

PRODUCTION OF PIGEON POX VACCINE FROM THE EGYPTIAN LOCAL STRAIN ON SPF EGGS

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Abstract

A local virulent Egyptian strain of pigeon pox virus was attenuated on specific pathogen free (SPF) eggs for production of pigeon pox vaccine. Twenty two serial passages for the virulent virus were applied on the chorioallantoic membrane (CAM) of SPF eggs. Titration of the serial passages were recorded as 106.8, 107.5, 107.8, 108.5 and 108.5 EID₅₀/ml for the 5th, 10th, 15th, 18th and 20th passages, respectively.

The pathogenicity of the 5th, 10th, 15th and 18th passages was tested by inoculation in susceptible pigeons, the 15th and 18th passages produced the most acceptable and mild post-vaccinal reaction. The 18th passage was considered the passage having the highest titre (108.5 EID₅₀/ml).

The quality control of the prepared vaccine was applied by testing sterility, safety and potency; the vaccine was proved to be sterile, safe and potent for pigeons which resisted the challenge with virulent pigeon pox virus.

Confirmatory serological tests (SNT and ELISA) were applied and recorded that the antibody levels after vaccination reached the protective levels (more than 1.5 index for SNT and more than 1.0 for ELISA).

INTRODUCTION

Pox is a common viral disease of domestic birds (chickens, turkeys, pigeons and canaries) and known as avian pox (Tripathy and Reed, 1997). The disease is worldwide distributed, and infects birds of all ages and breeds (Odend'hal, 1983).

Pigeon pox virus is one of Avipox viruses from the family Poxviridae, characterized by the development of discrete nodular proliferative skin lesions on the non-feathered parts of the body (cutaneous form), or fibrino-necrotic and proliferative lesions in the mucous membrane of the upper respiratory tract, mouth and oesophagus (diphtheritic form) (Tripathy *et al.*, 1981).

Pigeons affected with the cutaneous form of the disease are more likely to recover than those with the diphtheritic form involving the respiratory tract. Flock

mortality in pigeons is usually low, but in severe cases, it may be as high as 50% (Tripathy and Reed, 1997).

In Egypt, pigeon pox disease is controlled through vaccination with live attenuated vaccine prepared from the Hungarian strain.

The aim of this study is the attenuation of the local virulent strain of pigeon pox virus preparation of new pigeon pox vaccine from this local Egyptian isolate in SPF embryonated chicken eggs.

MATERIALS AND METHODS

1. Birds

Eighty pairs of susceptible pigeons, 45 day-old were used in this study, for evaluation of the prepared vaccine and monitoring its pathogenicity.

2. Embryonated eggs

Four hundred SPF embryonated chicken eggs, 9-11 day-old, were obtained from Kom-Osheem SPF Farm, Fayoum. They were used for propagation, attenuation and titration of the local strain of pigeon pox virus.

3. pigeon pox virus

A local virulent strain of pigeon pox virus was obtained from Pox Department, Veterinary Serum and Vaccine Research Institute, Cairo. It has a titre of 106.7 EID₅₀/ml. It was used for serial passages in embryonated chicken eggs, as well as challenging the potency of the prepared vaccine.

4. Pigeon pox antigen

A clarified virus suspension of pigeon pox antigen (Hungarian strain) obtained from Pox Department, Veterinary Serum and Vaccine Research Institute, Cairo, was used as antigen in ELISA technique.

5. Blood and serum samples

Heart puncture blood samples were collected for serum separation, before and after inoculation with different passages of pigeon pox virus.

6. Anti-avian IgG peroxidase conjugate

It was supplied by the National Diagnostic Scientific Office (Sigma), USA. It was used in solid phase ELISA.

7. Stabilizers

It was prepared as 5% lactalbumin hydrolysate and 2.5% saccharose powder. It was used for lyophilization of the prepared pigeon pox vaccine.

8. Adaptation of the virulent pigeon pox virus in SPF embryonated chicken eggs

It was applied according to Moss (1990), where 22 serial passages were done by inoculation of 0.2ml pigeon pox virus on the chorioallantoic membrane (CAM) of fertile SPF eggs. The passages were repeated by isolation of pigeon pox virus from the infected CAM and its reinoculation in fertile SPF eggs.

9. Titration of the adapted pigeon pox virus

Passages of the adapted pigeon pox virus were titrated in embryonated chicken eggs according to Dhillon *et al.* (1968).

10. Pathogenicity of adapted pigeon pox virus in susceptible pigeons

The pathogenicity of adapted pigeon pox virus passages was sequenced according to Woodward and Tudor (1973) on the passages 5, 10, 15 and 18. It was applied by inoculation of ten susceptible pigeons with the adapted pigeon pox virus by feather follicle method according to Winterfield and Hitchner (1965).

11. Lyophilization of the adapted pigeon pox virus

The selected passages were lyophilized after addition of lactalbumin sucrose stabilizer in a ratio (1:1). It was done according to Mayr (1962).

12. Detection of the field dose for the prepared vaccine

According to Winterfield and Hitchner (1965), three doses were prepared from pigeon pox vaccine as 10², 10³ and 10⁴ EID₅₀/ml. Ten pigeons were vaccinated with each dose with keeping three pairs of pigeons as non-vaccinated controls. Challenge was applied by inoculation of all pigeons with virulent pigeon pox virus by feather follicle route, then the results were recorded.

13. Evaluation of the prepared pigeon pox vaccine

13.1. Sterility test:

The prepared vaccine was tested for freedom from any bacterial or fungal contamination, using thioglycolate broth, nutrient, blood and McConkey's agars.

13.2. Safety test

Ten susceptible pigeons were inoculated with the selected field dose containing 10³ EID₅₀/ml of the prepared vaccine according to Winterfield and Hitchner (1965); and another ten pigeons were inoculated with 10 times the field dose. Five pigeons were kept as a contact control and another five were kept as an isolated control group. Challenge was applied with virulent pigeon pox virus one month after inoculation and the results were recorded.

13.3 Potency test and duration of immunity

Immunization of 40 susceptible pigeons took place by inoculation of 0.025ml from the prepared vaccine using feather follicle (F.F.) route according to Woodward and Tudor (1973), in addition to 20 non-inoculated control pigeons. Challenge test was undergone by inoculation of a dose 103 EID50/ml of virulent pigeon pox virus in 8 vaccinated and 4 susceptible control pigeons at 1, 3, 5, 6 and 7 months post-vaccination, with observation of gross lesions and collection of serum samples from the birds.

14. Serological assays

14.1. Serum neutralization test (SNT)

It was applied according to Buscaglia *et al.* (1985), for detection of the antibody level in the serum of vaccinated and challenged pigeons.

14.2. Enzyme linked immunosorbent assay (ELISA)

It was carried out according to Buscaglia *et al.* (1985) for measuring the antibody level of tested pigeons.

RESULTS

1. Adaptation and titration of the Egyptian virulent pigeon pox virus on CAM

Virulent pigeon pox virus was passaged on the chorioallantoic membrane of fertile SPF eggs resulted in compact, proliferative pock lesions that may be focal or diffuse. The severity of the reaction, as well as the titre of these passages was recorded in Table 1.

Table 1. Adaptation and titration of pigeon pox virus on chorioallantoic membrane of embryonated chicken SPF eggs.

Passage No.	Reaction	Titre in log ₁₀ *
1	++++	6.7
3	++++	6.8
5	++++	6.8
7	+++	7.0
10	++	7.5
12	++	7.5
14	++	7.8
16	+++	8.3
18	+++	8.5
20	+++	8.5
22	+++	8.5

+: Pock lesions on the CAM of inoculated eggs.

*: The titre was expressed in EID50/ml.

2. Pathogenicity of the adapted pigeon pox virus in susceptible pigeons

Passages No. 5, 10, 15 and 18 of the pigeon pox virus were tested in susceptible pigeons. The characteristic lesions of the cutaneous form of pox in pigeons were clearly observed in the 5th passage, and decreased by the 10th passage, and completely disappeared in the 15th and 18th passage. These lesions appeared as local epithelial hyperplasia involving epidermis and feather follicles, with formation of nodules that first appeared as white foci, then, rapidly increased in size and became yellow in colour.

3. Detection of the field dose of the prepared pigeon pox vaccine

Different doses 102, 103 and 104 EID₅₀/ml from the passage of choice (18th passage) were prepared and inoculated (by FF route) into pigeons, in addition to susceptible pigeons. They were challenged with virulent pigeon pox virus and the results were recorded in Table 2.

Table 2. Post challenge reaction of vaccinated and control pigeons

Dose*	Bird status	Inoculated	Control
102		-ve	+ve
103		-ve	+ve
104		+ve	+ve

+ve: Positive local and generalized pigeon pox lesions.

±ve: Mild pock lesion.

-ve: No local or general lesions.

*: Doses were prepared as by EID₅₀/ml

4. Evaluation of the prepared pigeon pox vaccine

4.1. Sterility

The prepared vaccine proved to be free from any bacterial or fungal contaminants.

4.2. Safety

Challenging the immunity of the pigeons which were previously vaccinated either with the field dose or 10 field doses showed that the vaccine is completely safe. The pigeons showed mild post-vaccinal and post-challenge reaction, while, the contact control birds did not resist the challenge with virulent pigeon pox virus and showed typical pigeon pox symptoms after the challenge. These results were recorded in Table 3.

Table 3. Safety of pigeon pox vaccine after challenge with virulent pigeon pox virus.

Dose \ Bird status	Inoculated	Contact control	Isolated control
Field dose	-ve	+ve	+ve
10x field dose	-ve	+ve	+ve

+ve: Local and generalized lesions of pigeon pox virus after challenge with virulent pigeon pox virus.

-ve: No local or general lesions.

*: Dilutions were prepared as EID₅₀/ml

4.3. Potency test and duration of immunity

The results of vaccination of four susceptible pigeons and their challenge with two susceptible controls using virulent pigeon pox virus at 1, 3, 5, 6 and 7 months post-vaccination were stated in Table 4.

Table 4. Potency and duration of immunity of pigeon pox vaccine.

Bird status \ M.P.V.	Positive reaction to challenge				
	1	3	5	6	7
Vaccinated	0/8	0/8	0/8	1/8	1/8
Control	4/4	4/4	4/4	4/4	4/4
Protection %	100%	100%	100%	88%	88%

M.P.V: Month Post Vaccination.

5. Serological assays

The immunological response of pigeon vaccinated with the prepared pigeon pox vaccine and challenged with virulent pigeon pox virus, was estimated by serum neutralization test (SNT) and (ELISA), the results were presented in Table 5.

Table 5. Results of serum neutralization test (SNT) and (ELISA).

WPI \ Test	SNT	ELISA
0 *	0.2	0.1
1	0.7	0.3
2	0.8	0.6
3	1.4	1.1
4	1.9	1.4
5	2.3	1.9
6	2.0	1.7
7	1.7	1.6
8	1.8	1.6

WPI: Weeks Post Inoculation.

*: Before vaccination.

SNT: Mean serum neutralization test results expressed by neutralizing index (NI) was considered protective when it was ≥ 1.5 .

ELISA: Mean ELISA readings expressed by optical density (OD) was considered protective when it was ≥ 1.0 .

DISCUSSION

Pigeon pox disease is one of the most important pox infections affecting birds (Schnitzlein *et al.*, 1988). Pigeon pox is controlled by using attenuated vaccine, which, not only have a direct protective effect on the vaccinated pigeon flocks, but also an indirect effect after vaccination of laying hens during egg production, as well as its use during fowl pox outbreak (Schuermann, 1958 and Tripathy and Reed, 1997).

The production of a new pigeon pox vaccine from the virulent local isolate, in specific pathogen free (SPF) embryonated chicken eggs, was the aim of this study.

Beginning by the propagation and attenuation of the virulent pigeon pox virus, 22 serial passages were applied on the chorioallantoic (CAM) of SPF embryonated chicken eggs; the virus produced focal or diffused pock lesions on the CAM (Hedao *et al.*, 1985). The severity of the reaction on the CAM was recorded in Table 1. and showed that it began high with remarked increase by the 7th and 16th passages which was revealed to the attenuation of the virulent pigeon pox virus by serial passages. As well, from the same table, it can be also observed that the titres of the different passages were not parallel to the pock lesion severity between the 10th and 14th passages, which meant that, by passaging the virus, the lesions appeared more clearly on the CAM and the titre was increased after the 14th passage (Cunningham, 1973).

The pathogenicity of different passages (5, 10, 15 and 18) was studied in susceptible pigeons and the results proved that more the virus was passaged, the least pathogenicity obtained (Pandey and Mallick, 1975); thus, the 18th viral passage was considered the passage of choice as it possessed the best titre with the least pathogenicity.

The adapted virus was lyophilized with lactalbumin sucrose stabilizer and the field dose was detected by testing different doses of the prepared vaccine (102, 103 and 104), where, it was found that 103 EID₅₀/ml was the minimum concentration which established satisfactory immunity (Winterfield and Hitchner, 1965).

Evaluation of the prepared pigeon pox vaccine took place where the vaccine proved to be sterile and free from any bacterial or fungal contaminants and safe enough as showed in Table 3. where the vaccine did not produce severe lesions in vaccinated

pigeons either by the field dose or 10 times the field dose (Tripathy and Reed, 1997), and was not transmitted to the contact non-vaccinated pigeons.

The potency test indicated that the vaccinated pigeons by the prepared vaccine when challenged one, three, five, six and seven months later by virulent pigeon pox virus resisted the infection, while, the control non-vaccinated pigeons showed local and generalized pigeon pox lesions while Table 4. showed that the protection percentage began to decrease (88%) at the 6th month post-vaccination and remained up to 7th month (the duration of the study) (Winterfield and Hitchner, 1965 and Michael *et al.*, 1986). Further studies should be applied on the duration of immunity by the producing department, for precise detection of the suitable time for revaccination, to provoke complete immunity for pigeons all over the rearing period.

Monitoring the antibody level in the serum of tested pigeons during the period of the experiment was applied through serum neutralization test (SNT) and ELISA (Buscaglia *et al.*, 1985). By following the results expressed in Table 5. we can confirm that antibodies against pigeon pox vaccine began to appear from the 2nd week post-vaccination and became protective from the 3rd week and reached its peak after challenge (Morita, 1973).

The previously obtained results proved the preparation of a safe, sterile and potent pigeon pox vaccine prepared in SPF fertile eggs from the local Egyptian isolate. This vaccine can be used, not only for controlling pigeon pox disease in pigeons, but also in decreasing the losses during fowl pox outbreak, as well as vaccination of laying hens during the egg production season (Schuermann, 1958 and Tripathy and Reed, 1997). All these advantages could be considered as forward steps for improving poultry industry against avian pox diseases in Egypt.

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إنتاج لقاح جدري الحمام من العترة المصرية على بيض خالى من المسببات المرضية

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تم إضعاف العترة الضارية المحلية المصرية من فيروس جدري الحمام على بيض خالى من المسببات المرضية لإنتاج لقاح جديد ضد جدري الحمام. تم تمرير الفيروس الضارى لعدد اثنين وعشرين تمريرة على الغشاء الألتوى لبيض خالى من المسببات المرضية. تمت معايرة التمريرات ٥، ١٠، ١٥، ١٨، ٢٠، وسجلت ٦،٨١٠، ٧،٥٠، ٨،٥١٠، ٧،٨١٠، ٨،٥١٠، ٨،٥١٠، ٨،٥١٠ على التوالي. تم اختبار الأثر المرضى للتمريرات الخامسة، العاشرة، الخمسة عشرة والثامنة عشرة بحقتها فى حمام قابل للعدوى، ووجد أن التمريرتين ١٥، ١٨ كانتا أفضل من حيث رد الفعل بعد التحصين، ولكن تم اختبار التمريرة الثامنة عشرة لحصولها على أعلى عيارية (٨،٥١٠) من بين التمريرات المؤقلمة.

تم إجراء اختبارات الجودة للقاح من حيث النقاوة، الأمان والكفاءة، ووجد أنه نقي، آمن وأثبت اللقاح المحضر كفاعله بصدده مرض جدري الحمام عند الحقن بالفيروس الضارى. تم إجراء التجارب السيرولوجية للتأكدية (التعادل المصلى والايذا)، وسجلت هذه التجارب أن مستوى الأجسام المناعية كان واقياً (أكثر من ١،٥ للتعادل المصلى وأكثر من واحد للأليزا).