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Original Article

Evaluation and Optimization of Chick Embryo Fibroblasts for Production of a Fowl Pox Vaccine Based on Cell Culture

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Abstract

Background and Aims: For more than half a century, the production of fowl pox vaccine at Razi Vaccine and Serum Research Institute, has been carried out by injection method in the chick chorioallantoic membrane (CAM) and the vaccine has a favorable and effective in poultry flocks and has provided a complete satisfaction to the poultry flocks owner. Fowl pox vaccine is also manufactured using chicken embryo cell (CEF) culture in other countries. The aim of this project is to develop a fowl pox vaccine based on CEF which is of vital importance and a requirement for Razi institute.

Materials and Methods: In this study, chicken fibroblastic cells were used as primary cell culture in Hanks or DMEM media supplemented with fetal bovine serum 10% (FBS). First, the cells were cultured and the cell count was determined. Subsequently, the virus was added to the cells. The virus that used to prepare the vaccine was initially grown up in the fibroblast cells and had a titer of 106.3 TCID50/ml. To determine the viral load, two methods plaque-forming unit (PFU) and TCID50 were used, safety and efficacy tests were performed on 10 chickens, and the potency test on 20 chickens and vaccinated chickens were challenged with wild fowl pox virus strain.

Results: The results of the tests showed that the vaccinated chickens had an adequate and sufficient resistance to the acute form of fowl pox virus.

Conclusion: In total, according to the OIE standard, the above experiments showed that cell culture-based fowl pox vaccine can generate good immunity response and was high efficacy.

Keywords: fowl pox vaccine; fibroblast cell culture; chick chorioallantoic membrane (CAM); Razi institute

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 DNA virus that comes under the genus Avipoxvirus of family Poxviridae and subfamily Chordopoxvirinae (1). FPV is brick shaped has a large size genome of approximately 288-300 k base pairs (Kbp). Replication

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and maturation of the virus occur in the cytoplasm of the host cell (2). The 4b core protein gene (p4b) of Avipoxvirus that encodes the protein with molecular weights of 75.2 kDa is usually chosen for comparative genetic analysis (3). On the other hand, amplification of the p4b of Avipoxvirus by PCR has often been used as a molecular tool for the detection of avian poxviruses (4) and is one of the most sensitive techniques for the routine diagnosis. The virus is spread by insects and wild birds. Clinically the affected birds show three forms of the disease namely; the cutaneous, diphtheritic, and systemic form (5).

In the cutaneous form, the bird shows a 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. 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 a secondary bacterial infection (5).

In native turkeys, weight loss is also important economically. In ornamental poultry, the septicemic form of the canary has a significant impact on the economy of canary breeders due to the high mortality in this form of the disease (6). For the appropriate diagnosis, viruses are isolated either in cell culture, or embryonated chicken eggs using the CAM route or by the combination of both techniques. Fowl pox is an emerging disease (7) and the variant FPV has been reported broadly. Disease treatments for fowl pox are not available. Propagation of avian poxviruses in cell cultures of avian origin (e.g., chicken embryo fibroblasts, chicken embryo dermis, and kidney cells, and duck embryo fibroblasts) has been accomplished. Also, the Japanese quail permanent cell line "QT 35" and liver cell line (LMH) are useful for the growth of some avian poxviruses after adaptation (8). Otherwise, isolation from turkeys and wild birds failed to grow in these cell lines even after repeated passages. While mammalian cells are believed to be abortive for infectious by avian pox also besides in new research showed that cultivation of three avian pox virus strains Syrian baby hamster kidney (BHK-21) cells were found permissive (9). Two types of vaccines, chicken embryo adapted (VacCE) and cell culture adapted (VacCC), were commercially available in the poultry industry. The "chick embryo origin" vaccine contains live FPV capable of producing serious disease in a flock if used improperly. 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 laying. Attenuated FPV 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 (10). Oral vaccination with an attenuated cell culture vaccine was reported to be effective in Germany by Mayr and Danner (11).

Successful immunization required 10^6 to 10^8 $TCID_{50}$/ml depending upon the vaccine virus used. Comparative immunity of FPV vaccines by intramuscular, feather follicle, oral, and intranasal routes in chickens of different age groups was evaluated by Sharma and Sharma (12). They reported that oral vaccination did not provide protection over 50%, and the other methods provided 80–100% protection. Nagy et al. (13) demonstrated that 1-day-old chicks can be vaccinated effectively against fowlpox through drinking water when the vaccine contains a sufficiently high concentration of virus (106 cell culture infective dose 50 per ml). In recent years, few outbreaks of fowl pox have occurred in all regions of the world in chickens that had been vaccinated with either fowl pox or pigeon pox virus vaccines, indicating their inability to provide adequate immunity (14). 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. Most of the field strains contain full-length reticuloendotheliosis virus (REV) in their genome. Experimental studies indicate that FPV containing integrated REV provirus

induces profound, but selective immuno-suppressive effects on infected chickens of younger age (15). Live virus vaccines are used for immunization of birds against pox.

Vaccines of fowl pox and pigeon pox virus origin are routinely used for vaccination of chickens and turkeys in areas where the disease is endemic. These should contain a minimum concentration of 10^5 EID_{50}/ml (16) to establish satisfactory takes for good immunity. Fowl pox and pigeon pox virus vaccines labeled "chick embryo origin" are prepared from infected CAM. FPV vaccine labeled "tissue culture origin" is prepared from infected chicken embryo fibroblast cultures. The success of a vaccination program depends on the potency and purity of the vaccine and its application under conditions for which it is specifically intended. Vaccination essentially produces a mild form of the disease (17). In the present study Razi Vaccine Research Institute fowlpox working vaccine seed strain were adopted in chicken embryo fibroblast cell culture of SPF eggs and prepared for experimental and hive industry vaccine that was aim of this study.

Methods

Specific pathogen-free eggs (SPF) eggs. The SPF embryonated chicken eggs were obtained from Razi Vaccine and Serum Research Institute. The SPF eggs were used for titration of egg-adapted fowl pox virus and preparation of primary chicken fibroblastic cell culture. The eggs were incubated at 37°C with 40-60% humidity.

Virus and vaccination. In this study, the Razi institute working seed of fowl pox vaccine strain was used for primary cell culture. Besides, this strain has been obtained from Chick chorioallantoic membrane (CAM) and being used as live attenuated virus vaccine using by wing web administration in chickens.

To prepare the vaccine, half of the volume of diluent (normal saline or sterile distilled water) was added into the vial containing the 2500 doses of the freeze-dried virus each dose of vaccine contained at least $10^{2.5}$ EID_{50} of virus (embryo infective 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 the vaccine.

Chicken embryo fibroblast cell culture. Primary fibroblast cells from 9 to 11 days old chick embryo were used for cell culture. The embryos were extracted from the SPF eggs. The fetal limbs were cut off and their viscera discharged and washed several times with PBS solution at pH 7.4. Then chopped into 1 mm pieces using scissors. The tissue pieces with trypsin (2.5% W/V) using Stirrer and Magnet apparatus. The trypsin was inactivated by FBS. The digestion process was repeated several times until the embryo was fully digested.

Finally, the suspension containing the digested cells was then filtered by a sterile tampon. The harvested liquid was then centrifuged at 1400 rpm to precipitate cells. The cells were washed twice with PBS and resuspended in 100 ml of the high glucose-DMEM cell culture containing L-Glutamine Sodium Pyruvate .

The medium was supplemented with 10% FBS. Antibiotics (penicillin and streptomycin) 0.1 mg were used to prevent infection.

To prevent fungal growth, the same amount of Nystatin was added. 5 ml of fibroblast cell suspensions were cultured in sterile 25ml-cell culture flasks for the production of monolayer fibroblast cells and stored in an incubator.

Adaptation of FPV in CEF cell culture. Monolayer CEF primary cells in DMEM media were cultured and after 70-80% confluency of the attached cells, the media was poured out. Cells were washed by 5-7 ml PBS gently then 0.2 ml of 10-fold serial dilution of 10% virus suspension cultured in CAM was added into mono-layered CEF cells and incubated at 37°C for 1-2 hours. For adsorption After removal of the unadsorbed virus DMEM growth medium containing 0.1% antibiotics and 2% FBS was added. The cytopathic effect

was seen after 3-5 days. In this stage, the culture was collected. The virus culture extract with the lowest amount of cytopathic effect was freeze-thawed twice then the virus culture in monolayer CEF primary cell was done again, until passage 4. After four consecutive passages, any passage should has been titrated. Because as the number of passages goes high the replication rate and virus titer gets better so when we reach passage no.8 we should be reached to the target virus titer. If this is not the case, a lower virus dilution should be used, to obtain $10^{6.3}$ $TCID_{50}$/ml at passage no.8. We used 10^{-3} (0.001) dilution and successfully reached to $10^{6.3}$ $TCID_{50}$/ml after 8 passages.

Virus titration. The titer of the Fowl Pox virus was calculated by 3 methods: $ECID_{50}$, $TCID_{50}$, and PFU.

$ECID_{50}$. SPF embryonated chicken eggs were inoculated via CAM rout for $ECID_{50}$ calculation. 0.2 ml ten-fold serial dilution of the cultured virus suspension was prepared. 5 embryonated eggs (10-12 days old) were inoculated for each dilution. The fatality within 24 hours post inoculations were not considered. The survived embryos were examined for evidence of the infection. Demonstrated pock lesions or generalized thickening of CAM, on the 5^{th} day post-inoculation considered as the infection signs. Finally, virus titration was performed using the Reed and Muench method.

$TCID_{50}$. Primary chicken embryo fibroblast cells were prepared in 6-well microplates after 70-80% confluency Virus dilution was prepared from 10^{-1} to 10^{-6}. 100µ of diluted virus suspension was then added to the wells for adsorption. One cell well was remained as control. The effect of the virus was monitored every day. On the fifth day PI, the $TCID_{50}$/ml was calculated according to the Spearman-Karber formula (M = xk + 1 / 2d-drl / n).

PFU. Plaque forming unit assay was also used to determine viral titer. The plaque assay showed the cytopathic effects of certain viruses on the cell culture medium, by counting the plaques viral dilutions from 10^{-1} to 10^{-6} were inoculated on a monolayer chicken embryo fibroblast cells after 1-2 hours the virus was removed and the cells were covered by a medium containing 2% FBS and 1% Agarose gel. It was noted that the temperature of the agarose suspension was not more than 42ºC, which causes cell death, and not less than 39º C, which may cause the gel to solidify. In this method, on the third day, 0.3% neutral red reagent was added to the. The plaques were counted and then 50% PFU was calculated.

After reaching the virus titer of $10^{6.3}$ EID_{50}/ml which was adapted to cell culture the virus was used for the production of the Fowl Pox Vaccine.

Safety test. Ten SPF chickens at four weeks of age were inoculated with the dilution of 1/20 of the prepared vaccine and five days later the immune response of chicken was checked on the injection site.

Challenge test. For this purpose, 20 non-vaccinated four weeks old SPF chicks were divided into 4 accidental groups of 5.3 groups were inoculated by 0.1¸1¸10 doses of FP vaccine, and one group of 5 chickens was kept as control. The chicks were monitored for 4 weeks to check nonspecific reactions. In the next stage, all vaccinated and control chicks were challenged with a virulent fowlpox virus at $10^{6.5}$ $TCID_{50}$/ml titer by the crown route (Fig. 1).



Fig. 1. The Challenge test.

Results

The working seed of fowl pox virus was propagated successfully on the chorioallantoic membrane of specific pathogen-free (SPF)

embryonated chicken eggs and clear pock lesions were observed (Fig. 2).

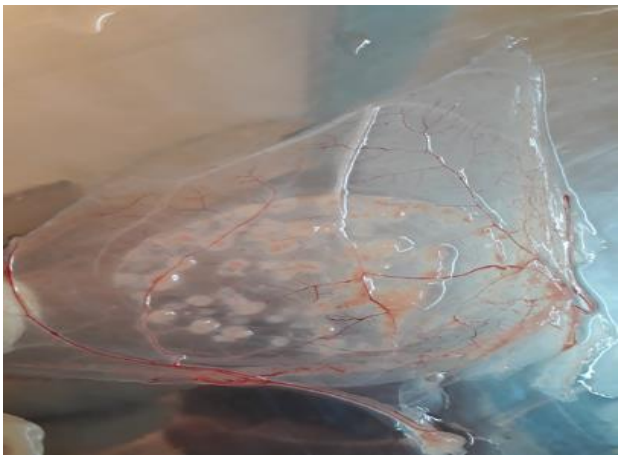


Fig. 2. Display of fowlpox on the chorioallantoic membrane of the chick embryo.

Results of cell culture and cytopathic effect. Chicken embryo fibroblast cell culture. As a result of this study, it was observed that the vaccine strain of FPV, was propagated successfully in CEF culture.

The CEF inoculated with FPV showed no characteristic CPE up to the second passage level. At third passage level CEF cell culture showed aggregation of cells which progressed rapidly and appeared as floating cells at 72 h PI. 48 hours after primary fibroblast cell culture in the 6-well plates, the cells became fusiform (Fig. 3).

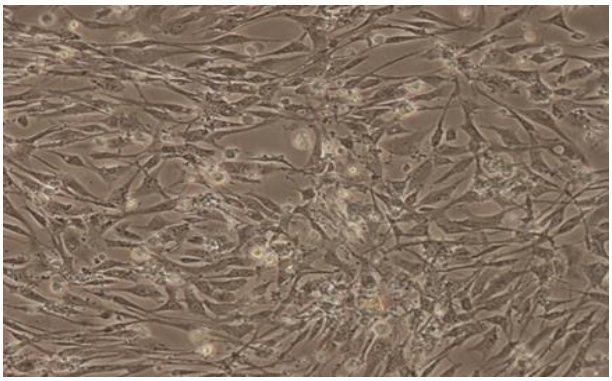


Fig. 3. Chick embryo fibroblast cell infected with virus.

The cytopathic effect of fowl pox virus on fibroblast cells was seen as rounding and scarring of the cells wall and accumulation of the cells at one point. There were rounding and degeneration of the cells and CPE appeared as "bunch of grapes." The remaining cells became elongated whereas corresponding uninfected controls showed no such changes.

After culturing of the diluted virus suspension in the primary CEF cells from second to eighth passages, the virus titer was reached to $10^{6.3}$ $TCID_{50}$/ml (Table 1).

The method of determining the virus titer in cell culture was based on the Reed and Muench formula.

The virus titer results by plaque-forming unit (PFU) assay was 5×10^5. The plaques were formed within 96 h PI (Fig. 4).

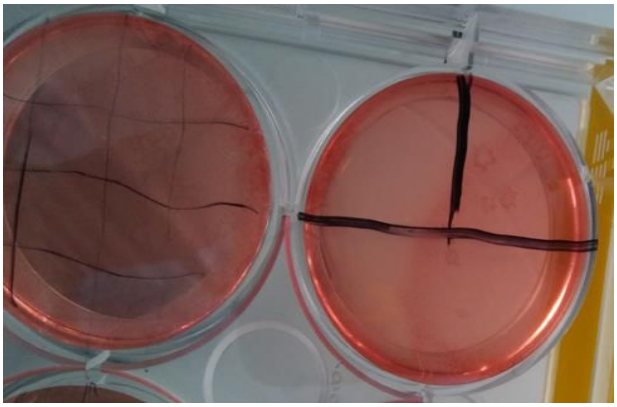


Fig. 4. The effect of the virus on the cells in the plaque forming units (PFU).

Vaccination result

The results of vaccination of the Razi and vaccine vaccines by fowlpox vaccine culture with three dilutions (E- 0.1) and (H5-1) and (C-10) showed that 100% of the chicks were positive for TB (Fig. 5). In the control group receiving PBS alone, no signs were seen.



Fig. 5. Observation of take after vaccination with the 10-dose (C) cell culture vaccine.

Table 1. Results of dilution and determination of the titer by the TCID50 method.

Dilutions	duration of effect CPE	percentage of cell lysis	passage number 2, 3 and 4	passage number 5	passage number 6	passage number 7	passage number 8
10^{-1}	3 days	100% cell lysis	Failed	-	-	-	-
10^{-2}	3 days	100% cell lysis	Failed	-	-	-	-
10^{-3}	4 days	70% cell lysis	Failed	$10^{5/1}$	$10^{5/1}$	$10^{5/9}$	$10^{6/3}$
10^{-4}	4 days	40% cell lysis	Failed	$10^{2/8}$	10^{3}	$10^{3/2}$	10^{4}
10^{-5}	5 days	25% cell lysis	Failed	$10^{1/4}$	$10^{1/8}$	$10^{1/9}$	10^{2}
10^{-6}	14 days	Circle the cell	Failed	-	-	-	-

Challenge test results

After injection, the challenge test results are divided into two parts: the first part involves the embryo fibroblast cell culture (Fig. 6), and the second part the control experiments on the SPF chick (Fig. 7).



Fig. 6. No scar observation after vaccination with the cell culture vaccine

Discussion

The emergence of the poultry industry in the twentieth century and its progress towards a high-yielding international system has drawn the attention of operators to increasing density and maximizing physical space utilization. This has created an artificial environment for poultry breeding that is less in line with the physiological structure of their bodies, which has led to the emergence of various viral and bacterial diseases. One of these diseases is avian fowlpox which causes negative economic effects and biodiversity (18).

Disease caused by FWPV is one of the important diseases in commercial poultry production and can produce significant problems when conditions are favorable for transmission, especially by mosquitoes, and the best control of disease is the prevention of transmission and by vaccination (19).



Fig. 7. Control groups showed signs of scab on the crown

In poultry, cutaneous fowlpox rarely has a significant mortality and economic impact, but its diphtheric form can cause up to 60% mortality in unvaccinated chickens. Natural diseases in wild and caged birds range from a dry form and tend to be mild and self-limiting to severe disease with high mortality in wet form (diphtheritic) (20). The severity of the disease is influenced by the strain of the virus, route of infection, and the species of bird (21).

A comparison in vivo of a field strain of FPV, its genetically modified progeny (in which all REV sequences were deleted) and a rescue mutant (in whose genome the REV provirus was inserted in its previous location) indicated that elimination of the provirus sequences correlated with reduced virulence (22). In young herds, the outbreak of fowlpox usually lasts about 6 to 10 weeks, which can have a significant economic cost for egg production

(23). Ordinary diagnosis of avipox infections is carried out by histopathological examination to show the presence of the virus in infected tissue samples, electron microscopy, virus isolation in cell culture, or on chorioallantoic membranes (CAM) of embryonated chicken eggs (24).

Due to the lack of definitive treatment, the only way to control and prevent the outbreak is to ensure proper hygiene and timely vaccination. However, it does occasionally become a problem even in countries that are at the forefront of control and prevention programs. Therefore, sufficient knowledge of the disease agents in each region and molecular identification of common viruses in the region can be of great help in targeted and correct use of vaccination to effectively prevent fowlpox (25).

The flock should be examined about 7–10 days after vaccination for evidence of "takes." A "take" consists of swelling of the skin or a scab at the site where the vaccine was applied and is evidence of successful vaccination. Immunity will normally develop in 10–14 days after vaccination. If the vaccine is properly applied to susceptible birds, the majority of the birds should have taken. In large flocks, at least 10% of the birds should be examined for takes. The lack of a take could be the result of the vaccine being applied to an immune bird, the use of a vaccine of inadequate potency (after the expiration date or subjected to deleterious influences), or improper application. This test has been done in this study in experimental chickens and positive results have been obtained from the test following vaccination with cell culture fowl pox vaccine (26).

The success of a vaccination program depends on the potency and purity of the vaccine and its application under conditions for which it is specifically intended. Vaccination essentially produces a mild form of the disease. Directions for use of vaccine as supplied by the producer should be followed explicitly. The vaccine should not be used in a flock affected with other diseases or in generally poor condition. All birds within a house should be vaccinated on the same day. Other susceptible birds on the premises should be isolated from those being vaccinated. If pox appears in a flock in an initial outbreak with only a few birds being affected, nonaffected birds should be vaccinated (21).

Cell cultures generally are not employed for the initial isolation of avian poxviruses. Adaptation of the virus to this host system is sometimes necessary because not all strains produce CPE on initial inoculation.

For antigenic and genetic characterization of an isolate, propagation in cell culture is more convenient than the use of CAM. A suspension of a pox virus suspected specimen from a dermal or diphtheritic lesion is inoculated on the CAM of 9-12-day-old developing chicken embryos from an SPF flock; 5–7 days after inoculation, the CAM is examined for pock lesions (see Figure Number 3). Occasionally, some isolates fail to grow on the CAM of chicken embryos (27). Characteristic CPE produced by the avian poxviruses in chicken embryo fibroblasts and QT 35 cells is characterized by an initial phase of rounding of the cells followed by the second phase of degeneration and necrosis.

The quantitative assay is by the cell culture dose-50% method based on CPE. Also, Differences in the plaque-forming ability of avian pox viruses have been observed.

Adaptation of the virus in cell culture is necessary because not all strains produce plaques. In monolayers of chicken embryo fibroblast cell cultures by some avian poxviruses was shown to be characteristic and is considered as an aid in differentiation (29).

Plaques are evident by 3-4 days PI in quail cells with certain avian poxviruses after adaptation. Mayr and Kalcher (11) found that the fowl pox virus produced plaques on chick embryo cell cultures. Development of plaques was slow and visible plaques could not be seen until about 8 days after infection;11days were required to be for be all of the plaques become visible. Feeding of the cells at 3-day intervals was necessary to maintain the cells for a long period of time under agar.

The tissue culture adapted strain was assayed either by its cytopathic effect (CPE) or as plaque-forming units (PFU). A bifurcated needle was placed in Eagle's serum-free

medium containing108 PFU/ml virus (30). Repeated inoculations were made into the wing web. About 50 ul of fluid were used for each chick. The mean dose was 5 x 10s PFU per chicks similar to our investigation but the inoculations amount was different and we used 0.1,1 and 10 for every chicken. In one study the plaque-forming ability differences in avian pox viruses have been observed. Adaptation of the virus in cell culture is necessary because not all strains produce plaques. Plaque formation in monolayers of chicken embryo fibroblast cell cultures by some avian poxviruses was shown to be characteristic and is considered as an aid in differentiation (30). Plaques are evident by 3- 4 days PI in quail cells with certain avian poxviruses after adaptation.

In Iran, the production of poultry vaccine has been carried out in Razi institute by SPF embryos for a long time and regarding the production of poultry vaccine in different ways such as cell culture and genetic recombination, comprehensive research is needed. In different countries, research has been done on the production of fowlpox vaccine, which is mentioned in the following lines. Pock forming ability of field strain and vaccine strain of fowl pox virus (FPV) in the chorioallantoic membrane (CAM) of embryonated chicken eggs and its adaptation in chicken embryo fibroblast (CEF) cell culture was carried out. Infected CAM showed intracytoplasmic inclusion bodies. The CEF inoculated with FPV field isolate as well as a vaccine strain showed characteristic CPE at the third passage level (31) but in our study at the eight passage level with the titer was obtained in 10 6.3 TCID50 / ml.

Newcastle wild strain (velogenic) were cultured on chick embryo fibroblast cells and their changes were examined over 50 passages by Mohan et al in 2005 (32). On the other hand, Barhouna and Hanson reported that the embryo's fibroblast cell is one of the cells that can be used to adapt to viruses, multiply, and spread the virus that causes fowlpox. However, Vero cells are also used to propagate the virus (33). In 1991, RT-PCR was used to identify the infectious titer of the virus for EID50 calculation based on OIE guidelines using the Reed and Manch calculation method, and based on this method, 100%. Infected eggs were calculated (26). Vaccination with Quil Pox live attenuated vaccine (Bio-pox Q) was carried out by Fatunmbi and his colleagues in 1996 at the 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, their immunity was not enough and appropriate. Adaption of the Beaudette strain in embryo fibroblasts cell culture and the producing vaccine was used on the farm. After evaluating the take reaction and exposing the chickens to the pathogenic strain, acceptable immunity was established (35).

That was proved by some 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 (10). The safety of intramuscular vaccination, feather follicles, oral and current intranasal vaccination was evaluated by Sharma in 1988. The results showed over 50% protection in the oral method and 80-100% in other methods. They reported that oral vaccination did not provide protection over 50%, and the other methods provided 80–100% protection (36). In 1964, Waterfield and his colleagues examined the safety of chickens with fowlpox and pigeon's vaccines. Vaccine safety evaluation was performed by observing Takes in chicks with different dilutions and showed immunity of between 20 and 60% at 10 4.5 EID / 50 per dilution but at a grade above 105.5 EID / 50 per 50 ml had good results. They saw the prevalence of the disease in herds vaccinated with fowlpox or pigeon vaccine in the United States indicates that these vaccines are unable to provide adequate immunity. In most cases, fowlpox and pigeon's vaccines are mixed and used in herds (37). Nagy et al. 1990, (13,38) demonstrated that 1- day-old chicks can be vaccinated effectively against fowlpox through drinking water when the vaccine contains a sufficiently high concentration of virus (106

cell culture infective dose 50 per ml). Khalsi et al. 2019 (39) Evaluation of Efficacy of Razi Fowl Pox vaccine in comparison with the commercial fowl pox vaccine in SPF chickens by challenge test showed fowl pox vaccine Razi Institute induces high immunity and has efficacy similar to imported vaccines. The immune response of two commercial fowl pox vaccine chicken embryo adapted (VacCE) and cell culture adapted (VacCC), were performed by blastogenesis assay at 2 and 8 wk of age chickens. The results of this study showed that in the birds which are vaccinated with VacCC at 8 weeks age the titer rate was higher than other groups at 21 days postvaccination (PV) and 7 post-challenge (PC) (40).

Conclusion

In this study vaccinated chickens had a sufficient and adequate resistance to the acute form of fowl pox virus. In total, according to the protocols of the European Pharmacopeia and OIE standard, the above experiments showed that cell culture-based fowl pox vaccine can generate good immunity response and have high efficacy. Considering the positive results of the experiments conducted in this study, it can be acknowledged that the success of laboratory production of fowl pox vaccine by cell culture can be a small step towards the self-sufficiency of this type of vaccine.

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Conflict of interest

The authors declare that they have no conflict of interest.

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