination for the presence of takes at the site of vaccination. The other parameter was determined by measuring HI antibody titer induced by the used commercial vaccines under test at 3 weeks post vaccination. All birds in Group (1-8A) at 3rd week post vaccination were challenged with 100 ul of FP challenge virus at dose of 3.0 log 10 EID_{50} (egg EID)/0.1 mL administered through the wing web at the left wing (opposite wing for the vaccination site). Following challenge, all birds were observed daily for clinical signs attributable to FP infection and determination of in vivo immune response to the used commercial vaccines.

Results

Determination of chicken immune response in vitro

At the 3rd-day post vaccination (DPV); right wings of all birds from Groups (1-7) were examined for the presence of takes; (9 birds from 20 in G5); (13 birds from 20 in each G2 and 4) and (14 in G6); respectively has inflammation as shown in Table-1 while the remaining birds appeared normally. Number of birds that had inflammation with developed small circular lesions with mild swelling on the right wing increased to (16; 17; 18 and 19) in Groups (G5; G4 and 7; G1 and 3 and G2 and 6) respectively at the 5th DPV. At the 7th DPV all birds in Groups (1, 2, 3 and 6) had taken (swelling of the skin or a scab) at right wings where the vaccine was applied. Birds in Groups 4; 5 and 7 had takes with 95% and

Table-1: *In vitro* monitoring of chicken immune response to FP vaccines.

Chicken group	Type of used vaccine	Vaccine titer ID_{50}	Numbers and percentage of takes/DPV (%)			
			3 DVP	5 DVP	7 DVP	10 DVP
G1	Diftosec	4.2	(9/20) 45	(18/20) 90	(20/20) 100	(20/20) 100
G2	Hiprapox	4.0	(13/20) 65	(19/20) 95	(20/20) 100	(18/20) 90
G3	Izovac	4.0	(10/20) 50	(18/20) 90	(20/20) 100	(18/20) 90
G4	Avipox	3.8	(13/20) 65	(17/20) 85	(19/20) 95	(19/20) 95
G5	Vaiol-Vac	3.8	(12/0) 60	(16/20) 80	(19/20) 95	(19/20) 95
G6	Poxine	4.2	(14/20) 70	(19/20) 95	(20/20) 100	(19/20) 95
G7	Fowlpox	3.8	(10/20) 50	(17/20) 85	(18/20) 90	(18/20) 90

*Titer of vaccine calculated as EID_{50} in case of egg adapted vaccine and $TCID_{50}$ for tissue culture vaccine, *DPV: Day Post Vaccination, *NB: The vaccine should contain at least of $10^{3.0}$ EID_{50}/dose at the time of use. EID_{50}: Embryo infected dose fifty, ID_{50}: infective dose, FP: Fowl pox

90%, respectively. These takes became scabby and started to disappear at the 10th DPV ranging from 5% to 10%.

The results showed that the PH antibody titres in all vaccinated groups with different commercial vaccines under test as in Table-2 as GMT were ranging between 5.6 and 9.60 log2 with a significant difference (p<0.05) higher than the control chickens in Group 8B.

PCR of genomic DNA extracted

PCR amplification: M a 100 bp DNA ladder. A&B 305 bp length amplicon was amplified for (KF 314718) challenge virus in the right. In left 578 bp length amplicon was amplified for seven FP polymerase gene vaccines under test.

Discussion

In this study, the efficacy of some living TC and egg adapted FP vaccines used in Egyptian farms was evaluated after the challenge with the recent field isolated virus strain (FPV isolate ch.08Tk) isolated from laying flock in Egypt in 2012. Three parameters were used for the evaluation of the protection including measuring the presence of takes from 3 to 10 DPV. Antibody levels against FPV were monitored by PH inhabitation test at 3 week's post vaccination. The last parameter was detecting protection percent against Egyptian isolated strain during 2012. All vaccines used in this study were identified by PCR as described OIE [19] as in Figure-1. The arrows of the seven commercial pox vaccines used in our study refer to the estimated size of the band at 578 bp product. Our results agree with Ha *et al* and Offerman *et al*. [9,22] which reported PCR amplification with primers specific to the 4b core protein gene of the avian FP vaccines developed.

In vitro monitoring of chicken immune response

According to Tripathy and Reed [8], 6 weeks old chicken vaccinated with different types of commercial vaccines were used in the Egyptian field. All birds were examined from 3 to 10 DPV for presence of FP vaccine reaction at site of infection, according to [8,17-19]. Skin thickness at the inoculation sites was measured, and the development of additional skin lesion was monitored according to Hye Jeong *et al*. [2].

Table-2: Monitoring of FP antibody and percentage of protection induced in chickens by different fowl pox commercial vaccines.

Chicken groups	Used vaccine	GMT-PH antibody titer (log2)	Protection (%)
G1	Diftosec	9.60	100
G2	Hiprapox	8.12	100
G3	Izovac	7.60	95
G4	Avipox	5.60	90
G5	Vaiol-Vac	6.60	90
G6	Poxine	9.50	100
G7	Fowl Pox	5.90	90
G8A	Control +ve non vaccinated and challenged	2.9	
G8B	Control −ve non-vaccinated non-challenged	2.9	0

*GMT: Geometric mean titer, *PH: Passive Hemagglutination. *Significant difference at p≤0.05, *Protection% not<90%. FP: Fowl pox

Birds in all groups at the 3rd DPV had inflammation at the site of inoculation ranging from 45% to 70%. The inflammation increased to 80-95% on the 5th day. Takes appeared in all vaccinated groups in the ratio between 90% and 100% at the 7th and the 10th DPV. Our results agreed with Hye Jeong *et al*. [2], who reported that FP vaccination developed small circular lesions (<3 mm in diameter) on the wing on the 3rd DPV and became scabby starting from approximately 10 DPV. The difference between skin thickness in control and experimentally vaccinated groups was significantly different (p<0.001), while the difference in skin thickness between vaccinated groups was not significant (p>0.1). Our results also agree with Tripalhy and Reed [23] who reported that the flock should be examined till 10 days after vaccination for evidence of takes. A take consists of swelling of skin and it is evidence of successful vaccination.

Antibody levels against FPV

Antibody levels against FPV were monitored by PH. Our results in Table-2 show mean log2 HA titers for different FP vaccines under test (9.60; 8.12; 7.60; 5.60; 6.60; 9.50 and 5.90) agreed with Tripalhy *et al*. [21] who reported that a PH test will detect antibodies in
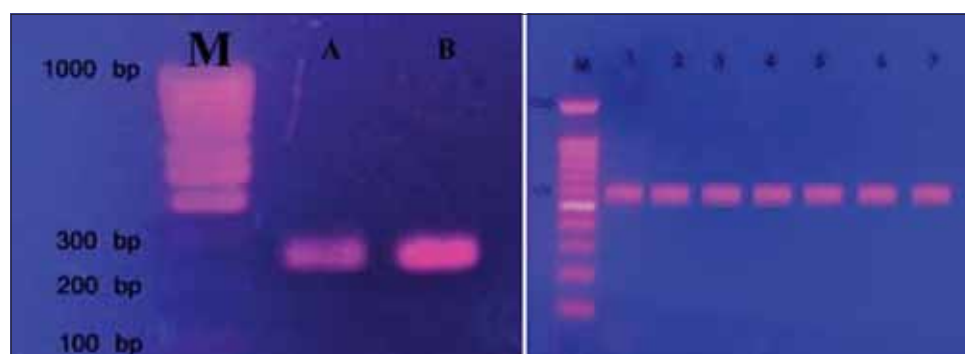
Figure-1: The polymerase chain reaction amplification of the spike gene of fowlpox polymerase gene vaccines under test.

the serum of FPV infected chickens earlier than the immundiffusion test.,However, this test is very sensitive and requires tanned horse or sheep red blood cells for sensitization to soluble poxvirus antigens. Furthermore, we agree with Tripathy and Reed [8] who showed that PH antibodies are detectable in some sera of infected birds as early as 1 week after inoculation and many persist for 15 weeks; that are longer than precipitating antibodies cross-reactions which occur among avian pox viruses [24]. It was shown that the PH antibody titers in vaccinated groups were comparable and significantly higher than the control chickens. It was further revealed that 14 days after vaccination HA GMT of ≥2 log2 was recorded in chickens vaccinated by oral and wing web stab routes whereas 35 days after vaccination, the HA antibody titers reached 5.6 log2 and 6.3 log2, respectively.

Detection of the immune response *in vivo*

At the 3rd-week post vaccination; birds in Groups (1-8A) challenged via wing web at left wing (Table-2) shows that the protection percentage ranged from 90 to 100% in all groups against the Egyptian isolated strain.

Our results in Tables 1 and 2 indicate that birds vaccinated with diftosec; hiprapox and poxine had 100% takes at the 7th DPV, and GMT HA antibody titer (9.60; 8.12 and 9.50) with 100% protection against recent field Egyptian isolated strain (KF 314718). All birds in G3; which were vaccinated with IZO VAC; showed takes; 95% protection with antibody level 7.60 log2 PHA antibody titer. Birds vaccinated with avipox; vaiol-vac or FP 2/2013 were also protected against field isolate (90%) with takes ranging between 95 and 90% and antibody levels (5.60; 6.60 and 5.90); respectively. Our results agree with OIE [19] who reported that 90% of challenged non-vaccinated control birds should show characteristic FP lesions and at least 90% of vaccinated birds should remain normal without evidence of any FP lesions.

Furthermore, Wambura and Godfrey [24] showed 100% protection against challenge virus at 35 days after vaccination.

Conclusion

Based on the data presented in this study, we conclude that, under experimental conditions the commercial FP vaccines used in the Egyptian field protect chickens (90-100%) against infection and disease following challenge with local FP which was recently isolated in Egypt in 2012. This confirms that under field conditions we can use vaccination programs to reduce the economic losses caused by FP infection virus in Egypt.

Authors' Contributions

C.M. was responsible for serological tests. S.S.E planed, drafted and supervised the research, data collection and analysis as well as results evaluation. Both authors read and approved the final manuscript.The manuscript was revised by the research committee, Veterinary Serum and Vaccine Research Institute.

Acknowledgments

Competing Interests

The authors declare that they have no competing interests.

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