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Original Article

Evaluation of Efficacy of Razi Fowl Pox Vaccine in Comparison with the Commercial Fowl Pox Vaccine in SPF Chickens by Challenge Test

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Abstract

Background and Aims: Fowl pox vaccine is produced in the Razi Institute for almost half a century and has a favorable and productive yield in poultry flocks and has provided complete satisfaction to the poultry breeder. In terms of comparing the efficacy of this vaccine with imported vaccines, the following research was conducted.

Materials and Methods: In this study, based on the latest protocols of European Pharmacopeia and OIE, 100 SPF chicks were divided into five groups: the first group was given 20 SPF chickens at the age of 8 to 10 weeks of domestic vaccine (Razi Institute: Live attenuated (inoculation in the wing with the needle of the twin branches); also in the second to fourth group: to 60 SPF chicks (in three groups of 20) at the age of 8 to 10 weeks; imported vaccines (I, C and H) were injected. Finally, in the fifth group, 20 chicks were considered as controls and did not receive vaccine. Response of the immune system was observed 7 to 10 days after vaccination by observing nodules at the injection site (Takes reaction). At 21 days (three weeks), all four groups vaccinated with acute pathogenesis of fowl pox strain were challenged. Chickens were observed daily for 21 days after vaccination and the results of vaccination immunization were evaluated and analyzed by statistical analysis.

Results: The results of the experiments indicated that after vaccination, 100% of the vaccinated chickens were positive by takes responses and after being challenged in four groups vaccinated in the Razi, I, C vaccines 100% and vaccine H, 95% of the immune responses were observed lesions in the Crown of the birds, and in the control group, there were symptoms of cartilage like in 100% of the birds.

Conclusions: In general, according to the OIE standard, the above experiments showed that fowl pox vaccine Razi Institute induces high immunity and has efficacy similar to imported vaccines.

Keywords: Efficiency, fowl pox vaccine, Razi institute, challenge test.

Introduction

Fowl pox is a contagious disease of domestic and wild birds of all ages, sexes and breeds which is caused by fowl pox virus (FPV)a . The virus is a DNA virus that comes under the genus Avipoxvirus of family Poxviridae and subfamily Chordopoxvirinae. FPV is brick shaped, has large size genome approximately 288-300 kbase pairs (Kbp) (1). Replication and maturation of the virus occur in the cytoplasm of host cell. Virus is spread by insects and wild birds (2).

Clinically the affected birds show three forms of the disease namely; the cutaneous,

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diphtheritic and systemic form. In the cutaneous form, bird shows nodular lesion on unfeathered parts of the body. The characteristics feature of a diphtheritic form is fibronecrotic lesions in the mucous lining of the oropharyngeal route and the internal tissues are found to be most affected in the third form (3).

The great concern is needed as the disease causes heavy economic loss. The mortality rate increased up to 50% when the diphtheritic form is accompanied by secondary bacterial infection. For the appropriate diagnosis, viruses are isolated either in cell culture, or embryonated chicken eggs using CAM route or by the combination of both techniques. Fowl pox is an emerging disease and the variant FPV has been reported broadly. Disease treatments for fowl pox are not available (4).

Differential diagnosis of avian pox viruses with restriction fragment length polymorphism (RFLP) has been carried out. It was reported that isolated avian pox virus from turkey and hen have same genotype pattern but canary and pigeon have different genotype pattern and RLFP is the best technique to differentiae avipox viruses (5). Molecular detection of avian pox virus from nodular skin and mucosal fibrinonecrotic lesions of Iranian backyard poultry was done by Gholami-Ahangaran et al. 2014 (6). Their study revealed that polymerase chain reaction (PCR) is a valuable tool for identification of an avian pox virus and that the frequency of pox infection in backyard poultry in western areas of Iran was high (6).

Replication of avian pox viruses appears to be similar in dermal or follicular epithelium of chickens, ectodermal cells of the chorioallantoic membrane (CAM) of developing chicken embryos and embryonic skin cells. Differences in the host cell and virus strain, however, may be reflected in the time scale of replication and virus output (7).

For the first time in Iran, isolation, identification and characterization of pox viruses from turkey has been performed by Ebrahimi et al., (2006) (5). The results of or their study indicated that turkey pox viruses isolates from different provinces of Iran have similarities in antigenic and genomic opinions and may be the fowl pox viruses (5).

The disease has been reported in more than 200 avian species. Fowl pox, in commercial poultry, is worldwide in distribution.

The incidence, however, is variable. In high density areas where multiple age birds are raised under confined conditions, the disease tends to persist for a long time despite preventive vaccinations (8). In recent years, several outbreaks of the diphtheritic form of fowl pox have been encountered in previously vaccinated chicken flocks (9).

The primary purpose of all poultry vaccination is to induce protective immune response that could prevent or reduce the economic lost caused by viral infection and diseases (10).

Vaccination against FPV is indicated under three conditions: a) When a flock on the premises was infected the previous year, all young stock produced on the premises or introduced from other sources should receive fowl pox vaccine. b) If pox was present the previous year and pigeon pox vaccine was used, birds should be revaccinated with fowl pox vaccine, because immunity from pigeon pox vaccine is not of long duration. c) In areas where pox is prevalent, fowl pox vaccine should be used for protection against infection from neighboring flocks (11).

As mentioned above, the fowl pox virus is a highly resistant virus and remains in the environment for a long time and can cause the disease, which is why it is difficult to fight, but because of a safe and effective vaccine, prevention is done to a great extent. Fowl pox is present in almost all parts of the world and almost all provinces in Iran. Therefore, from the State Veterinary Organization, the vaccine control program has been developed through vaccination in laying flocks, mothers and ancestors. The most commonly used vaccine in all parts of the world is a live vaccine that has been activated, which provides active and powerful immunity to the disease (12).

Due to the fact that, apart from the live attenuated fowl pox vaccine produced at the Razi Institute, foreign brands of fowl pox vaccine are available in poultry farms in Iran. Therefore, comparing the efficacy of this vaccine with imported vaccines in SPF chickens seems to be useful and valuable.

Methods

Vaccine. Razi institute fowl Pox vaccine is a chicken embryo propagated, freeze-dried, live attenuated virus vaccine for wing web administration in chickens. To prepare vaccine, half of the volume of diluent (normal saline or sterile distilled water) was added into the vial containing the 2500 doses of freeze-dried virus each dose of vaccine titer was at least 102.5 EID50 embryo infective dose) per dose. The partly dissolved vaccine was added into the diluent bottle to mix with the rest of the diluents and was shaken vigorously until the vaccine was dissolved completely. The vaccine was now ready for administration by the wing-web method. For administering the vaccine, the underside of one wing spread outward. The double needle applicator was spread into the vaccine bottle, wetting or charging both needles. The web of the exposed wing was pierced with the double needle applicator charged with vaccine.

B) Three imported vaccines (I, H, C) were also used, as described above.

Chickens. Experimental chicks: 100 SPF chicks were divided into five groups, and treated as follow:

Step One: In four groups of 20, SPF 8 to 10 weeks old, we used four types of vaccine as mentioned above and 20 other chicks were used as control. Step two: The immune response was observed 7-10 days after vaccination by observing nodules at the injection site (Takes).

Challenge Test. To test the challenge, wild fowl pox virus of was injected into both groups (vaccinated and control) and inoculation was carried out as scratching at the crown (Figure. 2,3., table 1).

Results

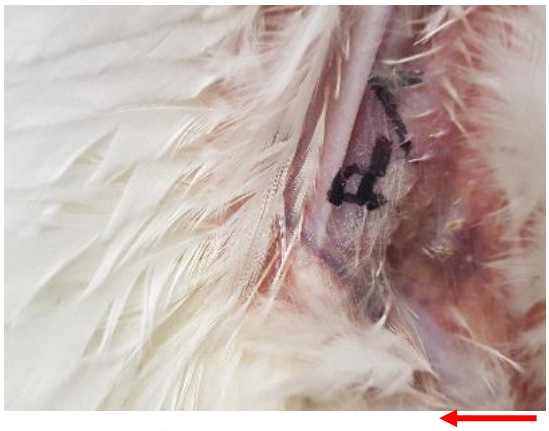

Fig. 1. View of take reaction after vaccination with Razi fowlpox vaccine.

Vaccination results. The results of vaccination of the domestic vaccine (Razi) and three imported vaccines (I, H, C) showed that 100% of the chicks were positive and they showed

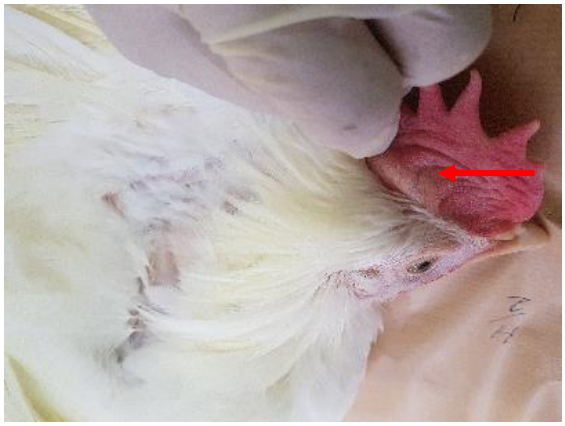

Fig. 2. Observation scars after the challenge in the H group.

nodular lesion at the site of inoculation (Take reaction. (Figures ,1) in the control group receiving only PBS no symptoms were seen and no visible lesion developed at inoculation site. (Figure, 1).

The results of challenge in vaccinated groups. After vaccination and evaluation of take reactions, the challenge test was taken in both groups that were tested 7 days after inoculation by pathogenic virus. In the first group or the group that received Razi vaccine and other vaccines, they all had no symptoms of disease but they showed scar in their crown exception one check in H group (Figure 2).

Results from the challenge of the control group. In the second group or control group,

all of them showed thickness symptoms in the crown (Figure 3).

The results of this study summarized in Table 1.

Table 1. Results of vaccination and challenge in experimental chickens

Group number	Vaccine name	Number of chicks per (10-8 weeks)	Take observation 10-7 days after vaccination	Observation or not the scars or lesions in the crown after the challenge test
1	Razi	20	100 percent observation Compatible with OIE	100 percent scar observation
2	I	20	100 percent observation Compatible with OIE	100 percent scar observation
3	H	20	95 percent observation Compatible with OIE	100 percent scar observation
4	C	20	100 percent observation Compatible with OIE	100 percent scar observation
5	control	20	100 percent no observation Compatible with OIE	100 percent lesions observation

Fig. 3. Observation scars after the challenge in the control group.

Discussion

Fowl pox outbreaks are reported throughout the world, but the incidences vary according to geographical areas however vaccinating susceptible birds prior to the time the disease is could be occur is the best way to immunize birds against pox (13). During spring and summer in areas where the disease occurs in fall and winter vaccination program is carried out. However, in large complexes containing multiple age birds and in tropical climates, where the disease may occur throughout the year, vaccination may be performed at any time when warranted without regard to the season (12).

Vaccination against FPV is indicated under three conditions: a) When a flock on the premises was infected the previous year, all young stock produced on the premises or introduced from other sources should receive fowl pox vaccine. b) If the disese was present in the previous year and pigeon pox vaccine was used, birds should be revaccinated with fowl pox vaccine, because immunity from pigeon pox vaccine is not of long duration. c) In areas where pox is prevalent, fowl pox vaccine should be used for protection against infection from neighboring flocks (4). The “chick embryo origin” vaccine contains live FPV capable of producing serious disease in a flock if used improperly (14).

Fowl pox vaccine is commonly applied by the wing-web method to 4-week-old chickens and to pullets about 1–2 months before egg production is expected to start. It is also used to revaccinate chickens held for the second year of egg production. The vaccine is not to be used on hens while they are lying (14).

A development of a multiplex polymerase chain reaction for differential diagnosis of canary pox has been reported (15,16,17). The results of the present study indicate the m-PCR assay holds potential to be versatile, rapid, and

sensitive for detection of CPV and differentiation of the virus from the other APVs (15,16,17).

In recent years, several outbreaks of fowl pox have occurred in all regions of the United States in chickens that had been vaccinated with either fowl pox or pigeon pox virus vaccines, indicating their inability to provide adequate immunity (18). Often combined fowl pox and pigeon pox virus vaccines have been used in chicken flocks with variable results. In this regard, field isolates of FPV from vaccinated flocks show variable pathogenicity in chickens (Singh et al.,2006) (18).

That was proved by a number of researchers that FPV attenuated vaccines of cell culture origin can be used effectively on chicks as young as 1 day of age and have been used at times in combination with Marek's disease vaccine (14).

Isolation and molecular characterization of avipox viruses was carried out by polymerase chain reaction (PCR) method using 578 bp fragment of 4b gene avain pox isolated virus. The conclusion of the mention study showed that PCR assay is one the important technique to detect DNA of pox viruses (5).

In the present report in Iran based on the phylogenetic analysis, the isolated fowl poxvirus was classified in a different subclade far from other Iranian isolates and close to the isolates from Tanzania, Egypt and Germany (12). The evaluation of avian pox situation in backyard chickens is very important because of its economic losses in rural areas and their role as sources of infection in commercial poultry (12).

Moreover, the molecular study in Avipox-viruses in Iran, especially in exotic birds (canary and mynah) was done by Nayeri Fasaei et al 2012. The sequence analysis reveals that the Iranian isolates are within the cluster with highly conserved p4b core protein in different countries and species of birds. Concerning the distance between countries which is the origin of the studied isolates that are situated in the same cluster with our Iranian isolates, nearly the same identity (95-99%) of isolates in this cluster exist, and so potential of infectivity of the isolates in several species and regions, and the import and export of birds from all over the world can likely spread the virus to other countries (19).

Such as all poultry viral disease the most effective way to prevent fowl pox disease is vaccination of poultry farms. Temporary and limited research has been conducted to evaluate the fowlpox vaccine, but there is no complete information and written data.

Considering the importance of the fowl pox disease in the country, evaluating the immunity of Razi Institute fowl pox vaccine in SPF chicks is very important to control mentioned disease. Therefore, the aim of this project was to determine the efficacy of fowl pox vaccine produced by Razi Institute and its comparison with common foreign vaccines in Iran, which seems to be necessary. In addition, In Iran, there is no comprehensive and complete research on this issue. However, the evaluation of fowlpox pox vaccine (produced in the Razi Institute) in laying hens by Alamian in 2012 was carried out as a research project with positive results (in Publishing process). Evaluation of fowl pox vaccine, which is produced by embryonated chicken eggs or cell culture particularly its efficacy has reported by some studies that we mention in following paragraphs.

Vaccination with Quil Pox live attenuated vaccine (Bio-pox Q) was carried out by Fatunmbi and his colleagues in 1996 at United States of America in 3 weeks old chickens. They challenged experimental chicks with five virulent strains of fowl pox, which was isolated from the 92-93 farm and the result was that although the cross immunity of the vaccine was created in chickens, but their immunity was not enough and appropriate (20).

In 1964, the experimental chickens vaccinated with fowl pox and pigeon pox vaccine. Evaluation of efficacy of fowl pox vaccine was performed by observing Takes in chicks with different titer. In the titer of 10 4.5 EID 50 gave immunity between 20 and 60%, but at a higher titer 10 5.5 EID 50, they could get good immunity (21).

When combined in a multivalent vaccine, quail, psittacine, and fowl pox viruses induced excellent protection in chickens against

challenge with the three respective viruses. The presence or absence of "takes" or reactions following vaccination by the wing web route did not necessarily correlate with the presence or absence of immunity noted from challenge by feather follicle virus application. The role of quail and psittacine pox viruses as potential pathogens for poultry. Quail, chickens, and turkeys vaccinated with pigeon and fowl pox viruses were not protected against challenge of their immunity with quail pox virus and they developed severe cutaneous lesions of pox. When quail and chickens were vaccinated with quail pox virus and given pigeon and fowl pox challenge viruses, no protection was present (21).

Adaption of the Beaudette strain in embryo fibroblasts cell culture, and the producing vaccine was used in the farm. After evaluating the take reaction and exposing the chickens to the pathogenic strain, acceptable immunity was established (22). That was proved by a number of researchers that FPV attenuated vaccines of cell culture origin can be used effectively on chicks as young as 1 day of age and have been used at times in combination with Marek's disease vaccine (14).

Mayr and Danner in Germany reported that oral vaccination with an attenuated cell culture vaccine could to be effective. They found out that successful immunization required 106 to 108 TCID50 depending upon the vaccine virus used (23).

Evolutions of Comparative immunity of FPV vaccines by intramuscular, feather follicle, oral, and intranasal routes in chickens of different age groups was done by Sharma and Sharma in 1988.They reported that oral vaccination did not provide protection over 50%, and the other methods provided 80–100% protection (24).

Drinking water fowl pox vaccine has used by Nagy et al, 1990, in 1-day-old chicks when the vaccine contains a sufficiently high concentration of virus (10 6 cell culture infective dose50 per ml) (25). Recent success with in ovo administration of FPV vaccines in 18-day-old chicken embryos has provided. With increasing use of in ovo vaccination, the cost of vaccination and stresses associated with handling the birds will be reduced significantly (4), (26).

Pock forming ability of field strain and vaccine strain of fowl pox virus (FPV) in chorio-allantoic membrane (CAM) of embryonated chicken eggs and its adaptation in chicken embryo fibroblast (CEF) cell culture was carry out. Infected CAM showed intracytoplasmic inclusion bodies. The CEF inoculated with FPV field isolate as well as a vaccine strain showed characteristic CPE at third passage level (9).

Islam et al 2008 in Banghladesh demonstrated by passive hemagglutination test add challenge test that imported fowl pox vaccine (PoxineÂ ®) and domestic fowl pox vaccine (DLSFPV) in their country are equally suitable and the chicks of nonvaccinated origin might be vaccinated with DLS-FPV at day 18 or 22 and 36 in case of PoxineÂ® fowl pox virus vaccine (11).

Relationship between Values of Fowl pox enzyme-linked immunosorbent assay (ELISA) and the Presence of "Takes" After Vaccination was proven by Barreda in 2006(4). In their study four experiments were conducted where the birds were bled once a week before and after vaccination and then were examined simultaneously for evidence of "takes." This study showed that there is a relationship between the ELISA values to the fowlpox vaccine that are considered positive and the presence of post vaccination lesions (4).

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. In addition, in mentioned study 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 day post-vaccination DPV with a maximum elevation of takes at the 7th DPV (90-100%). Geometric mean titer of passive hemagglutination (PH) 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% (27).

The experiments of this study were summarized in two sections. The first stage of

vaccination of chicks and evaluation their immunity, and in the second phase involves the challenge testing that was carried out in the vaccinated-chickens.

In the first stage, the fowl pox vaccine was prepared in the department of research and production of poultry viral vaccine of Razi Institute and three imported vaccines (I, H, C) in the chicks. After vaccination, the vaccine should be evaluated 7 to 10 days after vaccination to assess the effects of the vaccine.

The swelling of the skin or the presence of ulcers (Take reaction) in the vaccinated area is a proof of the success of the vaccination campaign, which we found to be the result of this study. Normally, immunization from the vaccine is obtained 10 to 14 days after vaccination. Most vaccinated birds should show the effects of the vaccine, however, In large herds, at least 10% of birds should be evaluated to assessment the vaccination operation.

According to the OIE protocol, after vaccination, about 10% the herd should be inspecting within 7-10 days. If birds infected, the inoculum site is full of stuffy skin and swollen skin, and if inoculated with one or two wings, dunedol is seen as rice or chickpea. Otherwise, vaccination should be renewed. The vaccine immunity obtained after 2 weeks of vaccination and peaked after 4 weeks and for one year and sometimes for a lifetime (28). In the second phase of this research, the challenge with the pathogenic fowl pox virus in the control group all the birds (100%) showed symptoms of the disease such as dermatitis their crown, and in other groups were protected and the presence of scars in all cases was evident except for one case and in the vaccine H, which, as mentioned above, was not likely to receive adequate vaccine from the bird.

Also, according to the OIE and European Pharmacopeia protocols for satisfactory immunization, at least 90% of the controls should have lesions of fowl pox and at least 90% of the vaccinated birds should not (29). In addition, according to other studies of colleagues (Allamiyan final report of research project), Razi fowl pox vaccine can definitely be considered as one of the most effective and the most quality vaccines available in the market for producing vaccines, which have high immunity and efficacy against the pathogenic agent.

Ethics

I hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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References

1. Afonso CL, Tulman ER, Lu Z, Zsak L, Kutish GF, Rock DL. The genome of fowlpox virus. J Virol. 2000;74 (8):3815-31.

2. Tripathy DM, Hanson LE, Myers WL. Passive hemagglutination test with fowl pox virus. Avian Dis.1970;14(1):29-38.

3. Winter field RW, Reed W. Avian pox: Infection and Immunity with Quail, Psittacine, Fowl, and Pigen pox Viruses. Poult Sci.1985; 64:65-70.

4. Barreda CB. Relationship Between Values of Fowlpox ELISA and the Presence of "Takes" After Vaccination. Avian Dis. 2016; 60 (1):67-9.

5. Ebrahimi MM, Shahsavandi S, Masoudi S, Pourbakhsh SA, Momayez R, Ebrahimi SR.

Isolation and molecular identification of avipox viruses. 14th Iranian Veterinary Congress. 2005; Tehran, Iran.
6. Gholami-Ahangaran M, Zia-Jahromi N, Namjoo A. Molecular detection of avian pox virus from nodular skin and mucosal fibrinonecrotic lesions of Iranian backyard poultry.Trop Anim Health Prod. 2014;46 (2): 349-353.
7. Baxi MK, Oberoi MS. Comparative evaluation of cell culture-adapted and chicken embryo-adapted fowl pox vaccine strains. Avi Dis. 1999;43 (1):16–21.
8. Tripathy DN, Reed WM. Pox. In: Calnek WB, Barnes HJ, Beard CW, McDougald LR, Saif YM, editors. Diseases of Poultry. 10th edition. Iowa State University Press; Ames, 1997;643–659.
9. Gilhare VR, Hirpurkar SD, Kumar A, Naik SK, Sahu T. Pock forming ability of fowl pox virus isolated from layer chicken and its adaptation in chicken embryo fibroblast cell culture. Vet World. 2015;8(3):245-50.
10. Wambura PN, Mzula A. A novel rapid direct haemagglutination-inhibition assay for measurements of humoral immune response against non-haemagglutinating Fowlpox virus strains in vaccinated chickens. Heliyon. 2017;31;3(10):e 00428.
11. Islam MR, Khan MSR, Islam, MA, Kayesh MEH, Karim MR, Gani MO, et al. Comparative efficacy of imported fowl pox virus vaccine with locally produced one in backyard chicks. Bangl J of Vet Med. 2008;6(1):23-26.
12. Norouzian H, Farjanikish Gh. Pathological and molecular diagnosis of avian pox in backyard chickens in Khorram Abad. Vet Res Biol Prod. 2016;117:2-10.
13. Wang J, Meers J. Spradbrow PB, Robinson, WF. Evaluation of immune effects of fowlpox vaccine strains and field isolates. Vet Microbiol. 2006;116:106–119.
14. Siccardi FJ. The addition of fowlpox and pigeonpox vaccine to Marek's vaccine in broilers. Avian Dis. 1975;19:362–365.
15. Ebrahimi MM, Shahsavandi S, Masoudi S, Ghodsian N, Hashemi A, Hablalvarid MH, et al. Development of a Multiplex Polymerase Chain Reaction for Differential Diagnosis of Canary Pox Virus . Iran J Virol. 2012;6(3):19-23.
16. Ebrahimi MM, Shahsavandi S, Masoudi S, Hashemi A, Hablalvarid MH. Differential diagnosis of canary pox virus by using molecular method. 17th Iranian Veterinary Congress. 29 Apr-1 May 2012, Tehran, Iran.
17. Ebrahimi MM, Shahsavandi S, Momayez R, Hablalvarid MH. Identification of canary pox virus using histopathological and molecular assays. 3rd International Veterinary Poultry Congress. 2012; Tehran, Iran.
18. Singh GK, Singh NP, Garg SKC. Studies on pathogenesis of fowlpox: Virological study. Acta Virol. 1987;31:417-423.
19.Nayeri Fasaei B, Madadgar O. Ghalyanchi Langeroodi A , Ghafari MM. Molecular detection and phylogenetic analysis of Avipoxvirus strains isolated from different bird species. Iran J Vet Res. 2014;15(1):40-44.
20.Fatunmbi OO, Reed WM. Evaluation of a Commercial Quail Pox Vaccine (Bio-Pox Q™) for the Control of "Variant" Fowl Poxvirus Infections. Avian Dis. 1996;40(4):792-797.
21.Winter field RW, Reed W. Avian pox: Infection and Immunity with Quail, Psittacine, Fowl, and Pigen pox Viruses. Poult Sci.1985;64:65-70.
22. Weli SC, Traavik T, Tryland M, Coucheron DH , Nilssen O. Analysis and comparison of the 4b core protein gene of avipoxviruses from wild birds: evidence for interspecies spatial phylogenetic variation. Arch Virol. 2004;149:2035-2046.
23. Mayr A. Danner K. Oral immunization against pox. Studies on fowlpox as a model. Dev Biol Stand, 1976;33: 249-59.
24. Sharma DK, Sharma SN. Comparative immunity of fowl pox virus vaccines. Zentralbl Veterinarmed B. 1988; 35:19–23.
25. Nagy E, Maeda-Machang'u AD, Krell PJ, Derbyshire JB. Vaccination of 1-day-old chicks with fowlpox virus by the aerosol, drinking water, or cutaneous routes. Avian Dis. 1990;34(3):677-82.
26. Peebles ED. In ovo applications in poultry: A review, Poult Sci .2018.1;97(7):2322-38.
27.Elemahdy SS, Mikheal C. Efficacy of fowl pox vaccines against Egyptian isolated strain during 2012. Vet World. 2014;7 (9): 656-660.
28.OIE. Office Internationals Epizooties; Paris: 2016. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.03.10_FOWLPOX.pdf.
29.European Pharmacopeia 9.0.2016.
30.https://www.edqm.eu/sites/default/files/index_9th_edition_pheur.pdf .1064.
31. Khalesi B, Ebrahimi MM, Ghodsian N, Ebrahimi SR, Masoudei S, Kaffashi A.
32. Ebrahimzadeh MS, Alamian A, Salimi A. Evaluation of efficacy of Razi fowlpox vaccine in comparison of commercial fowlpox vaccine in SPF chickens by challenge test. 20th Iranian Veterinary Congress. 1-3 July 2018, Tehran, Iran.