IMMUNOLOGICAL AND INFLAMMATORY RESPONSES OF KILLED AND LIVE NEWCASTLE DISEASE VACCINES IN PIGEONS [+]

الاستجابة المناعية والالتهابية للقاح الحي والمقتول لمرض نيوكاسل في الحمام

Adil S. Ag'gar [*]

Abstract:

This trail was suggested to determine the correlation between the inflammatory and immune responses after the vaccination of domesticated pigeon with killed and live Newcastle disease (ND) vaccines, separately or in combination. For this purpose the evaluation of immune response based on Haemagglutination Inhibition antibody titer (HI-titer), while inflammatory response based on C-Reactive Protein (CRP) level because it's available commercially. A total of sixty domesticated pigeons were used in the experiment, then those pigeons were divided into four groups, 15 pigeon in each group, each group was propagated separately and had been given water and feed *ad libitum*. The first group (G1) was vaccinated with ND killed vaccine subcutaneously, the second one (G2) was vaccinated with ND live vaccine orally, while the third group (G3) was got the live vaccine then followed by the killed one within two days, the fourth group (G4) remained as a control group without any vaccine. Blood samples were collected from the pigeons of all groups to estimate the presence of an immunological or inflammatory responses to ND in their own sea within two days intervals for five times to follow up the acute phase proteins CRP and the HI titer. The results showed that the HI titer was elevated slowly but steadily in all vaccinated groups, this elevation was statistically significant at the level p>0.01 in G3, then G2 followed by G1; with no HI titer was detected in G4, while there was abrupt elevation in CRP level at about two days after the vaccination with live and combine vaccines only, no alteration was detected in the group that was vaccinated with killed ND vaccine in comparison with control group. The conclusion is that live ND vaccine induces sufficient level of CRP abruptly after vaccination; HI, as well, had been elevated significantly (p>0.01) in combination with vaccinated group then live vaccine group, and slow elevation in killed vaccine one.

المستخلص:

اقترحت هذه التجربة لتحديد وجود علاقة بين الاستجابة المناعية والاستجابة الالتهابية بعد تلقيح الحمام المستأنس بنوعي لقاح مرض نيوكاسل؛ اللقاح الحي والمقتول كلاً على حدة أو بالتعاضد. استخدم في البحث ٦٠ حمامة وزعت عشوائياً بالتساوي على أربعة مجاميع، كل مجموعة احتوت على ١٥ حمامة، وربيت تحت الظروف عينها وأُعطيت ماءً وغذاءً قدر الحاجة. لقحت المجموعة الأولى (م١) بلقاح مرض نيوكاسل المقتول حقناً تحت الجلد، في حين لقحت المجموعة الثانية (م٢) بلقاح مرض نيوكاسل الحي بالتجريع بالفم، أما المجموعة الثالثة (م٣) فقد لقحت بلقاح مرض نيوكاسل الحي متبوعاً بلقاحه المقتول بمدة يومين، والمجموعة الرابعة (م٤) فقد تركت بدون تلقيح كمجموعة

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[*] **Assit . Lech./ Technical Institute / Kut**

سيطرة. سحبت نماذج الدم لاستخراج المصل منها على فترات زمنية كل يومين ولخمس مرات لمتابعة ظهور الأضداد المناعية فيه (أضداد اثباط تلازن خلايا الدم الحمر) وبروتينات الطور الالتهابي الحاد. أظهرت النتائج ارتفاعاً بطيئاً ثابتاً في الأضداد المثبطة لتلازن خلايا الدم الحمر في كل المجاميع الملقحة عند التحليل الإحصائي لها مع تفوق معنوي عند مستوى $p>0.01$ في المجموعة الملقحة باللقاحين معاً (م٣)، تلتها (م٢) ثم (م١) في حين كان الارتفاع سُريعاً في بروتينات الطور الالتهابي الحاد في اليومين الأولين فقط بعد التلقيح باللقاح الحي وحده أو مع اللقاح المقتول في حين لم تظهر المجموعة الملقحة باللقاح المقتول أو مجموعة السيطرة ارتفاعا في هذه البروتينات.

Introduction:

Newcastle disease (ND) is a viral disease of wild and captive birds with high morbidity and mortality [1], this disease is caused by a non-segmented, negative-sense, single-stranded RNA virus called paramyxovirus [2], which is a member of the genus *Rubulavirus* in the family *Paramyxoviridae*, primarily because of their nonconserved intergenic junctions and lack of C-protein open reading frame, hallmarks specific to the *Rubulavirus* genus [3]. Newcastle disease virus (NDV) is synonymous with avian paramyxovirus 1 (APMV-1), while pigeon paramyxovirus 1 (PPMV-1) is a variant of NDV but the pathogenesis and pathogenicity in both chicken and pigeon is the same [4]. In nature ND virus is with different virulence, highly virulent ones cause the serious outbreaks and called (Velogenic), and the best known model is the (Visceral Velogenic ND –VVND), others are intermediate called (Mesogenic), their model is Roakin isolate, and the rest are non-pathogenic called (Lentogenic), and this group is the represented by most known vaccination isolates [5]. The virus stimulates host body to produce two types of antibodies after natural infection or vaccination; these are virus neutralizing (VN) and Haemagglutination Inhibition (HI) antibodies [6]. ND seems to be endemic in Iraq [7 & 8], ND outbreaks often occur once or twice a year at regular intervals affirming the endemicity of the virus [8]. Although pigeons appear to be fully susceptible to all strains of NDV, the virus responsible for the panzootic in racing and show pigeons all over the world showed sufficient antigen divergence that, it could be compartmentalised, more or less as a different virus [9]. Unlike chickens, pigeons may infect at faecal-oral route other than respiratory route due to unclose bird-to-bird association exist [10]. With less sever clinical signs [11].

Acute phase proteins (APP):

These are a group of plasma proteins (ß-globulin), mostly synthesized in the liver, their concentrations may increase several times (may reaches to 100-fold) as a part of the response to inflammatory stimuli. [12]. Three of the best known of these proteins are C-reactive protein (CRP), fibrinogen, and serum amyloid A (SAA) protein. CRP binds and promotes the phagocytosis of a variety of intracellular pathogens by polymorphnuclear cells [13]. This is carried out by binding to foreign pathogens and damaged host cells to initiate their elimination by interaction with phagocytic cells or activation of complement system. The acute phase responses include bacterial infection, viral infection, trauma, neoplasms, and burns. [14].

This study aimed to detect the precise role of the immune response to ND vaccines depending on the HI titer as well as the inflammatory response depending on acute phase proteins CRP to NDV.

Materials and methods:

Experimental design: The study was suggested to find out the correlation between the immune and inflammatory responses to vaccination with live and killed ND vaccine.

Pigeons: A total of sixty domesticated pigeons were divided equally into four groups, these pigeons were treated raised in well ventilated conditions, and provided with feed and water *ad libitum*, and treated as following:
Group 1 (G1): Pigeons in this group were vaccinated with killed ND vaccine subcutaneously.
Group 2 (G2): Pigeons in this group were vaccinated with live ND vaccine orally.
Group 3 (G3): pigeons in this group were vaccinated with live ND vaccine orally, followed by killed ND vaccine subcutaneously within two days.
Group 4 (G4): pigeons in this group remained as non vaccinated group and was considered as control group.

Viral vaccines:
Killed vaccine: A 2000 dose vial of clone 30 strain of ND-killed vaccine[1], each bird was given 0.1 ml of vaccine subcutaneously at the back of the neck, as being recommended by the manufacturer.
Live vaccine: A 1000 dose vial of clone 30 strain[†] of ND live vaccine, the whole content dispensed in 1 litre of distilled water, each bird was given 1 ml (EID_{50} was 10^7 / bird) of the dispensed vaccine orally, as being recommended by the manufacturer.
The birds were divided into four groups randomly; each group was given different ND vaccine programme (*see above*).

Serum collection:
Blood samples were collected from the medial metatarsal vein by using disposable and sterile syringes and needles, a quantity of about 2 ml of fresh blood put in a test tube without anticoagulant in slant position over night at room temperature to collect sera [15]. The sera then stored at freezing (20° C) till time of tests.

Haemagglutination-Inhibition Test (HI): The HI tests were done by the beta-procedure in V-bottom microtiter plate as described by [16].

Acute phase proteins (APP): the procedure based on CRP level because of commercial availability of the kit[2], a qualitative and quantitative procedures applied as directed by the manufacturer**.**

Statistical analysis: all data had been submitted to F-test for comparison between different quantitative values, and *Chi* square-test for qualitative values, this procedure was followed by application of space of confidences for all trails at 99% to detect the lesser differences between groups ($p > 0.01$) [17].

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[1] **(†): Commercially available vaccine, manufactured by "Intervet Company", Holland. Supplied by Al-Atheer Veterinary Clinic, Kut, Wassit province, IRAQ.**

[2] **(‡): Commercially available product, manufactured by "Plasmatec Laboratory Products Ltd", England. Supplied by Al-Affaq Bureau for Scientific Supplies, Kut, Wassit province, IRAQ.**

Results:

NDHI antibodies evaluation / CRP evaluation:

- HI titer after two days post vaccination: there were no detectable HI antibodies at that time in all groups (table 1).
- CRP quantitative level after two days post vaccination shows positive reaction in all groups with live viral vaccine (G2 and G3); (figure 3), the same groups were with reliable results in qualitative test (figures 1 & 2), but with no significant differences except when compared with control group (G4) and the group with killed viral vaccine (G1) (table 1).

Table 1: HI-titre and C-reactive protein two days post vaccination:

group	HI-titer Mean ± SE	CRP (quantitative level)	CRP (qualitative level) Mean ± SE
G1	0.0	-ve	0.0 A*
G2	0.0	+ve	3.72 ± 0.32 B
G3	0.0	+ve	4.11 ± 0.13 B
G4	0.0	-ve	0.0 A

(*): Different letters refer to significant differences among groups.

- HI titer after seven days post vaccination: there were significant differences in HI antibodies at $p > 0.01$ among groups, these differences were restricted to (G3), which elevated in titer than (G2) (table 2).
- CRP level after seven days post vaccination in quantitative test was –in somehow- still with positive results at the same groups of live viral vaccine (G2 and G3); in qualitative test the level begins to fade, despite being positive, once again the differences between live viral vaccine were not significant, until when compared with other two groups (G1 and G4) (table 2).

Table 2: ***HI-titre and C-reactive protein seven days post vaccination:***

group	HI-titer Mean ± SE	CRP (quantitative level)	CRP (qualitative level) Mean ± SE
G1	0.0 C *	- ve	0.0 a
G2	4.8 ± 0.8 A	± ve	1.87 ± 0.07 b
G3	14.0 ± 2 B	± ve	2.05 ± 0.15 b
G4	0.0 C	-ve	0.0 a

(*): Different letters refer to significant differences among groups.

- HI titer after fourteen days post vaccination: there were significant differences in HI antibodies at $p > 0.01$ among groups, these differences were restricted to (G3), which elevated in titer significantly than (G2); which overcomes (G1), and no HI antibodies were detected in (G4) (table 3).
- CRP level after fourteen days post vaccination; the qualitative test was with negative result for all groups, in qualitative test the level was not detectable for all groups too (table 3).

Table 3: *HI-titre and C-reactive protein level fourteen days post vaccination:*

group	HI-titer Mean ± SE	C-Reactive protein level	CRP (qualitative level) Mean ± SE
G1	2.2 ± 0.6 C*	-ve	0.0
G2	6.7 ± 1.2 B	-ve	0.0
G3	20.0 ± 6.3 A	-ve	0.0
G4	0.0 D	-ve	0.0

(*): Different letters refer to significant differences among groups.

- HI titer after twenty days post vaccination: significant differences in HI antibodies were detected at p > 0.01 among groups, these differences were noticed in (G3), which elevated in titer significantly than (G2); then be (G1) in recording, and no HI antibodies were detected in control group (G4) (table 3) (figure 4) .
- CRP level at both qualitative and quantitative tests was negative after twenty days post vaccination for all groups (table 4).

Table 4: *HI-titre and C-reactive protein level twenty days post vaccination:*

group	HI-titer Mean ± SE	CRP (quantitative level)	CRP (qualitative level) Mean ± SE
G1	5.8 ± 0.9 C*	-ve	0.0
G2	11.4 ± 2.3 B	-ve	0.0
G3	25 ± 6.3 A	-ve	0.0
G4	0.0 D	-ve	0.0

(*): different letters refer to significant differences among groups.

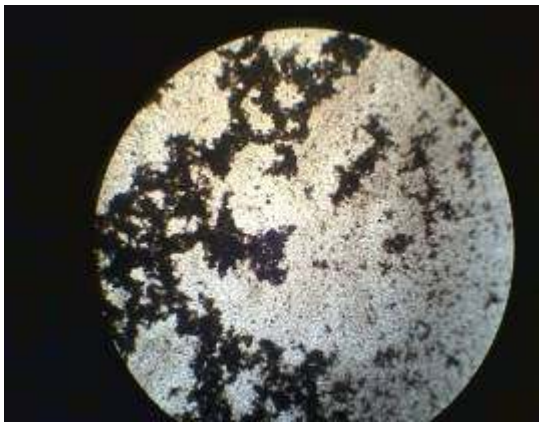

Figure1: CRP, the qualitative procedure (positive result).

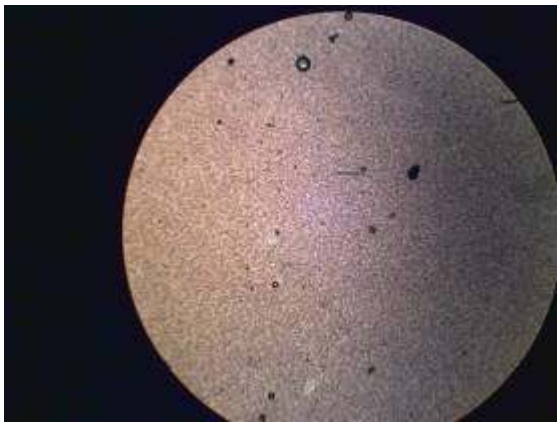

Figure2: CRP, the qualitative procedure (negative result).

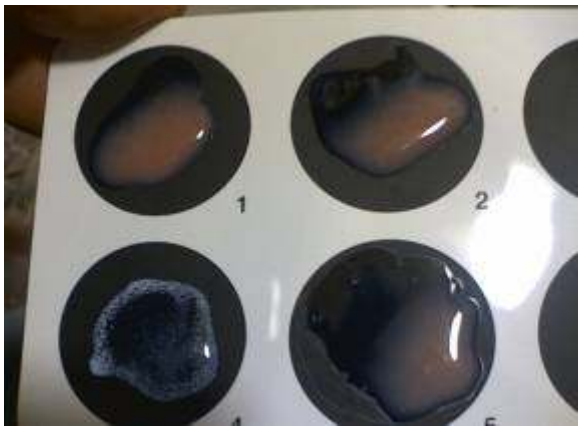

Figure3: CRP, the quantitative procedure (positive & negative results).

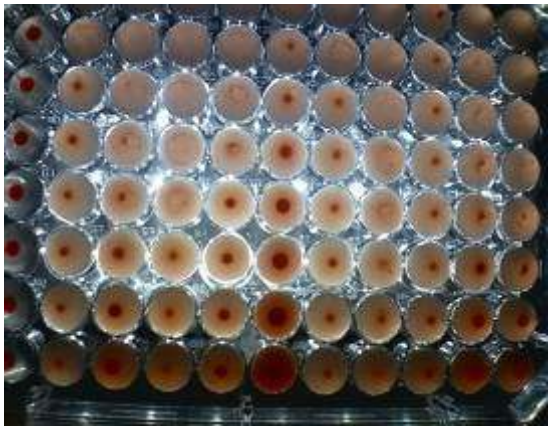

Figure4: Haemagglutination Inhibition test.

Discussion:

This study was suggested to determine the correlation between the immunological and inflammatory responses to the vaccination with ND vaccines in pigeons. The best known fact the vaccination of birds with the live ND vaccine is a usual mean for HI antibody elevation [15]. Oil- immersed vaccine was recently used with the same aspects [4], nowadays, the combination of killed and live ND vaccines gave a synergistic immune response as well [11].
No sufficient reports were found to support the inflammatory effects of ND vaccines at the level of serum proteins; the usual tool for judgment for these effects is still the histopathological changes in tissues [17]; and to emerge the precise role of the CRP in the inflammatory process proceed by vaccination.
The results showed that there were significant immune responses after vaccination with either killed or alive ND vaccine or both [16]. On the other hand the birds show only limited inflammatory responses after vaccination with live vaccine only [18]. These postulated data decided that live virus-infection (disease or vaccine) able to induce an inflammatory response; this induction can be detected by measuring CRP, the conclusion met with [18 and 4]; also its clear that the inflammatory response arise abruptly within two days post vaccination with live vaccines only; but the effective immune response may delay as far as two weeks average, this met with [4 and 5].
The conclusion is that live vaccines-based vaccination programmes can induce an inflammatory response as well as the immune response, while those programmes based on combination between live and inactivated vaccines were stronger in immune response induction, but the inflammatory response was the same, and no inflammatory response in killed vaccines-based vaccination programmes, also such programs were with limited immune response.
The recommendation is that domesticated pigeons can be successfully vaccinated with live and killed ND vaccines to well gain immune response at their sera; on the other hand only the live vaccines are able to induce an inflammatory effect in such pigeons, so that it need more studies to find out any other adverse effects of vaccines on other biological activities of birds.

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