Antigenic Comparative Studies Between Vaccinal and Virulent Strains of Pigeon Pox Virus

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ABSTRACT

Local pigeon pox virus (PPV) from naturally infected pigeon was isolated and identified. The isolated virus was inoculated on CAM of SPF and it produced specific pock lesion of pox virus. Pathogenicity test was applied by inoculating the isolated virus in susceptible squabs and it produced typical pox lesions. The isolated virus was identified by applying neutralization test and agar gel precipitation test using specific antisera. Comparative polypeptide analysis was carried out on SDS-PAGE to identify the difference between the vaccinal strain (Hungarian) and the local field isolate. The polypeptide ranged from 194.2 and 20.0 kDa with total molecular weight (MW) of 1363.598 and 1183.83 respectively for the two strains and the total viral protein of the two strains were estimated by using spectrophotometer and the values were 0.1353 g/dl and 0.1701 g/dl for the vaccinal and the local isolate strain respectively.

INTRODUCTION

Pigeon pox is one of the Avipox viruses affecting pigeon and cause pigeon pox disease with considerable economic losses *(1)*. The disease is commonly characterized by development of nodular proliferative skin lesion on the unfeathered parts of the skin (cutaneous form) and/or formation of fibrinonecrotic proliferative lesions on the mucous membrane of upper respiratory tract (diphtheritic form) *(2)*.

In Egypt, Hungarian vaccinal strain of pigeon pox is used for protection of pigeon against the disease. So, the aim of this study is to identify the differences between the vaccinal and the local isolate (virulent strain) of pigeon pox virus.

MATERIAL AND METHODS

Virus

Vaccinal strain of PPV

Egg adapted Hungarian strain of PPV (vaccinal strain) was obtained from Pox Dept., Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo in freezed-dried form. Its titre was $10^{6.5}$ EID_{50}/ml.

Local isolate (Virulent strain) of PPV

Virulent PPV isolated from locally infected pigeons (private owners) was identified according to *(3)*.

Embryonated chicken eggs (ECE)

Eleven, day-old, specific pathogen free (SPF) embryonated chicken eggs (ECE), were used for isolation, propagation and titration of the locally isolated virulent strain of PPV and for activation and titration of the vaccinal strain *(4)*.

Birds

Twenty squabs, 6 weeks old, susceptible for pigeon pox were used for inoculation and confirmation of the field isolated virus. They were divided into 2 groups each of 10 squabs. The 1st group was inoculated with the isolated virulent PPV. The 2nd group was kept as non-inoculated controls.

The squabs were inoculated with the PPV by feather follicle method and kept under observation for two weeks.

Samples

Skin samples

Skin nodules or scabs were taken from 10 clinically infected pigeons for virus isolation Isolation was carried out according to th previously described techniques *(3)*.

Chorioallantoic membrane (CAM)

ECE were inoculated with PPV strains and incubated at 37°C 5 days. CAMs with pock lesions as well as CAMs from non-inoculated control were collected and used for SDS-PAGE.

Hyperimmune serum against PPV

It was obtained from Pox Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. It was used for identification of the local isolate.

Virus isolation in ECE

Specific pathogen free ECE (SPF ECE) were inoculated with the supernatant fluid of the minced lesions as 0.2 ml/egg on the CAM *(4, 5)*, for the detection of specific pock lesions and incubated at 37°C for 5-7 days. In addition to 5 non-inoculated control eggs.

Identification of the field isolated virus

Neutralization test

A mixture of isolate and specific immune serum was incubated at 37°C for one hour and inoculated on CAM of SPF ECE and incubated for 7 days at 37°C *(6)*.

Agar gel precipitation test

Agar gel precipitation test (AGPT) was applied according to the previously described method *(7)*. Examination of the agar after was carried out 24, 48 and 72 hours for the detection of the precipitation.

Propagation of the PPV in ECE

Eleven-day-old ECE were inoculated with PPV (both strains) on CAM. CAMs were collected after 5 days and used for further passages and titration.

Titration of the PPV in ECE

Specific pathogen free ECE (SPF-ECEs) were used for titration of the isolated PPV *(4)* and results were calculated *(8)*.

Total protein quantitation by spectrophotometer

Protein estimation was applied for both the vaccinal and the field isolated PPV strains according to *(9)* by using kits of Biocon Company, Germany.

SDS-PAGE

SDS - SAGE was applied on the two strains of the PPV according to *(10 , 11)*. The viral protein was boiled for 5 minutes with lysis buffer and loaded in 10% polyacrylamide gel with the pre-stained standard protein marker. The gel was run and results were recorded using computer programs through scanner. The molecular weight (MW) of the protein bands were estimated by comparing their electrophoretic mobilities with those of known standard M.W. marker after electrophoresis in the same gel following staining by Coomassie blue stain.

RESULTS

Isolation of PPV

Clear large white pock lesions with thickening of the CAM were observed at the 5^{th} day post inoculation after five successive passages in ECE.

Identification of the propagated virus

Inoculation of squabs

Inoculation of squabs with the virulent isolated PPV showed characteristic lesions of PP disease.

Neutralization test

No pock lesion appear after inoculation of the mixed antigen with the hyperimmune serum of PPV.

Agar gel precipitation test

Positive known serum was used against isolated virus in AGPT showed clear white precipitation line between this known serum and the isolated virus.

Sterility

The isolated virulent PPV was tested for sterility and it was free from fungal, bacterial, viral and mycoplasmal contamination.

Titration of the isolated PPV

The titre of isolated virulent PPV on SPF-ECE was 10^5 EID_{50}/ml.

Total protein estimation

The percent of total protein of the vaccinal strain of PPV and the local isolate (virulent strain) were 72.06 and 91.27 respectively. Results were recorded in Table 1. The estimated quantities of protein for the PPV vaccinal and isolated strains two strains using spectrophotometer were 0.1353 g/dl and 0.1701 g/dl respectively.

Table 1. Percentage (%) of protein in band of PPV vaccinal strain and PPV virulent strain

Rows	Lanes			
	Lane (1)	Lane (2)	Lane (3)	Marker
	Amount	Amount	Amount	Amount
r1	8.42	9.67	5.81	-
r2	-	6.05	-	-
r3	7.15	5.21	-	11.0
r4	3.15	3.81	2.19	6.39
r5	3.25	3.43	2.55	-
r6	2.62	2.88	1.82	-
r7	2.73	2.21	-	-
r8	5.55	5.23	3.9	4.54
r9	3.12	5.30	-	-
r10	3.01	2.60	-	-
r11	3.65	3.77	-	8.53
r12	4.64	4.28	-	-
r13	3.99	2.75	3.64	-
r14	3.51	3.55	-	-
r15	4.06	2.68	3.35	-
r16	2.47	2.02	-	-
r17	-	2.10	-	-
r18	5.09	3.25	-	6.96
r19	2.65	2.72	-	-
r20	-	2.39	-	-
r21	-	5.24	-	-
r22	3.00	3.37	-	-
r23	-	3.55	-	-
r24	-	3.21	-	3.54
Sum	72.06	91.27	23.26	40.96

Lane (1): Local isolate (virulent strain of PPV)
Lane (2): Vaccinal strain of PPV
Lane (3): Control non-vaccinated SPF eggs

SDS-PAGE analysis

The electrophotometric characterization of PPV strains by SDS-PAGE revealed that 24 and 18 polypeptides were detected after coomasie blue staining for the vaccinal strain and isolated virulent PPV respectively (Table 2 and Fig. 1). The M.W. of polypeptides ranged between 194.2 and 20.00 kDa with total M.W. of 1183.83 and 1363.598 respectively for the two viruses.

Table 2. The molecular weight of pigeon pox vaccine and PPV virulent strain

Rows	Lanes			
	Lane (1)	Lane (2)	Lane (3)	Marker
	M.Wt.	M.Wt.	M.Wt.	M.Wt.
r1	193.50	194.2	159.43	-
r2	-	163.5	-	-
r3	173.5	93.79	-	116
r4	81.60	85.707	80.60	97
r5	76.467	80.573	73.467	-
r6	73.214	78.521	72.321	-
r7	71.308	75.79	-	-
r8	63.977	61.754	62.541	66.2
r9	57.308	58.79	-	-
r10	55.314	56.23	-	-
r11	41.824	43.969	-	37.3
r12	37.193	36.124	-	-
r13	34.841	34.754	37.193	-
r14	32.253	33.238	-	-
r15	30.151	31.848	32.49	-
r16	30.120	31.10	-	-
r17	-	30.23	-	-
r18	28.00	28.00	-	28.0
r19	27.58	27.49	-	-
r20	26.21	26.32	-	-
r21	25.32	25.41	-	-
r22	24.15	24.11	-	-
r23	-	22.15	-	-
r24	-	20.00	-	18.4
Sum	1183.83	1363.598	518.042	362.9

Mol. W.: Molecular weight (kDa)
Lane (1): Local isolate (virulent strain of PPV)
Lane (2): Vaccinal strain of PPV
Lane (3): Control non-vaccinated SPF eggs

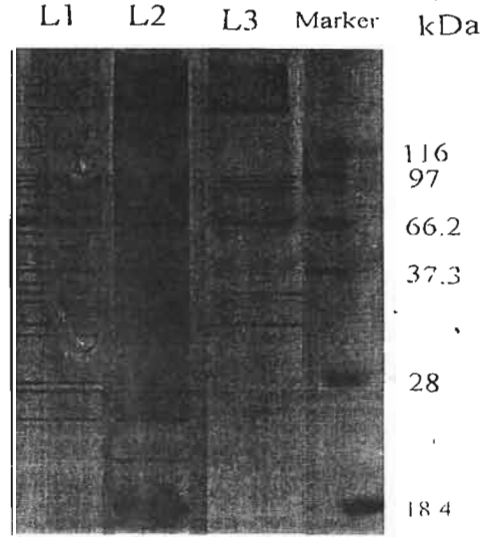

Fig. 1. Electrophoretic analysis of pigeon pox virus structural polypeptides
Lane (1): PPV local isolate (virulent strain) of PPV
Lane (2): Vaccinal strain of PPV
Lane (3): Control non-vaccinated SPF eggs

DISCUSSION

Pigeon pox virus infection is one of the most important pox infections. The vaccinated pigeons showed only takes post vaccination without clinical symptoms of the disease while the pigeons inoculated with the virulent strain showed atypical pigeon pox disease. By applying serum neutralization test to identify the local isolate using hyperimmune serum against pigeon pox no pock lesion appeared in mixed local isolate with hyperimmune serum of pigeon pox but in the inoculated eggs with the isolate only there were pock lesion appear, this results is in agreement with previously described investigation ***(12)***. White precipitate line appear when apply agar gel precipitation, this result this result consistent with that cited by ***Luthgen (13)***.

The literature concerning the pigeon pox protein profile seems to be scare, and unavailable so we used the literature concerning the fowl pox to compare the results of the two strains of pigeon pox with fowl pox as the most related poultry protein.

Results of total protein using spectrophotometer was 0.1353 g/dl for vaccinal strain and its percent using SDS-PAGE was 72.06 while for the local isolate strain the protein quantitative was 0.1701 g/dl, and its percent was 91.27 (Table 11). These results showed variation in the quantity of protein and its profile between the two strains.

The electrophoretic analysis of vaccinal and virulent pigeon pox strain polypeptides was subjected to 10% polyacrylamide gel. By this concentration of gel 20 and 24 polypeptide bands were appeared after coomassie blue staining for vaccinal and virulent pigeon pox strains respectively. These results are in agreement with ***Obijeski et al. (14)*** who used 12% gel concentration and showed a minimum 14 polypeptides for FPV. Also a similar finding identified 21 FPV polypeptides produce in response to FPV infection ***(15)***.

These results are nearly similar to the results carried out by several investigators of ***(5, 16)*** who identified up to 28.31 FPV specific polypeptides of which only few were described as major polypeptides. From this study, we concluded that the total protein quantitation and protein analysis using spectrophotometer and SDS-PAGE showed some variation protein profile f PPV vaccinal and PPV virulent strains. Also, this study proved that the little variation may be due to some differences in protein sequence.

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الملخص العربى

دراسة المقارنة الأنتيجينية بين عترة اللقاح والعترة الضارية لفيروس جدرى الحمام

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قد تم عزل وتعريف فيروس جدرى الحمام المحلى من حمام مصاب طبيعياً وتم حقن الفيروس المعزول على الأغشية اللفائفية فى بيض خالى من المسببات المرضية. ووجد أنه أحدث إصابات جدرية. وقد تم حقن الفيروس المعزول فى زغاليل قابلة للعدوى ونتج عن ذلك ظهور أعراض مرض جدرى الحمام.

الفيروس المعزول تم تعريفه بواسطة اختبار التعادل واختبار الآجار جيل الترسيبى بواسطة استعمال السيرم المرجعى الخاص بالفيروس. كما تم دراسة تحليل السلاسل الأمينية بواسطة **SDS-PAGE** لمعرفة الاختلاف بين عترة اللقاح (هنجرين) والعترة المعزولة محلياً. وجد أن السلاسل الأمينية تتراوح بين ١٩٤,٢ الى ٢٠,٠ كيلو دالتون وكان الوزن الجزيئى الكلى ١٣٦٣,٥٩٨، ١١٨٣,٨٣ على التوالى للعترتين وكان كمية البروتين للفيروس الكلى من العترتين عند قياسه بالتحليل الضوئى ٠,١٣٥٣، ٠,١٧٠١ جرام لكل ١٠٠ مللى على التوالى.