Evaluation of the immune response of pigeons to Newcastle disease and pigeon paramyxo virus vaccines

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A B S T R A C T

One hundred and twenty pigeons divided into 4 groups (30 birds/ group). Group one vaccinated with the local produced inactivated pigeon paramyxo vaccine using 0.5ml injected subcutaneous in the dorsal aspect of neck; Group two vaccinated with Hitchner B1 Newcastle disease vaccine through drinking water while third group vaccinated with oil inactivated Newcastle disease vaccine through injection of 0.5ml subcutaneously in the dorsal aspect of neck. The 4th group was kept without vaccination as control. The use of homologous virus in HIT; SNT and ELISA, revealed that all vaccinated pigeon groups exhibited detectable antibodies by first week post vaccination reached their peak by the 2nd month post vaccination then began to decline gradually to reach their lowest titers by the 12thmonth post vaccination. So, the use of PP virus, it was noticed that specific PP antibodies were higher than those non-specific antibodies induced by other ND vaccines. On the other side the use of ND virus showed that antibodies were lower than those induced by other ND vaccines. It was found PP vaccine provided 100% protection against the homologous PP virus and 90% against ND virus (group-1) while Hitchner-B1 protected pigeons against PP virus with 50% only and 100% against ND virus while inactivated ND vaccine protected 60% of challenged pigeons against PP virus and 100% against ND virus. But non-vaccinated challenged pigeons survived PP virus with 10% only and survived ND virus with 80%.

Key Words: Newcastle disease; pigeon paramyxovirus serotype-1; HA; HI

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1. INTRODUCTION

Pigeons (Columbia Livia) and other members of the Family Columbidae are known to be susceptible to infection with avian paramyxovirus serotype-1 (A/PMV-1) which includes Newcastle disease virus (NDV) [1]. The occurrence of paramyxovirus-1 (PMV-1) and other viral diseases in pigeons has already been reported [2], [3], [4] and [5] usually reported outbreaks amongst pigeons; particularly domesticated ones appeared with widespread epizootics of Newcastle disease among domestic poultry [6]. Since pigeon paramyxovirus serotype-1 infection is of wide spread, so prevention of infection looks important and needs thorough investigation. This could be achieved by vaccination of pigeo ns to minimize losses and hazard spread of infection to other birds and/or pigeons. Many trials were carried out using different strains of Newcastle diseases virus vaccines to protect pigeons against paramyxo virus infection where [7] and [8] found that Hitchner B1 and Lasota strains of Newcastle disease vaccines could not protect pigeons against the paramyxo virus infection while [9] concluded that homologous vaccine is in need to provide complete protection for pigeons against the disease. On the other side,[10]and[11]reported that Lasota and oil inactivated ND vaccines protect pigeons against paramyxo virus infection up to 6

months. Thirty-nine pigeons were randomly divided into three equal groups; pigeons of one group were vaccinated with ND vaccine (LaSota strain) intraocularly after 14 days of procurement, while the other two groups served as vaccinated and non-vaccinated controls. Birds of these two groups were challenged with velogenic strain of field isolate of NDV 7 days post-vaccination. Birds were kept under observation for 15 days post challenge. Haemorrhages and congestion were observed in trachea, lungs, liver, proventriculus and intestine of pigeons infected with NDV [12]. Complete protection that withstood the challenge of vaccinated pigeons with the virulent virus was obtained using homologous paramyxo vaccines [13, 14, 15, 16, and 17]. The present study aims to evaluate the vaccine of choice to be used to provide the highest protection rate and longest duration of immunity against pigeon paramyxo virus infection using heterogonous (Newcastle disease vaccines) and homologous (pigeon paramyxo vaccine) vaccines and to any extent the heterogeneous vaccines could be used in the absence of the homologous one.

2. MATERIALS AND METHODS

.1. Pigeons:

One hundred and twenty local bread squabs where screened before application of the experimental work using HI and SNT and found that they were free from PPMV type 1 antibodies. These pigeons were used for investigation of the efficacy of pigeon paramyxo and Newcastle disease vaccines to withstand the virus infection.

2.2. Viruses:

2.2.1.Virulent pigeon paramyxo virus:

A local isolate of PPMV was supplied by Veterinary Serum and Vaccine Research Institutes, Abbasia, Cairo. It had a titer of 108ED50 / ml and used for challenge of experimentally vaccinated pigeons.

2.2.2. Cell culture adapted pigeon paramyxovirus:

VERO cell adapted pigeon paramyxo virus [17] was used in serum neutralization test to monitor the levels of induced antibodies in vaccinated pigeons.

2.2.3. Cell culture adapted Newcastle disease virus:

VERO cell adapted ND virus was used in serum neutralization test to monitor the levels of induced antibodies in vaccinated pigeons.

2.3.Vaccines:

Local produced pigeon paramyxo; Hitchner B1 and inactivated oil Newcastle disease vaccines were supplied by Veterinary Serum and Vaccine Research Institute, Abassia, Cairo and used for vaccination of experimental pigeons.

2.4.African green monkey cell line (VERO):

The cells were established by [18] and kindly obtained from The Department of Pet Animal Vaccine Research, Abbasia. VERO cells were used in serum neutralization test.

2.5. Experimental design:

The included pigeons were divided into 4 groups(30 birds/ group)as follows:-

Group-1 vaccinated with the local produced inactivated pigeon paramyxo vaccine using 0.5ml injected subcutaneous in the dorsal aspect of the neck.

Group-2 vaccinated with Hitchner B1 Newcastle disease vaccine through the drinking water.

Group-3 vaccinated with the oil inactivated Newcastle disease vaccine through the injection of 0.5ml subcutaneously in the dorsal aspect of the neck.

Group-4 was kept without vaccination as control.

Blood samples were obtained from all groups on week intervals post vaccination up to 4 weeks then monthly up to one year to follow up the levels of induced antibodies using HI and SNT.

2.6.Challenge test:

Three weeks post vaccination; the challenge test was carried out for 20 birds from each group of vaccinated and non vaccinated squabs. Each bird was intramuscularly inoculated with 0.5 ml of virulent PPMV-1 having a titer of 106 EID50 /ml. The challenged birds were kept in a separate isolate and clinical signs; morbidity and mortality were recorded daily. Dead birds were necropsied for gross pathological changes. The other 10 birds of each group were kept to follow up the duration of induced immunity in vaccinated pigeons.

2.7. Haemagglutination inhibition (HI) test:

It was done using the Beta procedure (constant virus plus diluted serum). The test was carried out according to the standard method of examining poultry biologics [19].
2.8. Serum Neutralization test (SNT):

SNT was carried out using the micro titer technique according to [20] and the antibody titer was expressed as the reciprocal of the final serum dilution which neutralized and inhibited completely the CPE of 100 TCID50 of the used virus according to [21].

3. RESULTS

It was noticed that all vaccinated and non-vaccinated pigeon groups remain healthy all over the experimental period revealing that all used PP and ND vaccines safe for pigeons.

3.1. Evaluation of humeral immune response to pigeon paramyxo vaccine:

Using PP virus, it was found that all vaccinated pigeon groups exhibited detectable antibodies by the first week post vaccination (2 log2/ml by HI and 4 log10/ml by SNT), reached their peak by the 2nd month post vaccination (7 log2/ml by HI and 256 log10/ml by SNT) then 50% only and 100% against ND virus (group2), while inactivated ND vaccine began to decline gradually to reach their lowest titers by the 12th month post vaccination (6.7 log2/ml by HI and 32 log10/ml by SNT). It was noticed that specific PP antibodies were higher than those non-specific antibodies induced by other ND vaccines. Non-vaccinated pigeons remain sero-negative. These findings were obtained by HI and SNT in the same manner as demonstrated in tables (1) and (2).

3.2. Evaluation of humeral immune response to New Castle Disease vaccine:

It showed that using of ND virus, all vaccinated pigeon groups exhibited detectable antibodies by the first week post vaccination (2 log2/ml by HI and 4 log10/ml by SNT for Hitchner B1 vaccine and 2 log2/ml by HI and 2 log10/ml by SNT for oil inactivated ND vaccine), reached their peak by the 4th week post vaccination for Hitchner B1 vaccine (6.7 log2/ml by HI and 64 log10/ml by SNT) and by 2nd month post vaccination for oil inactivated ND vaccine (6.3 log2/ml by HI and 128 log10/ml by SNT), then began to decline gradually to reach their lowest titers by the 12th month post vaccination (4.5 log2/ml by HI and 8 log10/ml by SNT for Hitchner B1 vaccine and 4.8 log2/ml by HI and 16 log10/ml by SNT for oil inactivated ND vaccine). It was noticed that detected PP antibodies were lower; as non-specific antibodies; than those antibodies induced by ND vaccines; as specific antibodies. These findings were obtained by HI and SNT in the same manner as shown in tables (3) and (4).

3.3. Challenge and percent of protection:

Percent of protection calculated in vaccinated pigeons post challenge against PP and ND viruses showed that PP vaccine provided 100% protection against the homologous PP virus and 90% against ND virus (group1), while Hitchner-Bl vaccine protected pigeons against PP virus with

protected 60% of challenged pigeons against PP virus and 100% against ND virus

(group3). On the other side, non-vaccinated challenged pigeons survived PP virus with10% only and survived ND virus with 80%. Un-survived challenged pigeons and post mortem signs. These results were shown in table (5).

Table (1): Mean serum HI antibody titers in different pigeon groups using pigeon paramyxo virus type-1.

Intervals post vaccination	Pigeon paramyxo type-1 HI antibody titers (log2/ml) in pigeons			
	Group -1	Group -2	Group -3	Group -4
0	0	0	0	0
1WPV*	2	1.5	1	0
2WPV	4	3	2.5	0
3WPV	5.0	4.5	4.3	0
4WPV	5.8	5.0	5.1	0
2MPV**	7.0	6.1	6.5	0
4MPV	7.1	6.1	6.5	0
6MPV	7.0	6.0	6.4	0
8MPV	7.1	5.8	6.4	0
10MPV	7.0	5.4	5.8	0
12MPV	6.7	3.2	4.5	0

aWPV= week post vaccination. bMPV= month post vaccination. Group-1 vaccinated with inactivated pigeon paramyxo vaccine. Group-2 vaccinated with Hitchner B1 Newcastle disease vaccine.Group-3 vaccinated with the oil inactivated Newcastle disease vaccine.Group-4 was kept without vaccination as control.

Table (2): Mean serum neutralizing antibody titers in different pigeon groups using pigeon paramyxo virus type-1

Intervals post vaccination	Pigeon paramyxo type-1 serum neutralizing antibody titers*			
	Group -1	Group -2	Group -3	Group -4
0	0	0	0	0
1WPV**	4	2	4	0
2WPV	8	4	8	0
3WPV	32	8	16	0
4WPV	64	16	32	0
2MPV***	256	32	64	0
4MPV	256	32	128	0
6MPV	256	32	128	0
8MPV	128	32	128	0
10MPV	128	16	64	0
12MPV	32	4	16	0

a Serum neutralizing antibody titer = the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100 TCID50 of PPV.
bWPV= week post vaccination. cMPV= month post vaccination. Group-1 vaccinated with inactivated pigeon paramyxo vaccine. Group-2 vaccinated with Hitchner B1 Newcastle disease vaccine. Group-3 vaccinated with the oil inactivated Newcastle disease vaccine. Group-4 was kept without vaccination as control.

Table (3): Mean serum HI antibody titers in different pigeon groups using pigeon Newcastle disease virus

Intervals post vaccination	Pigeon paramyxo type-1 HI antibody titers (log2/ml) in pigeons			
	Group -1	Group -2	Group -3	Group -4
0	0	0	0	0
1WPV*	1.3	2	2	0
2WPV	2.0	3.2	2.8	0
3WPV	4.0	5.1	5	0
4WPV	5.5	6.7	6.3	0
2MPV**	5.4	6.6	6.3	0
4MPV	5.3	6.0	6.2	0
6MPV	5.0	5.4	6.0	0
8MPV	5.0	5.0	5.8	0
10MPV	4.9	5.2	5.5	0
12MPV	4.7	4.5	4.8	0

aWPV= week post vaccination. bMPV= month post vaccination. Group-1 vaccinated with inactivated pigeon paramyxo vaccine.Group-2 vaccinated with Hitchner B1 Newcastle disease vaccine.Group-3 vaccinated with the oil inactivated Newcastle disease vaccine. Group-4 was kept without vaccination as control.

Table (4): Mean serum neutralizing antibody titers in different pigeon groups using Newcastle disease virus

Intervals post vaccination	Mean ND serum neutralizing antibody titer*			
	Group-1	Group-2	Group-3	Group-4
0	0	0	0	0
1WPV**	2	4	2	0
2WPV	4	8	4	0
3WPV	8	16	8	0
4WPV	16	64	32	0
2MPV***	32	64	128	0
4MPV	32	64	128	0
6MPV	32	32	128	0
8MPV	16	16	64	0
10MPV	8	16	32	0
12MPV	8	8	16	0

aSerum neutralizing antibody titer = the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100 TCID50 of NDV bWPV= week post vaccination. cMPV= month post vaccination. Group-1 vaccinated with inactivated pigeon paramyxo vaccine. Group-2 Group-3 vaccinated with the oil inactivated Newcastle disease vaccine. Group-4 was kept without vaccination as control vaccinated with Hitchner B1 Newcastle disease vaccine.

Table (5): Protection of experimental pigeons against challenge viruses

Number of pigeons	Group - 1		Group - 2		Group - 3		Group - 4	
	PPV*	NDV**	PPV	NDV	PPV	NDV	PPV	NDV
Challenged	10	10	10	10	10	10	10	10
Survived	10	9	5	10	6	10	1	8
Protection%	100	90	50	100	60	100	10	80

Group-1 vaccinated with inactivated pigeon paramyxo vaccine.Group-2 vaccinated with Hitchner B1 Newcastle disease vaccine.Group-3 vaccinated with the oil inactivated Newcastle disease vaccine.Group-4 was kept without vaccination as control. [a]PPV= pigeon paramyxovirus.

4. DISCUSSION

Although the obtained result could be explained on the bases of homologous and heterogonous used viruses in applied serological tests, it indicate that pigeons respond well to both of PP and ND vaccines.

It was found that pigeons vaccinated with local inactivated PP vaccine exhibited detectable antibodies using HI and ELISA by the first week post vaccination, reached their peak nearly by the 2nd month post vaccination then began to decline gradually to reach their lowest titers by the 12th month post vaccination. These results were recorded in comparison with non-vaccinated pigeons that remained sero-negative. It was noticed that specific PP antibody titers obtained by HI and SNT were higher using pp virus than the titers calculated using ND virus. These results, come in agreement with the results mentioned that vaccination of pigeons with inactivated aqueous suspension of pigeon paramyxovirus type-1 (pPMV-1) vaccine (Colombovac PMV) induced high protective specific antibodies that persisted for at least a year [1], and result showed that oil emulsion pPMV-l vaccine gave higher antibody response of a mean 8.9 log2 HI titer at the third week after the second vaccination [15]. Also, our results agreed with that monitored in pigeons vaccinated with inactivated cell culture PP vaccine and

induced specific PP antibodies by the first week post vaccination (4 log10 SNT titer and 8 log2 HI titer) then increased to reach a titer of 32 log10 by SNT and 64 log2 by HI on the third week post vaccination then decreased to 16 log10 by SNT and 32 log2 by HI after application of the challenge test. Later on; PP antibodies recorded their peak titer (128 by SNT and 256 by HI) by the first month post challenge(about 2 month post vaccination) and still unchanged up to 9 months then began to decrease gradually to reach their lowest titers (8 log10 by SNT and 16 log2 by HI) on the 12th month [16, 17].

The results showed also that pigeons vaccinated with Hitchner B1 and oil inactivated ND vaccines demonstrated detectable antibodies using HI and ELISA by the first week post vaccination, reached their peak nearly by the 2nd month post vaccination then began to decline gradually to reach their lowest titers by the 12th month post vaccination. These results were recorded in comparison with non-vaccinated pigeons that remained sero-negative. It was noticed that specific ND antibody titers obtained by HI and SNT were higher using ND virus than the titers calculated using PP virus. These results, come in agreement with the results mentioned that an aqueous Lasota vaccine induced rapid antibody formation and these antibodies persisted for at least 6 months [l]. Our results came in a parallel manner with agreement with other results finding as who found that an inactivated ND vaccine in oil adjuvant had no adverse effect on antibody titers or resistance to pigeon paramyxo virus type-l (pPMV-l) infection [9], the result which suggested that, the preferred method for protecting pigeons against pPMV-l infection was by subcutaneous inoculation with inactivated oil emulsion ND vaccine which gives protection for more than 6 months [l0], [11]; the work that controlled pPMV-1 infection in 2800 carrier pigeons by immunization with an inactivated ND vaccine [22]; and the results which stated that inactivated and live ND vaccines were effective for immunizing healthy birds with immunity lasted for about a year [23].

Concerning the protection of vaccinated pigeons post challenge against PP and ND viruses, It was found that PP vaccine provided 100% protection against the homologous PP virus and 90% against ND virus. These results, come in agreement with that concluded, pigeons immunized with homologous oil emulsion vaccine against pPMV -1 infection, was highly protected than for pigeons given commercial ND vaccines for a year[24].

Challenge test of vaccinated pigeons showed that Hitchner-Bl vaccine protected pigeons against PP virus with 50% only and 100% against ND virus. These results come in agreement with similar results which found that Hitchner B1vaccine did not provide sufficient protection for pigeons against PP virus [7]; and other results which concluded that Hitchner B1vaccine could not protect pigeons against PP virus where it could not propagate in pigeon tissues and therefore could not induce suitable immunity [25].

Challenge test of pigeons vaccinated with inactivated ND vaccine protected 60% of challenged pigeons against PP virus and 100% against ND virus. These results coincided with the results which demonstrated that oil emulsion ND vaccine gave better protection for pigeons against pigeon pPMV-l than live ones[6]; the results recorded that oil emulsion ND vaccine protected pigeons against pPMV-1 for a year [22]; and the results proved that ND inactivated adsorbed vaccines had a good protective effect against pPMV-1 infection in pigeons and the inactivated vaccines were superior to live vaccines in their ability to induce demonstrable HI titers; as it found that inactivated ND vaccines induced relative high antibodies sufficient to protect pigeons against PP virus, but Hitchner B1 vaccine did not provide sufficient protection for pigeons against PP virus [26, 27]. However, specific homologous PP vaccine is the

preferable and recommended one to obtain the maximum protection level [1, 15, 16, 17, 24, 28].

Non-vaccinated challenged pigeons survived PP virus with10% only and survived ND virus with 80%. Unsurvived challenged pigeons against PP virus showed typical PP clinical and post mortem signs. The recorded signs on un-survived pigeons challenged against PP were characterized by initial excessive thirst with watery diarrhea which becomes green followed by nervous signs; torticollis, circling movements and inability to stand. The determined postmortem findings came in agreement with that reported and represented by hemorrhages in the brain and congestion of most internal organs [4, 5, 6, 16, 17, 29, 30, 31].

From the obtained results it could be concluded that pigeons may play a part in transmission of ND although they not show clear signs of the disease or deaths but they attract the virus and induced specific antibodies the thing which may directs the attention toward vaccination of pigeons with ND vaccine to avoid the virus shedding to chickens or as anon specific vaccine against pigeon paramyxo virus. So, further studies are in requirement. It is preferable to use the specific PP vaccine to protect pigeons against the disease but in case of such vaccine absent inactivated ND vaccine could be used in a preferable suggestion than Hitchner-B1 vaccine.

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تقييم استجابة الحمام المناعية للقاحات النيوكاسل والباراميكسو

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الملخص العربى

تم إجراء هذه الدراسة على عدد مائة وعشرون حمامة ،قسمت إلى أربعة مجموعات،كل مجموعة بها عدد 30 طائر ،تم تحصين المجموعة الأولى بكمية 5, مل من اللقاح المثبط لـ باراميكزو الحمام تحت جلد الرقبة،بينما تم تحصين المجموعة الثانية من خلال ماء الشرب باللقاح ((هتشنر ب 1)) ،بينما المجموعة الثالثة تم حقنها باللقاح الزيتى المثبط لمرض النيوكاسل بكمية 5, مل تحت جلد الرقبة ،والمجموعة الرابعة تم وضعها بدون تحصين كضابط للتجربة ،وعند فحص الأجسام المناعية المتكونة بواسطة إختيار منع قوة التلازن الدموى ،وإختيار المصل المتعادل ،وإختبار مختصر مقايسة الممتز المناعى المرتبط بالإنزيم ،وجد أن مستوى الأجسام المضادة المتكونة يبدأ فى الزيادة اعتباراً من الأسبوع الثانى للتحصين ليصل إلى أعلى مستوى له فى الشهر الثانى للتحصين ثم يبدأ فى الهبوط تدريجياً ليصل إلى أقل مستوى فى الشهر الثانى عشر بعد التحصين. كما وجد أن الحمام المحصن باللقاح المثبط لـباراميكزو الحمام أظهرت مناعة بنسبة 100% مع إختبار التحدي لـمرض الباراميكزو الحمام. بينما الحمام المحصن باللقاح المثبط لمرض النيوكاسل أظهر مناعة بنسبة 90% بينما الحمام المحصن باللقاح هتشنر ب 1 أظهر مناعة ضد الإصابة بمرض الباراميكزو الحمام بنسبة 50% وأظهر مناعة بنسبة 100% ضد مرض النيوكاسل. بينما الحمام المحصن باللقاح الزيتى المثبط لمرض النيوكاسل أظهر مناعة بنسبة 100% ضد مرض النيوكاسل وأظهر مناعة بنسبة 60% ضد إختبار التحدى لباراميكزو الحمام ،بينما وجد أن المجموعة الضابطة عند إجراء إختبار التحدى لمرض الباراميكزو الحمام أظهرت مناعة بنسبة 10% فقط، فى حين أن المناعة ضد مرض النيوكاسل فى الحمام وصلت إلى 80% .

مما سبق يتضح أن من المفضل تحصين الحمام باللقاح الخاص بمرض الباراميكزو الحمام وإذا لم يوجد فيفضل تحصين الحمام بلقاح المثبط لمرض النيوكاسل وهو أفضل من التحصين بلقاح هتشنر ب1.

(مجلة بنها للعلوم الطبية البيطرية: عدد 25(1):148-156, سبتمبر 2013)