Trials For Attenuation Of Pigeon Paramyxovirus-1 In Mice

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ABSTRACT

A preliminary attempt for the possibility of attenuating a local isolate of pigeon paramyxovirus-1 (PPMV-1) was investigated by passaging in weanling mice, aiming for using it as live immunizing agent. After five passages by intracerebral (I/C) route, the virus showed significant reduction of its virulence for SPF chicken embryos and susceptible pigeons as judged by mean death time (MDT), intravenous, and intracerebral pathogenicity index (IVPI and ICPI). Diminished pathogenicity was observed without losing its immunogenicity as proved by developing antibody after 21 days. Titre in inoculated pigeons reached 6.8 $\log_2$ and 6.5 $\log_2$ on the fourth and the fifth passages respectively. Such titer level was satisfactory enough to resist inoculation with the virulent strain. Achieved results were highly promising as successful attempt for encouraging further investigation aimed to prepare alive attenuated immunogenic, low cost and effective (PPMV-1) vaccine.

INTRODUCTION

Pigeon paramyxovirus-1 (PPMV-1) is one of the viruses within the genus paramyxovirus which includes many viruses infecting avian species including pigeons *(1)* and causing great losses *(2)*.

PPMV-1 type isolate comprises a unique subset of avian paramyxovirus. They included in a single group based on monoclonal antibody (MAb) binding and frequently have biological properties that overlap the classical NDV pathotypes *(3)*.

Many Egyptian research workers could frequently isolate avian paramyxovirus serotype-1 from field disease problems showing nervous manifestations among pigeons *(4)*. Despite Veterinary Serum and Vaccine Research Institute **(VSVRI)** had produced an effective and potent inactivated pigeon paramyxovirus-1 vaccine using different inactivators and adjuvants *(5)*. However for mixmum protection priming of pigeon by an attenuated PPMV-1 vaccine should be highly recommended. So the present study aimed to take the first step in attempting the attenuation of the local strain of PPMV-1 via several passages in weanling mice for the purpose of producing a local live attenuated safe vaccine

MATERIAL AND METHODS

Laboratory hosts

Pigeons

Fifty squabs of 3-4 weeks old of native breeds were purchased from local market. After rearing in isolated cages for one week and checked for absence of materanal antibodies against PPMV-1. Blood samples were obtained from wing vein and serum was separated. Such birds were used for experimental inoculation and serological tests.

Chickens

Ten, one day old, SPF chicks were obtained from SPF Farm, Koum Osheim, Fayoum, Egypt. They were fed on a balanced ration. These birds were used for determination of ICPI of local isolate and another Ten chickens 6 weeks old were used for IVPI determination.

Mice

Seventy, 14-16 day old,(25-28 gm weight) mice were obtained from the Laboratory Animal Department, **(VSVRI)** Abbasia, Cairo. They were used for virus attenuation experiments.

Fertile chicken eggs

SPF embryonated chicken eggs were obtained from SPF Farm, Koum Osheim, Fayoum, Egypt and used for virus propagation and titration.

Virus

Pigeon Paramyxovirus-1

The virus strain was kindly provided by Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo locally isolated from diseased pigeons. Its EID_{50} was $10^{7.5}$/ml when titrated in chicken eggs with MDT of MLD 56 hours.

The virus was identified by the Central Laboratory for Evaluation of Veterinary Biologics, Abbasia, Cairo. And used for viras attenuation in weanling mise.

Methods

Virus Titration and MDT in chicken embryos

Virus titration and MDT in chicken embryos was carried out *(2).*

Rapid Haemagglutination (HA) test

Standard quantitative plate method and rapid slide method was performed *(2).*

Determination of the intracerebral (IC) and intravenous (IV) pathogenicity index

It was carried out as previously described *(2)* the observation period was 10 days.

Intracerebrally serial passage through weanling mice

Five serial passages of the stock PPMV-1 virus were carried out intracerebrally through weanling mice each were inoculated intracerebrally with 10^{-2} of infective material using 0.03 ml/mouse and observed for 10 days PI. Mice which developed nervous signs during the observation period were sacrificed and their brains collected aseptically and kept at -20°C until further processing. Infective amnio allantoic fluid (AAF) of the stock virus diluted in sterile saline to contain approximately $10^{7.0}$ EID_{50}/ml used as inoculum for the 1st mouse passage. For subsequent passages, a 1:20 brain suspension in sterile saline containing penicillin and streptomycine antibiotic mixture was used after being allowed to stand for 30 minutes at room temperature. The brain suspension of the mice passages were inoculated into 9-11 embryonated chicken eggs each by the allantoic route for virus propagation. (AAF) harvested from embryo which died 3-5 days PI were used for virus titration in embryonated chicken eggs and assessment of MDT as well as for estimation of the IMPI in pigeons *(7).*

Pigeons inoculation

Ten susceptible squabs aged 3-4 weeks were used per determination. The virus in the form of allanto-amniotic fluid was diluted 1:10 in sterile saline antibiotic mixture (200µgm penicillin and 200µgm streptomycin per ml). The sample was injected IM 0.25 ml per bird. The birds were observed for signs and death for 3 weeks post inoculation.

Pigeon inoculated with the 5th mouse passage that remained normal for 3 weeks PI were tested for HI antibodies and then IM challenged. with 0.2ml 10^6 EID_{50}/bird of the local isolate. A group of 5 susceptible pigeons served non vaccinated.

Samples for virus reisolation

Internal organs (spleen, liver, brain) of the dead challenged pigeons were collected under complete aseptic conditions in screw capped bottles containing PBS pH 7.5 with antibiotics and kept at – 70°C till used for virus reisolation.

Histopathological examination

For histopathological studies, brain samples were collected from inoculated and non-inoculated control mice with pigeon paramyxovirus-1 such, Samples were fixed in 10% neutral buffer formalin, embedded in paraffin sectioned and finally stained with haematoxylin and eosin by standard technique *(8).*

RESULTS

Table 1. ICPI, IVPI and MDT for the local isolate of PPMV-1

Recorded Data				Pathotype
EID50/ml	MDT	ICPI	IVPI	
$10^{7.5}$	56 hours	1.5	1.6	Velogenic

EID_{50}: 50 % infective dose
MDT: Mean death time for minimal lethal dose
ICPI: Intracerebral Pathogenicity Index.
IVPI: Intravenous Pathogenicity Index.

Table 2. Clinical squeal of PPMV-1 after intracerebral passage through mice (n= 10)

No. of Passages	Pathogenicity for mice				
	Sick / Inoculated*	Onset of symptoms per day	Symptom s degree	Days post Inoculation / Deaths	Dead / Inoculated
Original	10/10	3 DPI	++++	5	10/10
1	10/10	3 DPI	+++	5	10/10
2	8/10	5 DPI	++	7	8/10
3	6/10	7 DPI	+	10	5/10
4	0/10	-	-	-	0/10
5	0/10	-	-	-	0/10
Control negative	0/10	-	-	-	-

* Symptoms: (Rough coat, paralysis in lower limb, off-food)
DPI: Days Post Inoculation
N.B.: Observation period for mice 10 days

Table 3. Pathogenicity of PPMV-1 in mice chicken embryo

Mouse passage	Pathogenicity for mice		Pathogenicity for chicken embryo	
	Sick/Infected	Dead/Infected	MDT hours	EID_{50}/ml
Original	10/10	10/10	56	7.5
1	10/10	8/10	60	7.0
2	8/10	8/10	73	6.8
3	5/10	2/10	80	-
4	2/10	0/10	90	-
5	0/10	0/10	90	6.3
Control	0/10	0/10	90	6.3

Table 4. result of the pathogenicity testing of PPMV-1 virus to pigeon

Passages	Pathogenicity for pigeons				
	No. of inoculated pigeons	On set of symptoms	Sick/Inoculated	Dead/Inoculated	No. of survivors 21 DPI
Original	5	1	5/5	5/5	0
1	5	3	4/5	5/5	0
2	5	3	4/5	5/5	0
3	5	10	2/5	2/5	3
4	5	-	1/5	0/5	5
5	5	-	0/5	0/5	5

Table 5. the pathogenicity of mice passages PPMV-1 virus for susceptible pigeons

Passage	No. of birds inoculated	Symptoms at the following days post infection											
		1	2	3	4	5	6	7	8	9	10	15	21
Original	5	N	N	S	S	S	S	S	D	D	D	-	-
1	5	N	N	S	S	S	S	D	-	-	-	-	-
2	5	N	N	N	N	N	S	S	S	S	D	-	-
3	5	N	N	N	→	→	→	→	→	→	S	D	-
4	5	N	N	→	→	→	→	→	→	→	→	→	→
5	5	N	N	→	→	→	→	→	→	→	→	→	→
Non-inoculated control	5	N	→	→	→	→	→	→	→	→	→	→	→

Dose and route: 0.25ml/bird intramuscularly
N: Normal
S: Symptoms (paralysis, dropping wings, tremor)
D: Dead

Table 6. Results of Seroconversion and virus reisolation of inoculated pigeons

Passage	Mean log_2 HI titre / DPI			Virus reisolation		
	7	15	21	Brain	Spleen	Liver
Original	7	-	-	+ve	+ve	+ve
1	7	-	-	+ve	+ve	+ve
2	6.0	-	-	+ve	+ve	+ve
3	6.0	7.0	-	+ve	+ve	-ve
4	5.8	6.3	6.8	-ve	-ve	-ve
5	5.5	6.2	6.5	-ve	-ve	-ve
Control	-	-	-	-	-	-

DPI: Days Post Inoculation

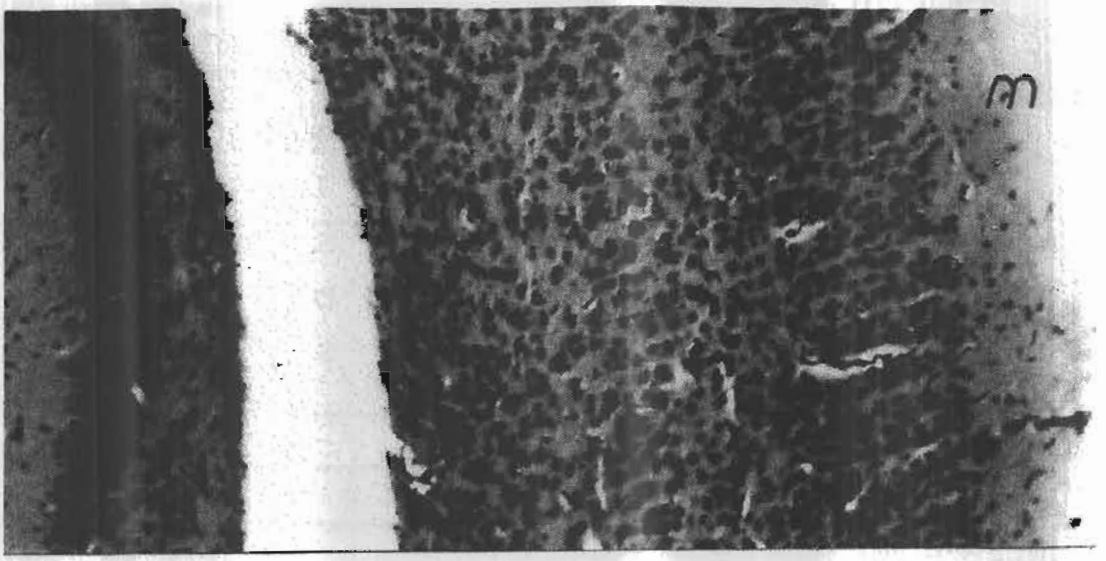

Fig. 1. Photomicrogaph of mice brain showing normal histopathological structure of the meninges (m), cerebral cortex (c) and hippocampus (h) (H&E,X40)

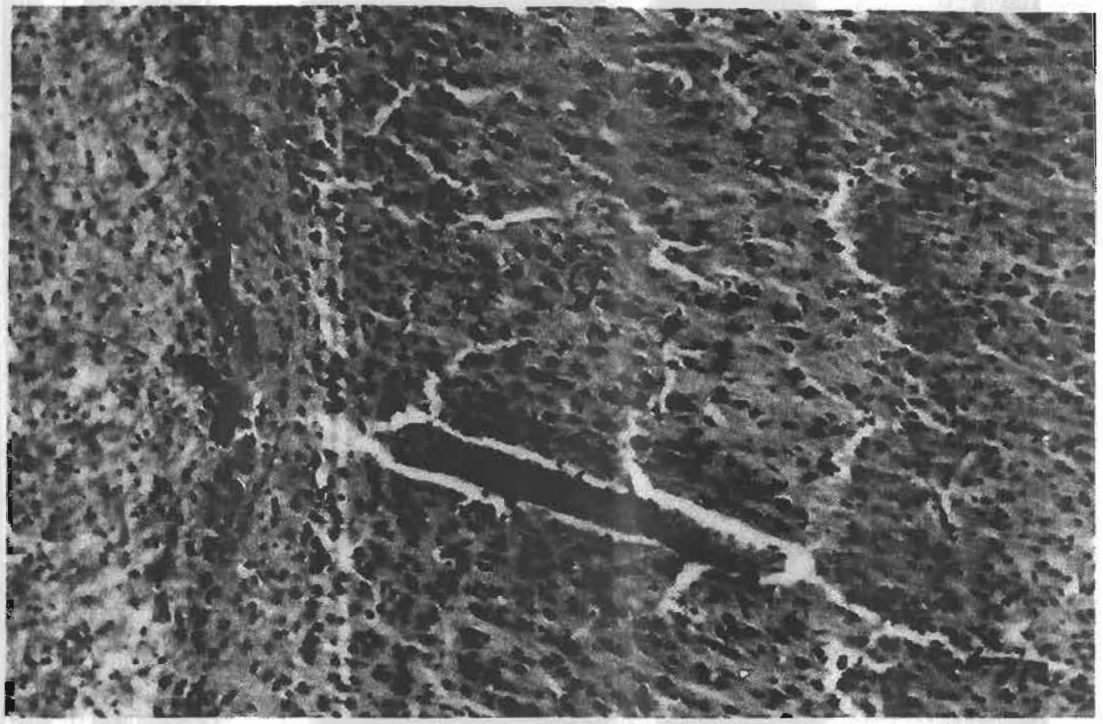

Fig. 2. Photomicrogaph of mice brain weanling laboratory mouse which I/C inoculated with PPMV-1 for first passage showing congestion in the cerebrum (v) with diffuse gliosis(g) (H & E, X40)

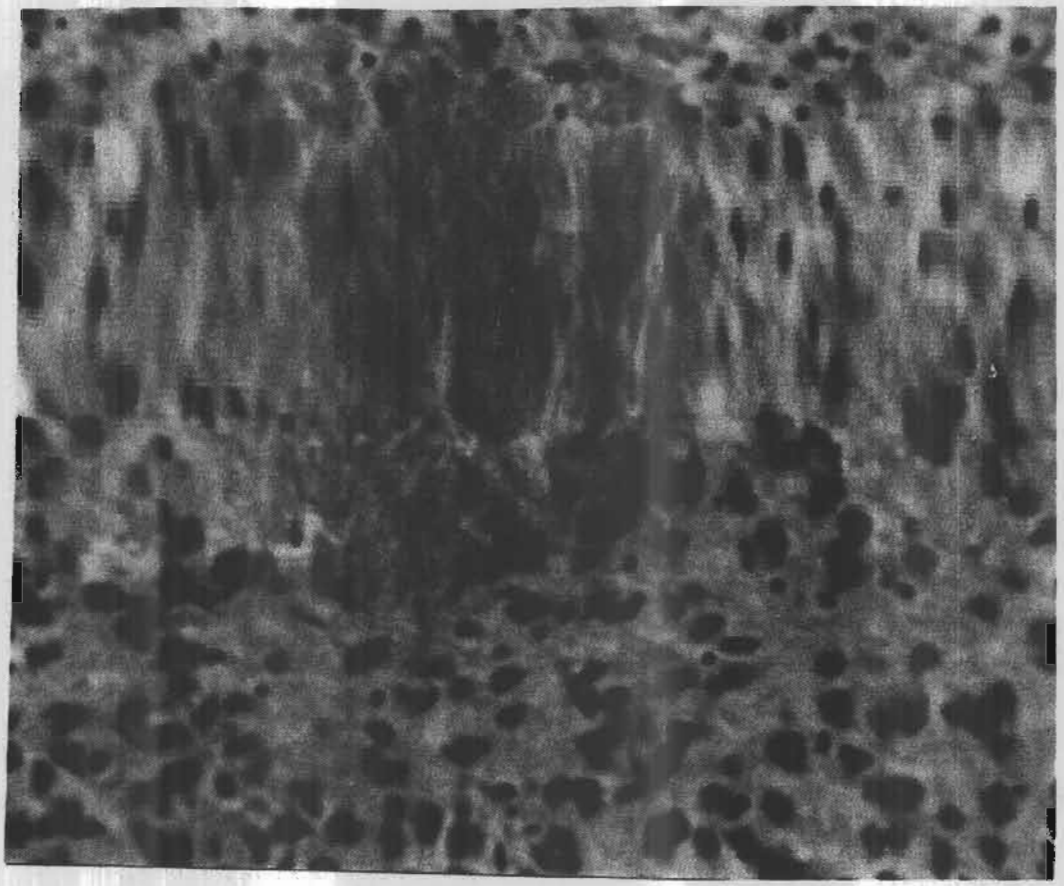

Fig. 3. Photomicrogaph of mice brain which I/C inoculated with PPMV-1 for third passage showing focal haemorrhagic in stratium (h) (H & E, X40)

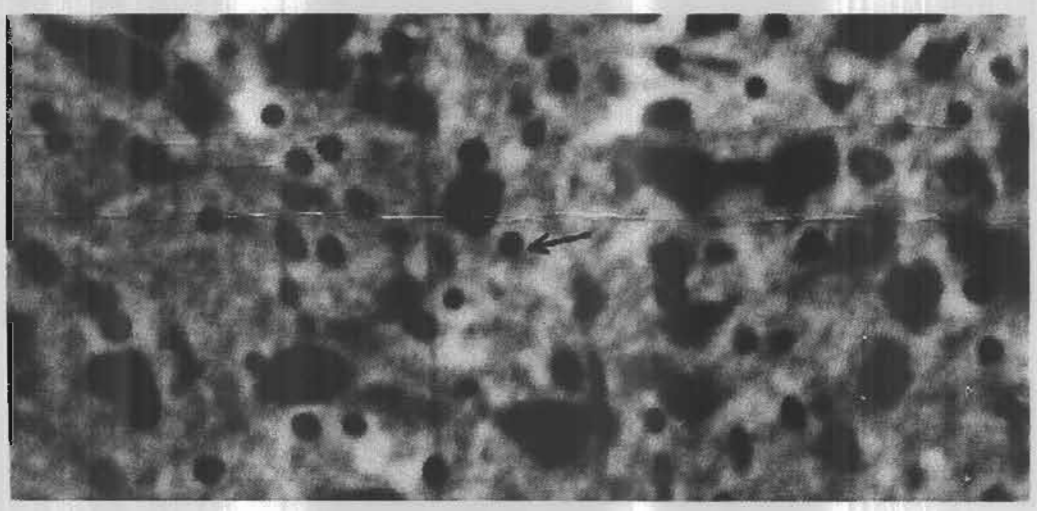

Fig. 4. Photomicrogaph of mice brain which I/C inoculated with PPMV-1 for fourth passage showing diffuse gliosis in medulla oblongata (arrow) (H & E, X40)

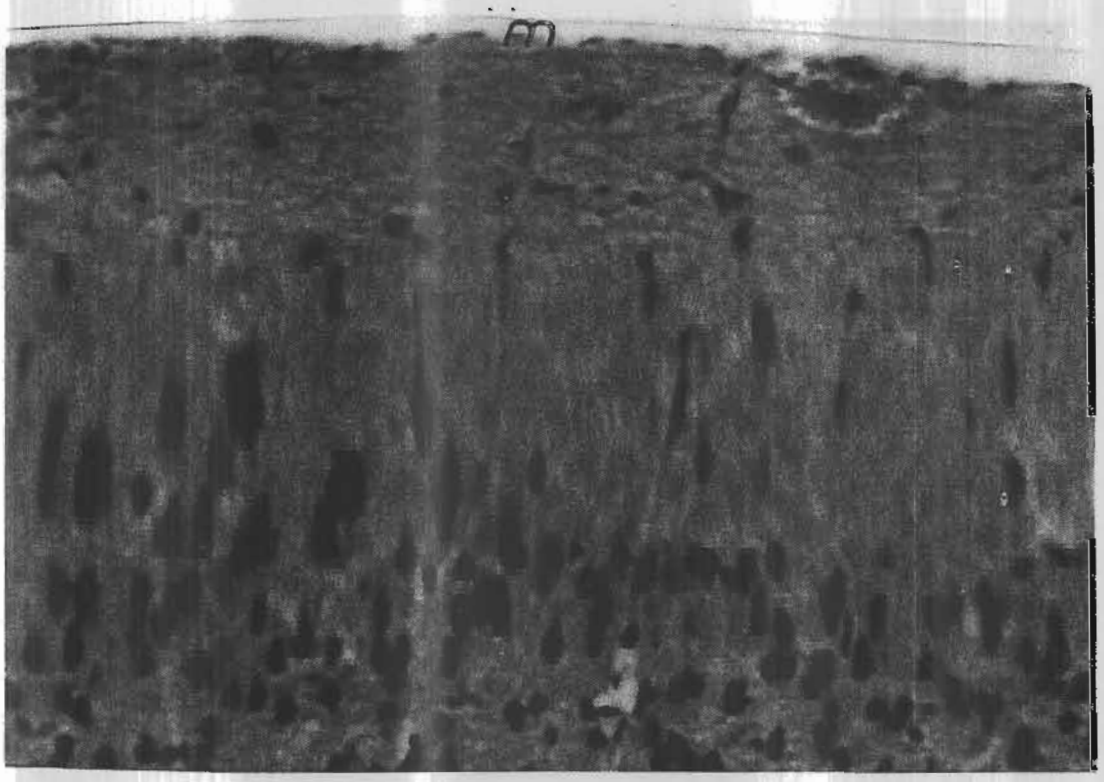

Fig. 5. Photomicrogaph of mice brain which I/C inoculated with PPMV-1 for fifth passage showing congestion in the menings (m) &(v) (H & E, X40)

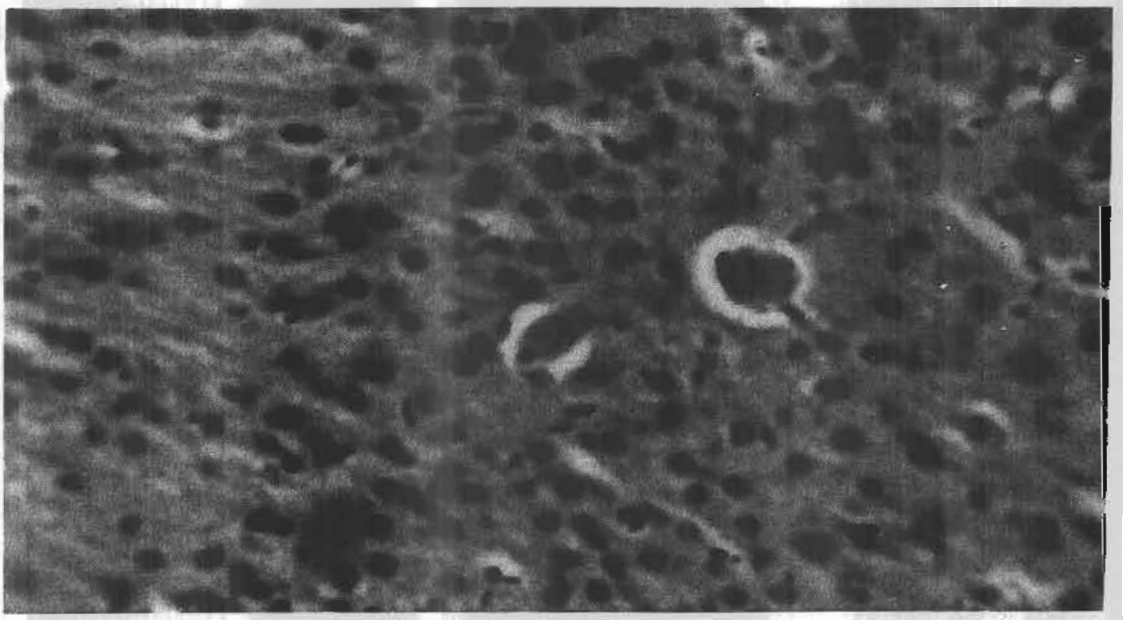

Fig. 6. Photomicrogaph of mice brain which I/C inoculated with PPMV-1 for fifth passage showing congestion in blood capillaries of the cerebrum(v)

DISCUSSION

Pigeon PMV-1 type isolate comprises a unique subset of avian PMV-1. They are included in a single group based on monoclonal antibody (MAb) binding and frequently have biological properties that overlap the classical NDV pathotypes *(3)*. In Egypt, PPMV-1 had been frequently isolated from pigeons lofts suffering from an outbreaks characterized by neverous symptoms and high mortality *(9,10)*

An inactivated locally prepared vaccine had been produced successfully by VSVRI, Abbasia, Cairo since 2002. Despite the performance of such vaccine under field condition is highly accepted however its usage is labor and time consuming and can not lie recommended for emergency vaccination in contrast to the live attenuated vaccine, Moreover the advantage of using live attenuated vaccines is to establish a priming infection in the flock for preparing the immune system to respond rapidly and effectively to boostering by inactivated vaccine. It is worth to state also that live attenuated vaccine can be easily administered via the drinking water, and its production and usage is cost effective *(11,12)*

The first attempt for attenuation of paramyxovirus-1 for using as a live immunizing vaccine have been initiated *(5)* as early as 2001 using two types of cell culture (primary and cell line), both system used resulting in reduction of virulent of the PPMV-1 however unsatisfactory immune response were achieved when inoculated into susceptible pigeons. So in this trial, weanling mice aged 14-16 days were used for attenuation of PPMV-1 isolate. For further Pathotyping of the local isolate as shown in Table 1 it is confermed that The isolate was velogenic strain its MDT was 56 hours , ICPI was 1.5 and its IVPI was 1.6, Table 2 showed that the onset of symptoms in mice was three days in the 1st passage and increased to 5&7 days post in oculation in the second and the third passage respectively while the forth and fifth passage showed no deaths or symptoms had been observed in mice.

Such results are generally agree with successful passage of other avian viruses in mice *(13)*.

Such virus begin to loss its virulence by the 3rd passage and gradually by the 5th passage the inoculated mice revealed none of the clinical symptoms and/ or deaths during the observation period of 10 days post inoculation. Egg inoculation by mice passaged virus as shown in Table 3 was parallel with pathogenisity in mice revealed a reduction in EID_{50} of the virus by 1.2 log_{10} from the 1st passage ($10^{7.5}$) compared to ($10^{6.3}$) at the 5th passage while the MDT slightly extended from 56 to 90 hour after 5th passage. Different passage of local PPMV-1 virus was lethal in a significant descending order for susceptible pigeons starting from 2nd passage till the 5th passage which was completely non lethal up to post inoculation *(7)*.

Table 6 Showed the seroconversion responses and efficiency of the selected passages 3rd , 4th and 5th estimated by haemagglutination inhibition test of the inoculated survival birds which recorded a titer of 7 log_2 , 6.2 log_2 and 6.3 log_2 respectively as determined post inoculation. While, pigeons inoculated with the 4th and 5th passages recorded 6.8 log_2 and 6.5 log_2 respectively three weeks post inoculation. such achieved titers were satisfactory protective *(14)*.

The PPMV-1 virus could be reisolated at a high rate from the internal organs (brain, spleen and liver) of pigeons inoculated by the 1st three passaged virus. While passage 4 & 5 showed no virus reisolation by the 5th passaged virus great margin of safety upon usage as a pigeon vaccine. The histopathological investigation of mice brain *(15)* as in Figs. 3 -5 showed that congestion in the blood vessels with diffuse gliosis were detected in the cerebrum , while the striatum showed focal haemorrhage . The medulla oblongata showed also diffuse gliosis. As compared with Fig. 6 of brain of mice after fifth passage that shows congestion in the blood vessels of the meninges, as well as in the blood capillaries of the striatum of cerebrum which is matched with Figs. 1,2 of the normal control mice brain. The above mentioned results confirm that the fifth mice pessage of PPMV-1

was safe and immunogenic agent for preparation of live attenuated vaccine and the capability of the 5th mice passage of PPMV-1 had been confirmed as immunogenic agent after further investigation.

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الملخص العربى

محاولات لتضعيف فيروس روشة الحمام فى فئران التجارب

إيمان احمد حسن على ، نادية محمد إبراهيم ، صفوت كمال روفائيل
معهد بحوث الأمصال واللقاحات البيطرية - العباسية – القاهرة

أجريت محاولات في تلك الدراسة بهدف أمكان استضعاف معزولة محلية من فيروس البارامكسوا - 1 باستخدام الفئران الرضع كنموذج اقتصادى متاح . حيث تم حقن فئران التجارب في المخ بخمس تمريرات متتالية ثم تم حقن كل من تلك التمريرات الفيروسية في البيض الخالى من المسببات المرضية وحقنة فيما بعد في الحمام القابل للعدوى وقد أظهرت النتائج نجاح الأنحسار التدريجى في ضراوة الفيروس بواسطة اختبارات ال (MDT, IVPI) وبواسطة التغيرات الهيستوباثولوجية المختلفة وعند حقن الحمام بالتمريرات المختلفة وقياس رد الفعل المناعى يواسطه اختبار قوه منع التلازن الدموى أظهرت النتائج مستويات مناعية بلغت $2^{6.3}$ ، $2^{6.5}$ بالنسبة للتمريرات الرابعة والخامسة على التوالى وعلية فأن النتائج التى توصلت اليها تلك الدراسة تعتبر مشجعة لمزيد من الدراسة التفصيلية بهدف تحقيق الهدف المنشود بانتاج لقاح حى مستضعف وآمن ضد باراميكسوا الحمام ليساهم مع اللقاح الميت المحلى في منظومة التحكم الكامل في الأصابه بهذا المرض الخطير