EVALUATION OF NEWCASTLE DISEASE VIRUS MATERNALLY DERIVED ANTIBODIES IN QUAIL CHICKS FOR ESTIMATION OF PROPER VACCINATION TIME.

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ABSTRACT

The present study was conducted to monitor the persistence of Maternal Derived Antibody (MDA) in quail chicks during first five weeks of their life and its effect on the immune response to Newcastle disease virus vaccination (LaSota and Hitchner B1).The obtained results showed that, the MDA HI titers ranged from 5 to 7 (log_2) with a geometric mean of 6.2 - 6.4 (log_2) at the end of first week. At the end of the second week of age (day 14) the MDA HI titers were decreased with a mean titer of 5.1 - 5.2 (log_2). At the end of the third week, only 40% of quail chicks (4/10) showed titers more than 5 (log_2), whereas 60% of the quail chicks titers less than 4(log2) in HI test. One week later (day 28), all of the quail chicks showed titers less than 4 (log_2) in HI test. At the end of the fifth week, (day 35), the mean titer was decreased to its minimum level equal to ($\leq$ 2.0 log_2). The obtained results revealed that, the mean value of HI antibody titers declined in the first two weeks post vaccination with (LaSota and Hitchner B1) when vaccination take place at 7 day old, whereas higher and persistence antibody response takes place when vaccination applied at 14 or 21 day old.

The findings of our study suggest that the 14th day of age is recommended as the proper time to start the first vaccination against ND in flocks of quail chicks with maternal antibodies.

Keywords: Maternal antibody, Newcastle disease, Quail

INTRODUCTION

Newcastle disease (ND) is a one of the most serious and highly fatal disease affecting poultry worldwide ***(Alexander, 1997)***. Newcastle disease Virus (NDV) causing ND is an avian paramyxovirus serotype 1 (APMV-1) virus, belonging to genus Avulavirus, subfamily Paramyxovirinae, family Paramixoviridae, order Mononegavirales ***(Moro de Sousa et al., 2000)***. Japanese quails (Coturnix Japonica) production is extensively expanded worldwide, as it is easily adapted to commercial management conditions with good performance in terms of meat and egg production. Japanese quails among 241 species of birds from 27 of the 50 orders of birds are susceptible to Newcastle virus infection ***(Kaleta and Baldauf, 1988)***. Natural outbreak of ND in Japanese quail manifested clinically by central nervous system dysfunction with 100% morbidity and mortality was reported by ***(Czirják et al., 2007)***. Because there is no effective treatment for ND in poultry, vaccination against NDV is considered the best way of protection against Newcastle disease ***(Miller et al., 2007)***. In practice, vaccination for protection quails from Newcastle disease have been practiced and different vaccination schedules have been recommended using live and inactivated oil vaccines ***(Lima et al., 2004)***. ***Paulillo et al. (2009)*** evaluated clinical and immunological parameters of vaccinated Japanese quails against Newcastle disease by (Ulster 2C strain), (B1 strain), (LaSota strain) and (LaSota strain inactivated and emulsified in mineral oil) and found that Japanese quails vaccinated with NDV LaSota strain inactivated and emulsified in mineral oil strain produced high antibody levels while Ulster 2C, B1 and LaSota live strains produced moderated

antibody levels and did not cause any clinical signs associated with post vaccinal reactions. Despite using many and different vaccination schedules against NDV, outbreaks of Newcastle disease are still recorded in flocks of Japanese quail ***(Chandrasekaran and Aziz, 1989; Islam et al., 1994; Momayez et al., 2007; Merino et al., 2009)***. The ability of hens to transmit antibodies to their off spring was documented in chicken many years ago ***(Giambrone and Ronald, 1986; Hamal et al., 2006)***. The level of maternally derived antibodies and its effect on the immune response to early vaccination with live vaccines had been extensively studied in chickens ***(Mondal and Naqi, 2001; Al-Natour et al., 2004; Kejun et al., 2012)***. Although maternal antibodies are important to protect young chicks in their early critical days of life against infectious diseases, it may interfere with life vaccines administered to chicks and neutralize the vaccine antigen resulting in vaccination failure ***(Awang et al., 1992)***. There is paucity in literatures dealing with the studying of maternal antibodies in quail and its effect on the immune response to Newcastle disease virus. ***Grindstaff et al. (2005)*** reported that, in captive Japanese quail the amount of dietary proteins affected egg number and size but not egg yolk immunoglobulin levels. ***Grindstaff, (2008)*** also, reported that, quail vaccinated with killed avian Reo virus vaccine provided offspring with passive humoral immune defense and in addition it allowed them to partially maintain growth during infection.

The object of the present study is to monitor and evaluate changes of NDV maternal antibody level in quail chicks during the first weeks of their life to estimate and recommend a proper vaccination program against ND in quail chicks.

MATERIALS AND METHODS

Newcastle disease vaccines:

- Live Newcastle Disease vaccine (LaSota strain).
- Live Newcastle Disease vaccine (Hitchner B1 strain).

Newcastle disease virus Antigen

- Newcastle Disease vaccine (LaSota strain).

It was prepared by inoculating live LaSota vaccine in Specific Pathogens Free (SPF) embryonated eggs, allanotic fluid from inoculated SPF eggs was used as antigens in HI test after measuring its Haemagglutinating activities.

Quails: A total of One hundred and twenty (120) day old Japanese quails were obtained from three commercial quail farms named A, B and C (40 quails from each) located in Riyadh, Saudi Arabia.

Farms A and B practiced vaccination against NDV, whereas the third farm (C) was not practiced vaccination against NDV.

All birds were reared on litter floor and supplied with feed and water *ad libitum*.

Quails in the three groups were further subdivided into four subgroups each of 10 birds (A 1- A 4, B 1-B 4 and C1-C4).

Haemagglutination Inhibition (HI) test.

- HI test was performed according to ***(Allan and Gough, 1974)***.
- The HI test was performed using 4 UHA LaSota antigen against each serum sample.
- Sera were separated and heat treated at 56 C°/ 30 minutes and stored at -20C° until tested.

- HI test were performed by using 1% chicken red blood cells.
- Results were recorded as $\log_2$ X values of the highest reciprocal of the dilution which showed complete hemagglutination inhibition.

Experimental design:

Experiment 1.

It was designed to monitor the persistence of Newcastle disease maternally derived antibodies levels in quail chicks over 35 day period.

Three groups each of ten quails were designed as follows:

A 1: Fifteen quails from farm A

B 1: Fifteen quails from farm B

C 1: Fifteen quails from farm C

Blood samples were collected from quails in days 7, 14, 21, 28 and 35 of age.

Ten blood samples were taken on each time.

Experiment 2.

It was designed to evaluate the effect of maternal antibody level on the immune response of quails vaccinated by live Newcastle disease vaccines (LaSota and Hitchner B1) at different time (Table 1).

Nine groups each of 10 quails were designed as follows:

A 2: Ten quails from farm A were vaccinated with LaSota vaccine at 7 day of age.

A 3: Ten quails from farm A were vaccinated with LaSota at 14 day of age.

A 4: Ten quails from farm A were vaccinated with LaSota at 21 day of age.

B 2: Ten quails from farm B were vaccinated with Hitchner B1 at 7 days.

B 3: Ten quails from farm B were vaccinated with Hitchner B1 at 14 day of age.

B 4: Ten quails from farm B were vaccinated with Hitchner B1 at 21 day of age.

C 2: Ten quails from farm C were vaccinated with LaSota vaccine at 7 day of age.

C 3: Ten quails from farm C were vaccinated with Hitchner B1 at 7 days.

C 4: Ten quails from farm C were kept as non vaccinated control.

Table (1): Vaccination design of quails with LaSota and Hitchner B1 vaccines.

Farm	Groups	No. of Quail	Age at vaccination	Vaccine type	Dose	Method of administration
A	A1	10	--	---	--	--
	A2	10	7	LaSota	One x chicken dose/quail	Eye drop
	A3	10	14			
	A4	10	21			
B	B1	10	--	--	--	--
	B2	10	7	Hitchner B1	One x chicken dose/quail	Eye drop
	B3	10	14			
	B4	10	21			
C	C1	10	--	--	--	--
	C2	10	7	LaSota	One x chicken dose/quail	Eye drop
	C3	10	7	Hitchner B1		
	C 4	10	--	--	--	--

Blood samples were collected from quails at 7, 14, 30, 45 and 60 days post vaccination.

Ten blood samples were taken on each time.

Sera were separated and heat treated at 56 C°/ 30 minutes and stored at -20 C° until tested.

RESULTS AND DISCUSSION

The present study was conducted to monitor the persistence of Newcastle disease virus maternally derived antibody (MDA) in young quails hatched from parent flock vaccinated with Newcastle disease vaccine, as well as made a comparative evaluation of immune response of quail chicks following intra-ocular vaccination with live Newcastle disease vaccines (LaSota and Hitchner).

In the first experiment, ten sera samples obtained from ten randomly selected young quails at day 7, 14, 21, 28 and 35 from each of three groups of quails designed A1, B1 (hatched from vaccinated dams) and C1 (hatched from non vaccinated dams) were collected and tested for NDV maternal derived antibodies. The obtained results (Table 2 and figure 1) showed that, the mean HI titers were (6.2, 5.2, 4.3, 2.8 and 2.1), (6.4, 5.1, 4.1, 3.1 and 2.0) and (< 2.0) at the age of 7, 14, 21, 28 and 35days in groups A1, B1 and C1 respectively. The MDA HI titers ranged from 7 to 5 (log_2) with a geometric mean of 6.2 and 6.4 (log_2) at the end of first week for groups A and B respectively. At the end of the second week of age (day 14) the MDA HI titers were decreased with a mean titer of 5.2 and 5.1(log_2) for groups A and B respectively, with (80%) of the samples for both groups showed titers ≥ 5 (log2). At the end of the third week, only 40% of quail chicks (4/10) showed titers more than 5 (log2) for both groups, whereas 60% of the quail chicks titers less than 4(log2) in HI test. One week later (day 28), all of the quail chicks showed titers

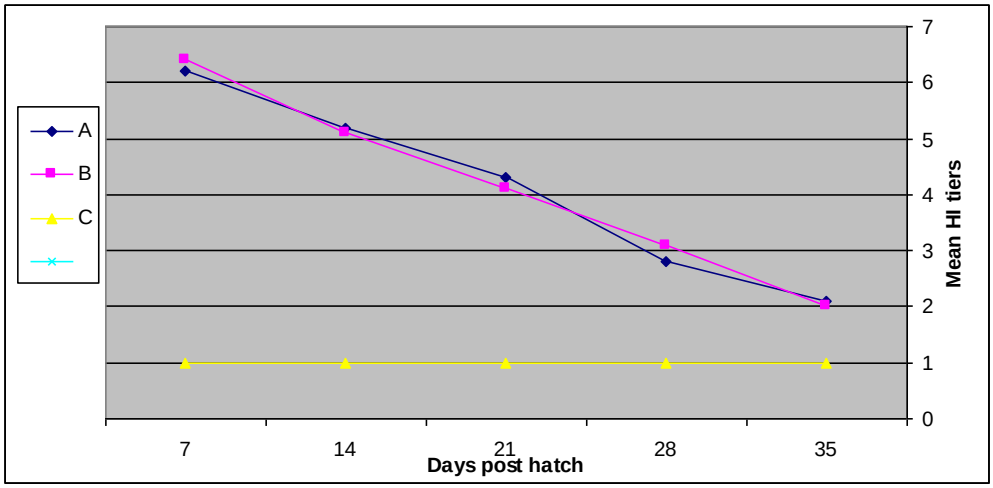
less than 4 (log2) in HI test. At the end of the fifth week, (day 35), the mean titer was decreased to its minimum level equal to (≤ 2.0 log2). The serological finding of group C (control group), ((< 2.0), confirmed that, no antibodies were elicited from either previous vaccination history or subjected to challenge virus during the experiment period. As compared with maternal antibody in chickens, our finding are slightly in agreement with results reported by ***(Saeed et al., 1988)***, who found that MDA declined to zero after the age of 25 days of chicks whereas, ***(Balla, (1986)*** reported that MDA persisted until day 27 of age of chicks.

There is a well-known correlation between NDV antibody HI titers and resistance to challenge by virulent NDV strains. A number of researchers have studied role of maternal antibodies in protection of chicks against virulent NDV in early ages, ***(Nagy et al 1991)*** reported that HI titers of ND antibody more than 4 (log2) are protective against mortality by virulent virus in chickens. Whereas, ***Jalil et al., (2009)***, reported that MDA titer of 128 or above protected chickens following challenge infection with virulent NDV. However, ***Erganis and Ucan, (2003)*** stated that a high HI titer more than 7 $\log_2$ of ND antibody may not totally correlate with protective immunity. On contrary, there is no available data on literatures on the role of maternal antibodies in protection of quail against challenge with virulent NDV. So, based on the obtained results in the present study, it can be assumed that at 21 days post hatching the quail were at high risk (60% of the quail chicks titers less than 4 ($\log_2$) in HI test) if they would expose to virulent NDV.

Table (2): Mean HI maternally derived antibodies titer (x log_2) of Newcastle disease in different groups of quail chicks for 35 days post hatch

Titer log2	Days post hatch														
	7			14			21			28			35		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	2	5	-	-	-	-	-	-	-	-	-	-	-	-	-
6	8	4	-	4	3	-	1	-	-	-	-	-	-	-	-
5	-	1	-	4	5	-	3	4	-	-	-	-	-	-	-
4	-	-	-	2	2	-	4	3	-	2	3	-	-	-	--
3	-	-	-	-	-	-	2	3	-	4	5	-	3	2	-
2	-	-	-	-	-	-	-	-	-	4	2	-	5	6	-
1	-	-	10	-	-	10	-	-	10	-	-	10	2	2	10
Mean	6.2	6.4	1.0	5.2	5.1	1.0	4.3	4.1	1.0	2.8	3.1	1.0	2.1	2.0	1.0

Fig. (1): Mean HI maternally derived antibodies titer (xlog_2) of Newcastle disease in different groups of quail chicks*



* Quails from farms A and B practiced vaccination against NDV, whereas the third farm (C) was not practiced vaccination against NDV.

In the second experiment, the effect of MDA on the immune response of quail chicks to vaccination with Newcastle disease vaccine LaSota and Hitchner B1 at different age were investigated for 60 days post vaccination and the results were presented in (table 3 and 4) respectively. The obtained results revealed that, the mean value of HI antibody titers in groups A2, A3, B2, B3, C2 and C3 were (4.9 and 4.6), (4.8 and 5.4), (4.5 and 4.2), (4.9 and 5.6) (4.3 and 5.9) and (4.8 and 5.2) which declined in the first week post vaccination in groups A2 and B2 compared to control group C2 and C3 (Hatched from non vaccinated dams). The drop in antibody titers in the first two weeks post vaccination may be attributed to the presence of maternal antibodies at a level that interfered with live vaccines in groups A2, A3, B2 and B3 compared to group C2 and C3. This finding agreed with results obtained by ***(Nasser et al., 2000)*** who observed that high percentage of chicken vaccinated against ND orally only once failed to produce protective level of antibodies and died against challenge. Whereas our results disagreed with finding obtained by ***Giambrone, (1985)***, who reported that, NDV HI titers were highest in chickens vaccinated at day old and revaccinated at day 14 with live vaccine by coarse spray. Later on, the mean value of HI antibody titers gradually increased from day 30 post vaccination onward for all vaccinated groups.

Table (3): Mean HI antibodies titer (x log_2) of Newcastle disease in quails chicks vaccinated with LaSota vaccine

Group*	Age at vaccination	Maternal antibody level	Mean NDV antibody HI titer (x log_2)				
			Days post vaccination				
			7	14	30	45	60
A 2	7	6.2	4.9	4.6	4.4	3.9	3.5
A 3	14	5.2	4.8	5.4	5.9	6.5	5.9
A 4	21	4.3	5.1	5.9	6.2	6.7	6.1
C 2	7	≤ 2	4.3	5.9	6.2	6.3	6.3
C 4	--	≤ 2	≤ 2	≤ 2	≤ 2	≤ 2	≤ 2

*A 2: Ten quails from farm A was vaccinated with LaSota vaccine at 7 day of age.

A 3: Ten quails from farm A was vaccinated with LaSota vaccine at 14 day of age.

A 4: Ten quails from farm A was vaccinated with LaSota vaccine at 21 day of age.

C 2: Ten quails from farm C was vaccinated with LaSota vaccine at 7 day of age.

C 4: Ten quails from farm C was kept as non vaccinated control

Table (4): Mean HI antibodies titer (x log_2) of Newcastle disease in quails chicks vaccinated with Hitchner B1 vaccine.

Group*	Age at vaccination	Maternal antibody level	Mean NDV antibody HI titer (x log_2)				
			Days post vaccination				
			7	14	30	45	60
B 2	7	6.4	4.5	4.2	4.6	3.9	3.5
B 3	14	5.1	4.9	5.6	5.9	6.3	5.7
B 4	21	4.1	4.7	5.1	5.4	5.9	6.4
C 3	7	≤ 2	4.8	5.2	5.6	6.1	5.5
C 4	--	≤ 2	--	--	--	--	--

* B 2: Ten quails from farm B was vaccinated with Hitchner B1 at 7 days.

B 3: Ten quails from farm B was vaccinated with Hitchner B1 vaccine at 14 day of age.

B 4: Ten quails from farm B was vaccinated with Hitchner B1 vaccine at 21 day of age.

C 3: Ten quails from farm C was vaccinated with Hitchner B1 at 7 days.

C 4: Ten quails from farm C was kept as non vaccinated control.

The results present in (table 3) revealed the, the mean HI titer of vaccinated quails were (5.9 & 6.1), (6.5 & 6.7), and (5.9 & 6.1) log2 at 30, 45 and 60 days post vaccination with LaSota vaccine when vaccination takes place at 14 and 21 days of age respectively. Similar results were obtained when vaccination takes place by Hitchner B1 (Table 4) which revealed that, there is no significant difference found when vaccination takes place at 14 or 21 days age.

The obtained results would be useful in order to estimate the proper time for the first vaccination date of quail against NDV. We could conclude that, the quail chicks are most susceptible to NDV around 21 days of age. Since maternally-derived antibodies can potentially neutralize the vaccine if done at a very younger age, the findings of our study suggest that the 14th day of age is recommended as the proper time to start the first vaccination against ND in flocks of quail chicks with maternal antibody.

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