,Assessment of the Immune Response using ND Clone 30 Vaccine through Eye Drop and Drinking Water in Ross 308 and Cobb 500

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Abstract

The study was designed to assess the immune response of Newcastle disease (ND) Clone 30 vaccine in Ross 308 and Cobb 500 breeds. Day old chickens, 105 Ross and 105 Cobb which were housed together, dietary and environmental conditions were identical in each six experimental boxes (A: Ross-ED, B: Cobb-ED, C: Ross-DW,D: Cobb-DW, E: Ross control and F: Cobb-control) 35 chickens for each box, the group A and B were vaccinated with Clone 30 through eye drop only at 8th day,18 day and 28 day, while C and D groups were vaccinated with Clone 30 through eye drop at 8 day and drinking water at 18, 28 day, and control E and F groups remain without vaccination. ELISA test was used to evaluate the level of immunity in serum of chickens in all groups at 5, 16, 26 and 36 days of age, the results of the tests showed that there were high level of maternal antibody titer at 5 days of age persist until 16 day of age. No significant differences (P>0.05) among the vaccinated groups and control group but a significant differences at 16, 26 and 36 days old chickens vaccinated eye drop or drinking water compared with control, the result tests showed higher level of immunity in chickens of group A and B as compared with groups C, D both induced protective local mucosal immunity. No significant differences was detected between two breeds (p > 0.05). We concluded that eye drop route as individual vaccination program followed by drinking water route results in a good immune response and maintaining high antibody level to ND Virus in both breeds Ross and Cobb can be used in area were ND risks are not controlled.

تقييم الاستجابة المناعية باستخدام لقاح نيوكاسل كلون 30 عن طريق التقطير بالعين وماء الشرب في روز308 وكوب 500

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الخلاصة

شملت الدراسة تقييم الاستجابة المناعية بإعطاء لقاح نيوكاسل كلون 30 بالطريقتين التقطير بالعين وماء الشرب لأفراخ سلالتي روز وكوب وضعت في نفس الظروف. تضمنت التجربة (210) فرخة منها 105 روز و105 كوب بعمر يوم واحد. تم تقسيمها الى مجاميع كل مجموعة تحتوي 35 فرخة في قاعة مهيئة في نفس الظروف البيئية والتغذوية. تم استخدام لقاح نيوكاسل كلون 30 مجموعتي (A و B) تم تلقيحهما بطريقة التقطير بالعين فقط في أعمار 8 و18و28 يوم أما مجموعتي (C و D) تم تلقيحهما بطريقة التقطير بالعين في عمر 8 أيام ثم التلقيح الثاني والثالث بطريقة ماء لشرب في عمر 18 و28 يوم وتركت مجموعتي (E و F) كمجموعتي سيطرة بدون تلقيح. تم قياس معيار الاضداد المصلية لتقييم الاستجابة المناعية بين المجاميع المختلفة من الأفراخ بعمر 5، 16، 26 و36 يوم باستخدام اختبار الاليزا. اظهرت النتائج ارتفاع مستوى عالي للمناعة الامية في عمر 5 أيام واستمرت لعمر 16 يوم مع عدم وجود فرق معنوي بين المجاميع الملقحة والسيطرة ووجود فرق معنوي بين المجاميع الملقحة بطريقتي

التقطير بالعين وماء الشرب مقارنة بمجاميع السيطرة في أعمار 16، 26 و36 يوم. كما بينت النتائج وجود مستوى عالي للمناعة في مجموعتي (A و B) الملقحة بطريقة التقطير بالعين مقارنة بمجموعتي (C و D) الملقحة بطريقة التقطير بالعين وماء الشرب وان كليهما أعطت مناعة موضوعية. ولم نلاحظ وجود فرق معنوي بين سلالتي روز وكوب. لذا تستنتج من خلال هذه التجربة بان طريقة التلقيح بالتقطير بالعين تتبعه التلقيح بطريقة ماء الشرب تعطي مناعة أعلى ومستمرة ضد فايرس نيوكاسل لسلالتي روز وكوب في المناطق موبؤة بمرض نيوكاسل والغير المسيطر عليها.

Introduction

Newcastle disease (ND) is a highly contagious viral disease, which affects almost all species of domestic and wild birds. This disease is caused by a virus of genus Avulovirus, family Paramyxoviridae, subfamily Paramyxovirinae (1). The disease was first rereported in Indonesia and England in 1926 (2) and Newcastle Disease viruses (NDV) is a worldwide spread. Vaccination is widely applied and is recommended method for prevention. The vaccines may be used by drops into the eye and through drinking water. In many viral infections humoral and cell mediated immune responses play a role in protection against such diseases (3). Both humeral and cell mediated immune responses are essential for complete protection (4). Several types of vaccines are available but the most widely used is the mild live virus vaccine as B1 and Lasota strains. Vaccines are provided in a standard dose and are not formulated to account for body weight or age (5). Vaccines can be administered using either individual or mass vaccination methods. Eye-drop and injection routes are individual methods used in the commercial poultry industry. Individual methods of application produce more consistent protection in more birds than mass vaccination methods (6). Immune responses of lymphoid organs to ND vaccines are influenced by routs (eye drop, drinking water) of vaccination. Eye drop vaccination stimulates Harderian gland to produce specific local antibodies (7) and significance increase in plasma cells in sections of Harderian gland (8) These findings suggest an important role for Harderian gland in protection immunity. Several Enzyme Linked Immunosorbent Assay(ELISA) kits are available commercially for the detection of antibodies against ND virus, and the main advantage of ELISA over more conventional tests, such as Haemagglutination Inhibition (HI) test, is that they can be semi-automated, results can be obtained rapidly, especially when sera are to be screened for antibodies against several viruses. ELISA for detection of NDV antibodies shows high reproducibility, with high comparative sensitivity and specificity and correlate well with HI test (9). Therefore this project was designed to assess the immune response of Ross and Cobb in the same rearing condition by using two routes (eye drop and drinking water) of vaccination in terms of protection against Newcastle disease virus.

Material and Method

Two hundred and ten chickens day old were obtained: 105 Ross from Ivan hatchery, 105 Cobb500 from Shemal company-Erbil. The chickens were housed together in six experimental boxes 35 chickens for each box, located in Girdarasha poultry farm- College of Agriculture- University of Salahaddin-Erbil. During April - May 2012.

- **Experimental Design:** Sex Group consist of replicate of A, B, C, D, E and F 35 chickens in each replicate either Ross or Cobb, ND Clone 30 vaccine H.I titer was between (1/64 to 1/128) when using 4HAU antigen from (Intervet Co.) against ND was

used through Eye drop or drinking water routes for A, B, C, D groups and Gumbo- L vaccine against IBD (Ceva Co.) was used also for A, B, C, D, E and F groups:

Group A (Ross): vaccinated in (eye drop) on 8 day, 18 day, 28 day old.

Group B (Cobb): vaccinated in (eye drop) on 8 day, 18 day, 28 day old

Group C (Ross): vaccinated in eye drop at 8 day and drinking water at 18 day, 28 day old

Group D (Cob): vaccinated in eye drop at 8 day and drinking water at 18 day, 28 day old

Group E (Ross): As control group.

Group F (Cobb): As control group.

Dietary and environmental conditions were identical in each of six groups. They were placed in separate boxes provided with litter in the form of (wood shaving). The chicks were supplied with basal scientific diet for commercial broiler chickens. TLC (Charm) analyses for Mycotoxins contamination showed in table (1).

Table (1) Feed analyses for Mycotoxins contamination

Toxins	Starter	Grower	Finisher
Aflatoxin	2 ppb	2 ppb	2 ppb
Ochratoxin	2 ppb	2 ppb	3 ppb
T2 toxin	8 ppb	7 ppb	14 ppb
Fumonisin	0.9 ppm	0.7 ppm	0.5 ppm

- **Blood collection:** Blood samples were obtained from 17% of each group's chickens on 5, 16, 26, 36 day. It must be noted that for obtaining blood samples from jugular vein on first 5 day the chickens were beheaded, but blood samples 1-2ml collected from brachial vein at the age 16, 26 and 36 days old (10). Blood samples was collected from chickens of different ages, were allowed to clot at room temperature then centrifuged at 2500 rpm for 15 minutes, and then the sera were collected, labeled and transported immediately to the laboratory for further analysis. Serum samples were analyzed by using kit Flock Check Idexx ELISA Test Kit (Idexx Laboratories, Beijing, China). The procedure was followed according to the manufacturer's directions.
- **Statistical analysis:** Statistical analysis data were expressed as mean ± SE. Data were compared and determined by Duncan's Multiple Range Test. Data processing was performed using the SAS statistical programme (2005), version 9.1(SAS Institute Inc., Cary, N. C., USA (21).

Results

The study was conducted to assess the level of antibody production in Ross and Cobb broiler chicks and persistence of maternally derived antibody (MDA) in broiler chickens, as well as, to compare the effect of routes in the level of antibody production in such birds following vaccination in different routes with ND-Clone30 vaccine. Birds of four groups A, B, C and D were administered with the above vaccine through eye drop and drinking water and five sera samples obtained randomly from each group on 5, 16, 26 and 36 days of age were subjected to ELISA test. Maternally derived antibody (MDA) was monitored on group-E Ross and group-F Cobb chickens starting from day 5 to day 36 and the titers are presented in Table 2. It was found that the MDA level persisted at a suitable level up to 16 days and it started to decline after 16 days and reached at a negligible level after 26 days. A comparative embodiment is illustrated in Table-2-. There was no significant difference between the antibody titers of the different group samples before vaccination (5 days old), the result show at age 16^{th} day in group A,B.C and D was (3.17±0.14, 3.18±0.17, 3.12±0.11, 3.04±0.16) respectively after 8 days post first dose compared with Control group E and F was (2.35±0.12, 2.38± 0.13), while the antibody titer at day 26 was elevated

in group A and B (3.89±0.17, 3.71±0.17) and declined in group C and D it show (2.81±0.08, 2.88±0.18) respectively after 8 days post first booster dose compared with control group E and F (2.23±0.08, 2.28±0.11) also the titers at age 36 in group A,B,C and D it showed (3.94±0.11, 3.82±0.06, 3.76±0.05, 3.58±0.08) respectively after 8 days post second booster compared with the antibody titer in control group E and F in same age was (1.85±0.11, 2.02±0.05). An illustrative of Mean (log10) ±SE ELISA titers at age 26 and 36 in group A, B, C and D compared with titer of non-vaccinated control birds group E and F is shown in table-2-. It was observed that (MDA) ELISA titer before vaccination stage of birds at five days of age of Ross chickens ranged (4.02±0.07 to 4.18±0.02) while for Cobb chickens ranged (4.04±0.07to 4.19±0.02).

Table (2) Mean (log 10) ±SE of post-vaccination ELISA titers obtained by eye drop and drinking water compared to unvaccinated control group among Ross and Cobb

Antibody Titer mean± Standard Error							
Age of birds	Day post vaccination	Group-A (Ross chickens)	Group-B (Cobb chickens)	Group-C (Ross chickens)	Group-D (Cobb chickens)	Group-E (Ross chickens)	Group-F (Cobb chickens)
		Eye drop Mean ±SE	Eye drop Mean ±SE	Drinking Water Mean ±SE	Drinking Water Mean±SE	Control Mean±SE	Control Mean ±SE
5	-	4.16± 0.03[a]	4.07± 0.08[a]	4.18± 0.02[a]	4.04± 0.07[a]	4.02± 0.07[a]	4.19± 0.02[a]
16	8	3.17± 0.14[a]	3.18± 0.17[a]	3.12± 0.11[a]	3.04± 0.16[a]	2.35± 0.12[b]	2.38± 0.13[b]
26	8	3.89± 0.17[a]	3.71± 0.17[a]	2.81± 0.08[ab]	2.88± 0.18[a]	2.23± 0.08[c]	2.28± 0.11[c]
36	8	3.94± 0.11[a]	3.82± 0.06[ab]	3.76± 0.05[ab]	3.58± 0.08[b]	1.85± 0.11[c]	2.02± 0.05[c]

SE=Standard Error; - =Not done

Discussion

Despite the widespread use of different sources and strains of NDV vaccines in different routes, ND continues to be a major threat to the poultry industry. One way of controlling this problem is improving efficacy and the way of vaccination by different routes. In this study an attempted to compare two vaccination programs (eye drop and drinking water) against ND using Clone 30 strain which were given in 8, 18 and 28 days of age and serum was collected at 8 days post-vaccination. When there is low titer of antibody particularly in endemic area, Veterinarian aloud to repeat a booster dose each 7 to 10 days to reached the peak level of antibody, this is accepted with (11). One of the result of the present study was maternal derived antibody titer shown in table-2, such antibody obtain, if the breeding hen vaccinated against ND, some resultant antibody produced and retained in yolk sac transmitted to the progeny chickens after hatching remains in chickens after 16 days of age following which declined (12). Based on obtained result from present study, maternal antibody titer in selected groups Ross chickens was (4.16± 0.03 to 4.07± 0.08) while for Cobb chickens was (4.18± 0.02 to 4.04± 0.07) in group A, B, C and D respectively, group A, B received eye drop vaccine and group B, C received vaccine through drinking water on 8 days and antibody titer calculated on 16 days of age the rate of which in six groups did not show meaningful difference ($P>0.05$) due to high level of MDA which neutralize virus vaccines, this is in line with (13). ELISA antibody titer of different groups at different days at 5 days of age and after 8 days post vaccination at age 16, 26 and 36 days is shown in table-2 as measured as log10 mean with standard error. The antibody titer gradually raised 8 days post booster dose and antibody titer increased significantly ($P>0.05$) in group A and B vaccinated via eye drop as shown in table-2- serum collected at day 26 of age either Ross and Cobb antibody titer was approximately 3.89± 0.17 and 3.71± 0.17 for Ross and Cobb respectively when compared with group C and D vaccinated through drinking water in the same age, duration and vaccine the antibody titer was 2.81± 0.08 and 2.88± 0.18 for Ross and Cobb respectively, and both are significant when

compared with control group E and F. A comparative study is illustrated in Table-2-. This is in line with spradbrow (14), who studied significant increase in plasma cell in section of Harderian gland and (15) suggested that eye drop vaccination of one day old give sufficient uniform immunity to protect chickens this is due to live vaccine replicates quickly in the mucosal membrane of the conjunctiva and Harderian gland. In the other hand our result also agreed with (16), who reported that gastric secretions provided a non-specific barrier against invaders and destroyed them. Thus some of vaccine virus given through drinking water cause denaturized and resulting in reduce antibody titer in group C and D. The result in the groups E and F chickens were susceptible to Newcastle virus after 16th days whereas group A, B, C and D chickens had proper immunity level on 26th day and afterward. A number of researchers have reported that live ND vaccines give better protection and health status (17,18). The use of live vaccines is preferred for priming the birds as it produces local immunity in the mucosal membrane of the conjunctiva, thus providing immediate protection on subsequent exposure with virus (18). Also it was found that live ND vaccines administered by eye drop or drinking water induce protective mucosal immunity (18). It is speculated that the results obtained in our study indicate that the high antibody titer in group A and B on day 36 is due to the second booster dose on (day 28) with ND Clone 30 vaccine, which replicated quickly in the mucosal membrane of the conjunctiva, inducing local immunity. It is preferable that priming vaccination of the birds should be carried out when the maternal antibody titer drops to the level where it does not interfere with the vaccine. In the current study, the initial vaccination was carried out at the time when the maternal antibody titer (log 10 mean SE) in Ross and Cobb was between (4.02±0.07) and (4.19±0.02) was diminishing (2.35±0.12) to (2.38±0.13) respectively, this is accepted with (19). We concluded that vaccination through eye drop followed by drinking water route give a good immune response for eliciting and maintaining high antibody level to NDV in both breed (Ross and Cobb).

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