STUDIES ON PROTECTION OF PIGEON SQUABS AGAINST INFECTION WITH PIGEON PARAMYXOVIRUS-1 USING DIFFERENT STRAINS OF NEWCASTLE DISEASE VACCINE IN THE PERIOD BEFORE THE AGE OF VACCINATION WITH PMV-1 VACCINE

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Abstract

Trial was carried out for primary vaccination of pigeon with different live Newcastle disease virus (NDV) vaccines at two weeks of age before and after boosting with inactivated pigeon paramyxovirus-1 (PPMV-1) at four weeks. Different strains of NDV live vaccines were used, including HB1 vaccine in drinking water, intraocular instillation of LaSota vaccine and intramuscular injection of Komarov vaccine. For comparison, a single dose of inactivated PPMV-1 vaccine was given at four weeks age without live NDV vaccine priming. The immune response of vaccinated birds was evaluated serologically using the haemagglutination inhibition test (HIT), as well as by challenge test. The obtained results revealed that, comparatively, better immune response can be achieved in pigeons primed with LaSota or Komarov vaccine before and after boosting with inactivated PPMV-1 vaccine. Than priming with HB1 vaccine. It was concluded that priming of pigeon squabs intraocularly with live LaSota vaccine at two weeks of age is recommendable for the practice.

INTRODUCTION

Pigeons are known to be susceptible to infection with avian paramyxovirus serotype-1 (A/PMV-1) which includes Newcastle disease virus (NDV) (Vindevogel and Duchatel, 1993).

Many Egyptian research workers could isolate avian paramyxovirus serotype-1 from disease problems of pigeons (Shakal, 1989 and Abou Hashem, 1993) other research workers prepared pigeon paramyxovirus-1 (PPMV-1) vaccine from local isolates which can be used safely for pigeon vaccination at an age of 4 weeks producing an immune response lasting for 4-5 months. At least two doses were used to achieve better and lasting immunity than a single dose (Hassan, 1997).

Weisman *et al.* (1984) and Tangredi (1985) found that young pigeons are more susceptible to PPMV-1 infection than older ones. They reported that the mortality was 100% in young pigeons, whereas, adult had much lower mortality and morbidity rates.

Alexander and Parsons (1984) reported that the economic importance of this disease is manifested by the high mortality and morbidity among young pigeons which reach up to 80%:100%. Since many years ago, research work was conducted to control infection of pigeons with pigeon paramyxovirus-1 by vaccination with NDV vaccines, but results were not always satisfactory (Duchatel *et al.,* 1986 and El-Zanaty *et al.,* 1992).
In this study, trial was made to investigate early primary vaccination of squabs at two weeks of age with different strains of NDV live vaccines in order to achieve the best immunological response before the age of vaccination with inactivated PPMV-1.

MATERIALS AND METHODS

1. Birds

Native squabs of about 2 weeks of age were purchased from the local markets. Serum samples were collected after being housed in isolated cages and screened for the absence of antibodies against PPMV-1. The birds were used for experimental vaccination, challenge and serological tests and were fed on ground grains, then, the ordinary whole grains used for pigeons feeding.

2. Viruses

Virulent strain of pigeon paramyxovirus-1

It was locally isolated and obtained from the Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo, Egypt, with a titre of 10^7 EID_{50}/ml. The virus was used for challenging the vaccinated and control pigeons.

3. Vaccines

3.1. Pigeon paramyxovirus-1 inactivated vaccine

Egyptian strain of PPMV-1 was obtained from VSVRI for preparation of inactivated alhydragel PPMV-1 vaccine, with a titre of 10^{10} EID_{50}/ml before inactivation with formalin.

3.2. Lentogenic Newcastle disease vaccine LaSota strain

Live LaSota vaccine, commercially produced locally by VSVRI, was used and had a titre of 10^{11} EID_{50}/ml.

3.3. Lentogenic Newcastle disease vaccine Hitchner B_1 strain

Live HB1 vaccine, commercially produced locally by VSVRI, was used and had a titre of $10^{10.5}$ EID_{50}/ml.

3.4. Mesogenic Komarov (K) strain of Newcastle disease virus

Live Komarov vaccine, commercially produced locally by VSVRI, was used and had a titre of 10^9 EID_{50}/ml.

4. Experimental design

one humored on and fifty squabs, about two weeks of age were used. They were found seronegative for both pigeon PMV-1 and NDV. The birds were divided into 5 equal groups of 30 birds each and were treated according to the following protocol:

Group (1) vaccinated with Hitchner B_1 vaccine in drinking water, after a mild degree of thirst by eliminating access to drinking water for approximately two hours prior to vaccination, procedure, followed by vaccination with the locally prepared alhydragel inactivated PPMV-1 vaccine two weeks later (0.5ml S/C containing $\geq 10^9$ EID_{50}/bird).

Group (2) Vaccinated with one drop of LaSota vaccine (eye drops containing 10^7 EID_{50}/bird), followed by vaccination with the locally prepared inactivated PPMV-1 vaccine two weeks later (0.5ml S/C).

Group (3) intramuscular vaccination with Komarov (K) vaccine 0.25 ml/bird containing 10^6 EID_{50}/ml, followed by S/C vaccination with the locally prepared inactivated PPMV-1 vaccine 2 weeks later (0.5ml S/C).

Group (4) subcutaneously vaccinated once with 0.5 ml/bird (containing not less than 10^9) of the locally prepared inactivated PPMV-1 vaccine at 4 weeks of age.

Group (5) non-vaccinated challenge control group.

Sampling

Serum samples were collected weekly for 12 weeks from vaccinated and non-vaccinated birds.

5. Challenge

Challenge test was applied to each of 10 birds 3 weeks post- primary vaccination and 4 weeks post- booster vaccination in vaccinated groups and to each of 5 birds in the control groups, using 0.25 ml (containing 10^7 EID_{50}/ml) of virulent PPMV-1 by I/M injection. Results of the challenge were recorded.

6. Serological tests

6.1. Haemagglutination Inhibition test (HI)

It was done using the Beta-procedure (constant virus plus two-fold diluted serum). Four HA units of pigeon paramyxovirus-1 were used as antigen for the test. It was carried out according to Anon (1971). This test was used for measurement of the serological response of pigeons to primary and secondary vaccination for 12 weeks post-vaccination.

RESULTS

Table 1. Experimental design for vaccination of pigeons wit various vaccines.

Group (h=30 bird/group)	Primary vaccination At 2 weeks old		Secondary vaccination At 4 weeks old	
	Type of vaccine	Route	Type of vaccine	Route
1st	HB1	D/W	PPMV-1	S/C
2nd	LaSota	Eye drop	PPMV-1	S/C
3rd	Komarov	I/M	PPMV-1	S/C
4th *	-	-	PPMV-1	S/C
5th	Non-vaccinated control			

*Birds received only one vaccine dose at 4 weeks of age.

Table 2. Serologic response of pigeons after vaccination with various vaccines as measured by haemagglutination inhibition test (HI).

Group	Mean $\log_2$ HI titre at weeks post- vaccination											
	1	2	3	4	5	6	7	8	9	10	11	12
1	2	2.8	3.1*	3.0	2.7	2.5	2.0	2.6	2.3	2.0	2.1	2.0
			3.5**	4.0	4.8	6.0	7.0	7.5	7.3	7.3	7.0	7.0
2	1.8	3.0	3.8	4.5	4.3	4.0	4.0	3.5	3.2	3.0	2.0	2.0
			4.0	5.2	7.2	7.0	7.5	8.0	8.2	8.2	8.0	8.0
3	1.5	3.0	3.5	4.0	5.0	4.7	4.5	4.3	4.0	4.1	4.0	4.0
			4.0	5.8	7.2	8.0	9.1	8.7	8.7	8.5	8.3	8.2
4	-	-	-	-	2	3.4	5.5	7.8	7.5	7.3	7.0	6.8
5	1	0	0	0	0	0	0	0	0	0	0	0

*Pigeons received only one vaccination dose of different vaccinal types.
**Pigeons were revaccinated at 4 weeks of age with the inactivated pigeon paramyxovirus-1 vaccine (0.5ml S/C).

Table 3. Protective efficacy against challenge with virulent PPMV-1 of vaccinated pigeons 3 weeks post -primary vaccination with live NDV vaccines.

	Group	No. of birds	No. of survivors	Protection % *
1	HB1 vaccine	10	2	20
2	LaSota	10	4	50
3	Komarov	10	5	50
5	(Unvaccinated Control)	5	0	0

$$\text{* Protection \%} = \frac{\text{No. of survivors}}{\text{Total No. of challenged birds}} \times 100$$

Table 4. Protective efficacy against challenge with virulent PPMV-1 of vaccinated pigeons4weeks post- secondary vaccination.

	Group	No. of birds	No. of survivors	Protection % *
1	HB1 vaccine	10	8	80
2	LaSota	10	10	100
3	Komarov	10	10	100
4	PPMV-1 *	10	9	90
5	Unvaccinated Control	5	0	0

* Received only one vaccine dose at 4 weeks of age.

$$\text{Protection \%} = \frac{\text{No. of survivors}}{\text{Total No. of challenged birds}} \times 100$$

DISCUSSION

PMV-1 is one of the viruses within the genus Paramyxovirus which includes many viruses infecting avian species including pigeons (Alexander and Parsons, 1984). Pigeon PMV-1 type isolates 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 (King, 1996).

The role of primary vaccination for initiation and activation of the immune response is well documented (Tizzard, 2000).

Usually, live virus vaccines are preferable for primary vaccination. They infect the host cells and undergo replication, and the infected cells process the virus as an endogenous antigen. In this way, live viruses trigger a response dominated by cytotoxic T-cells (CTL), one of the most important functions of CTL is the elimination of virus infected cells. Cytokines, on the other hand, bind to specific receptors on the surface of target cells and regulate immune response by signaling between cells. Receptor-bound cytokines and other membrane-associated molecules often act together to stimulate the effector function in a target cell. T-cells, B-cells, macrophages, and denteritic cells all secrete cytokines (Sharma, 2003).

Many trials for attenuation of PPMV-1 were done, but till now it has yet not been produced commercially. So, priming by NDV vaccines was suggested for early initiation and activation of the immune response in pigeons, which is mainly due to cell-mediated activation, then, the humoral immune response follows later (Sharma, 2003).

In the present work, the immune response to primary vaccination with each of three different types of live NDV vaccinal strains given to corresponding groups of two-week-old squabs and boosting with inactivated alhydragel PPMV-1 vaccine two weeks later was studied. An additional group was vaccinated with a single dose of the inactivated PPMV-1 at four weeks of age was included and another group served as non-

vaccinated challenge control. The immune response was weekly monitored serologically by HI test and the degree of protection against virulent PPMV-1 was determined by challenge test. It is well recognized that the immune response increases as the pathogenicity of the live vaccine increases as cited by Sharma (2003).

Results of the humoral immune response as shown in Table 2 measured by haemagglutination inhibition test (HI) revealed that four weeks post-priming of groups (II and III) with the lentogenic LaSota or the mesogenic Komarov vaccine respectively higher mean antibody titres ($2^{4.5}$,$2^{4.0}$) were determined than in group (1) primed with HB1 lentogenic vaccine (2^3). subsequently, group I and II) showed a gradual drop in mean HI values to reach (2^2) by the 12^{th} week post- priming.

On the other hand, groups (III) revealed relatively higher mean HI titres than at 4 weeks post -priming and was still higher than groups (I and II) at 12 weeks. In subgroups from group (I, II and III) boosted with inactivated PPMV-1 vaccine after 2 weeks, the mean $\log_2$ HI titres increased by 4 $\log_2$ six weeks post- booster vaccination (8 weeks post-priming), values of $2^{7.5}$, 2^8, $2^{8.7}$), respectively. With respect to group (IV) which received only one dose of inactivated PPMV-1 at 4 weeks of age, serological response was low in the first two weeks post vaccination (2^2, $2^{3.4}$), then, subsequently increased to reach a maximum mean value by the 4^{th} week ($2^{7.8}$), then, gradually decreased to reach ($2^{6.8}$) by the 8^{th} week post- vaccination (12 weeks of age). The protective efficacy of the applied vaccination program was evaluated three weeks post - primarily vaccination as shown in Table 3. It is evident that groups (II and III) gave higher, but unsatisfactory, protection (50%) than group (I) (20%). However, significantly, higher and practically acceptable protection was achieved four weeks post - booster vaccination of subgroups (II and III) (100% and 100%) and 90% for group IV 4 weeks post- primary vaccination with inactivated PPMV-1.

On the other hand, subgroup (I) revealed comparatively lower (80%) protection than other subgroups.

Although partial unsatisfactory protection was achieved by early primary vaccination of pigeon squabs at two weeks of age with lentogenic or mesogenic NDV vaccine strains when challenged three weeks later with virulent PPMV-1; yet, this protection may be needed in endemic areas since boosting with inactivated PPMV-1 vaccine two weeks post- primary resulted in satiafactory protection (80-100%) when the birds were challenged with virulent PPMV-1 four weeks post- booster vaccination (i.e. at 8 weeks of age). From the results and from the practical view point; a vaccination program for pigeons primary vaccination with live ND LaSota vaccine intraocularly at two weeks of age and booster vaccination with inactivated PPMV-1 vaccine subcutaneously at four weeks, is highly recommendable

REFERENCES

1. Abou Hashem, N. A. 1993. Studies on some paramyxovirus infection in pigeons. Thesis, M. V. Sc. Dept. Fac. Vet. Med., Zagazig Univ.
2. Alexander, D. J. and G. Parsons. 1984. Avian paramyxovirus type-1 infection of racing pigeons: Pathogenicity experiments in pigeons and chickens. Vet. Rec., 114 (19): 466-469.
3. Anon. 1971. Methods for examination of poultry biologics and for identifying avian pathogens. National Academy of Science, Washington, D.C., P. 83-87.
4. Duchatel, J. P., Leroy p. and H. Vindevogel. 1986 Duration of protection after vaccination of pigeons against paramyxovirus infection by subcutaneous injection of inactivated vaccine. Vlaams Diergenkd, Tijdschr, 55 (3): 146-152.
5. El-Zanaty, K., Abdel T. Y., Motilib M. Seifedien and B. Salem. 1992. Vaccination against paramyxovirus type-1. Assiut Vet. Med. J., 26 (52): 265-271.
6. Hassan, E. A. 1997 Some trials for vaccine production against paramyxovirus. Thesis M. SC., Alex. Univ.
7. King, D. J. 1996 Avian paramyxovirus type-1 from pigeons: isolate characterization and pathogenicity. Avian Dis., 40: 707-714.
8. Shakal, M. A. 1989 Studies on paramyxovirus infection in pigeons in Egypt. Thesis Ph. D. Cairo Univ.
9. Sharma, J. M. 2003. The avian Immune System. In: Diseases of Poultry (11^{th} Ed.), pp. 5-16. Y.M. Saif (Edition). Am. Asso. Avian Pathologists, Iowa State Univ., Ames, Iowa.
10. Tangredi, B. P. 1985. Avian paramyxovirus type-1 infection in pigeons. Clinical observation. Avian Dis., 29: 252-255.
11. Tizzard, I. 2000. A Book of Veterinary Immunology, 3rd Ed., p. 238.
12. Vindevogel, H. and J. P. Duchatel. 1993. Paramyxovirus type-1 infection in pigeons. In: Virus Infection of Birds, Chapter 24: 363-374.
13. Weiesman, Y., A., Aropovici M., Malkinson E. Shihmanter and M. Lipkind 1984. Isolation of paramyxovirus from pigeons in Israel. Vet. Rec., 115 (23): 605.

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دراسات على وقاية زغاليل الحمام من الإصابة المبكرة بمرض باراميكسو الحمام باستخدام لقاحات النيوكاسل الحية فى الفترة الغير قابلة للتحصين باللقاح المثبط باراميكسو-١ الحمام

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أجريت دراسة للتحصين الأولى للحمام باستخدام بعض اللقاحات الحية الخاصة بفيروس النيوكاسل قبل تحصينه بلقاح باراميكسو-١ المثبط الخاص بالحمام.

استخدم لقاح الهتشنر B1 فى مياه الشرب واستخدام لقاح لاسوتا بالتقطير فى العين واستخدم لقاح كوماروف بالحقن فى العضل كتحصين أولى عند عمر اسبوعين ولقاح باراميكسو-١ المثبط والخاص بالحمام عند عمر ٤ أسابيع. تم تقييم الاستجابة المناعية للطيور المحصنة باستخدام اختبار منع التلازن الدموى (HI) طوال فترة التجربة واختبار التحدى لمرتين.

أظهرت النتائج وجود استجابة مناعية جزئية لحماية مبدئية للطيور بعد ٣ أسابيع من تحصين الأولى باللقاحات الحية وقدرتها للتصدى للفيروس الضارى بعد ٤ أسابيع من التحصين الثانى باللقاح المثبط بحماية الطيور بنسب ٨٠%، ١٠٠%، ١٠٠% فى المجموعات الأولى والثانية والثالثة على التوالى، وكانت ٩٠% فى المجموعة الرابعة التى حصنت باللقاح المثبط بجرعة واحدة عند ٤ أسابيع.

كما أوضحت النتائج أن استخدام لقاح لاسوتا بالتقطير فى العين كتحصين مبدئى قبل لقاح الباراميكسو المثبط هو الأفضل بالمقارنة بباقى المجموعات وذلك لأنه أكثر أماناً واسهل تطبيقاً بالنسبة للطيور.