

## **Immune response in day old broiler chicks vaccinated against Newcastle disease virus**

**A. Z. Al-Zubeedy**

Department of Microbiology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

### **Abstract**

One day old broiler chicks from four groups of broiler breeder chickens were immunized by different routes of vaccination. (1: orally), (2: intraocular), (3: a single injection of oil emulsion vaccine), (4: unvaccinated). The first two groups were vaccinated with live NDV vaccine. Serological antibody titers were determined to study the correlation between the different groups using ELISA test and leukocyte migration inhibition test to study the cell-mediated immune response in different groups. By results of this study vaccination of one day old chicks is very important to enhance the maternal derived antibody response also the cell-mediated immunity play an important role in increase the level of immunity beside the humeral one.

**Keywords:** Maternal immunity, Cell mediated immunity, ND, Vaccine, ELISA test.

Available online at <http://www.vetmedmosul.org/ijvs>

### **الاستجابة المناعية لأفراخ اللحم الملقحة بعمر يوم واحد ضد مرض النيوكاسل**

**أنوار زكي داؤد**

فرع الاحياء المجهرية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

### **الخلاصة**

تم دراسة الاستجابة المناعية لأفراخ لحم بعمر يوم واحد بعد تقسيمها الى اربعة مجاميع حيث لقت بطرق مختلفة الاولى عن طريق الفم والثانية عن طريق التقطير بالعين والثالثة عن طريق الحقن باللقاح الزيتي المبطل والرابعة لم تلقح باي لقاح وقد كانت المجموعة الاولى والثانية قد لقت بلقاح نيوكاسل الحي المضعف. تم قياس معيار الاضداد المصلية لدراسة العلاقة بين المجاميع المختلفة باستخدام اختبار الاليزا كما استخدم اختبار تثبيط هجرة خلايا الدم البيضاء لدراسة المناعة الخلوية للمجاميع المختلفة. اشارت نتائج هذه الدراسة ان تلقيح الأفراخ بعمر يوم واحد ضد مرض النيوكاسل وبطرق مختلفة جدا مهم لتعزيز المناعة الامية اضافة الى الدور الذي تلعبه المناعة الخلوية في رفع مستوى المناعة بجانب المناعة الخلطية.

### **Introduction**

Newcastle disease (ND) Caused by ND virus (NDV) which is an Avulavirus, is one of the most important disease encountered in the poultry industry (1,2). Vaccination for protecting chickens from Newcastle disease is routinely practiced throughout the world (3). Today there are commercial live and inactivated oil adjuvant vaccines (IOAV) which are very effective as immunization antigen. The live ones are produced from lentogenic and mesogenic virus strains namely Mukteswar and Komarov are

commercially available (4) various kind of live vaccination techniques namely, oral administration through drinking water, course spray, eye drop, intranasal installation, subcutaneous and muscle injection (4). Vaccination one day old broiler chicks which possess natural maternal antibodies show pronounced immunity between 3 and 4 weeks of age, the ability of mothers to transmit antibodies to their off spring was documented in both mammals and birds over 100 years ago (6-8) maternal antibodies is protective and during the vaccination the maternal antibodies neutralize the vaccine antigen rendering the vaccine in effective (9)

also the age of chicks at vaccination and the level of maternal antibody greatly influence immune response of broiler chickens to the vaccinal antigen (9). Immune system of poultry is a complex network of different cell types and soluble factors that give rise to an effective response to pathogenic challenges (10) both cellular and humeral response have been suggested to play important roles in the hosts defense against NDV infection (11,12).

The objective of the present study was to know the effect of different vaccination routes of one day old broiler agaisned NDV on immunological response (humeral and cellular).

## Materials and methods

### Experimental chicks

A total of 160 day old broiler chicks were housed and kept adlibidum feed on concentrated feed. These chicks were not given any types of antibiotics or vaccine and were divided into four groups each having 40 birds; the first group: forty chicks were vaccinated at one day, 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> days old with 0.1 ml (one vaccinated dose) of live attenuated Newcastle disease virus vaccine orally. The second group: forty chicks were vaccinated at one day 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> days old with oil and of live attenuated Newcastle disease virus (LNDVV) by eye drop. The third group: forty chicks were vaccinated at one day old with 0.1 ml of inactivated ND vaccine subcutaneously.

### Blood collection and serological test

Blood samples from test and control were collected at 1<sup>st</sup>, 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> days of age, all the blood samples obtained from the heart, serum were separated and stored at -20°C until the analyzed test preparing the serum sample were analyzed by indirect ELISA (enzyme linked immunosorbant assay) to detection antibodies against ND (13-15). For migration inhibition test blood sample with EDTA of all group were studied capillary migration inhibition test the migration inhibition index was calculated using the following formula:

$$\text{Migration index (MI)} = \frac{\text{Distance of migration with antigen}}{\text{Distance of migration without antigen}} \times 100$$

MI of  $\leq 70\%$  was considered as significant result, the migration index represent the mean of four reacting (11,12,16).

Statistical analysis data of all experimental were expressed as mean  $\pm$  SE. data were compared two analysis of variance. Significant differences determined by Duncan's Multiple Range Test. All statistical analysis performed by sigma state (Jandel scientific software V3.1)  $P < 0.05$  was considered as statistically significant.

## Results

Results of antibody titers against ND virus in different groups are presented in table 1. significant difference was found between the antibody titers of vaccinated groups (first and second group with the third group) 7 days post vaccination and significant difference was found between the vaccinated groups 14<sup>th</sup> days post vaccination also the antibody titers between the vaccinated groups and control group were different significantly in contrast the titer was significant in first group 21 days post vaccination and 7<sup>th</sup> days old post vaccination in second group while the antibody titers were significant in 14<sup>th</sup> days post vaccination in third group, the control group referred to significant result, 1<sup>st</sup>, 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> days of age, these results more explained in figured the result of cell-mediated immunity by migration inhibition test gave an inhibition of migration of leukocyte with significant index and this inhibition is increase in three vaccinated groups but the third groups was more one of them (Table 2).

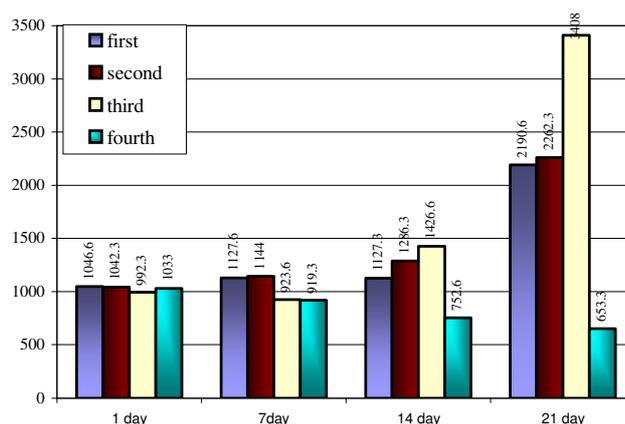


Figure (1): ELISA antibody titers in experimental groups.

## Discussion

Among the infectious diseases, Newcastle disease is deadly viral disease of poultry due to its high contagiousness and rapid spreading among chicken and other domestic and semi-domestic species of birds (3,17).

The result of the present study revealed that antibody tires of different vaccinated groups in 14<sup>th</sup> days' post vaccination were different significantly. This variation according to the type of route of vaccination, so the reason

Table (1): ELISA antibody titers against ND virus in experimental groups.

Group	NDV antibody titers (Mean±SE)			
	1 day	7 day old	14 day old	21 day old
First	1046.6	1127.6	1127.3	2190.6
	± 27.4 A a	± 52.3 A a	± 105.8 A a	± 68.8 A b
Second	1042.3	1144.0	1286.3	2262.3
	± 24.8 A a	± 31.1 A a,b	± 33.7 A b	± 74.1 A c
Third	992.3	923.6	1426.6	3408.0
	± 27.1 A a	± 56.4 B a	± 48.7 B b	± 140.7 B c
Fourth	1033.0	919.3	752.6	653.3
	± 21.9 A a	± 56.4 B a,c	± 45.9 A b,c	± 28.3 C b

A,B,C: values within a column followed by different letters are significantly different (P < 0.05).

a,b,c: values within a raw followed by different letters are significantly different (P < 0.05)

Table (2): Results of migration indices in different vaccinated groups.

Group	Age			
	1 day	7 day	14 day	21 day
First	0.9 NS	0.4 S	0.3 S	0.2 S
Second	0.8 NS	0.5 S	0.5 S	0.3 S
Third	0.9 NS	0.8 NS	0.8 NS	0.3 S
Fourth	1 NS	0.8 NS	0.8 NS	0.7 S

S = significant, NS = Non significant.

of high antibody response in third group (oil emulsion vaccine) that was inactivated vaccine was highly immunogenic compared to that of live vaccine (3) also inactivated vaccine was more capable of eliciting an immune response in the face of existing antibody in spite of generally slower onset of immunity (18) in contrast the second group (eye drop vaccination) of one day old give sufficient immunity to protect chickens this due to live virus vaccine replicates quickly in the mucosal membrane of the conjunctiva and nostrils also the virus strain replicate in the harderian gland and induce the IgA in the tears (1,19) as a source of local immunity all these reasons come from using the live vaccine by eye drop and the high antibody titers by this method remain as boosting dose using for longer-lasting immunity (20), the antibody titres of first group (orally vaccinated) was the lower one it was more susceptible one with maternal immunity although ability to neutralize the live virus vaccine and induction the immunity with the present boosting dose (1,18) the choice of method vaccination affected with the other factors than

maternal antibody these were type of production, bird species, size of flock, length of production cycle, general health status vaccines to be applied and costs (21) the antibody titers of control group (unvaccinated) declined gradually from one day old to 21<sup>st</sup> days of age and accepted with there of (12,18,21,22). The cellular beside the humeral response have been suggested to play important roles in the hosts defense against NDV infection cell-mediated immunity has been reported as the first immunological response, being detected as early as 2-3 days after ND vaccination (10,12,23,24). By result of the present study cell mediated immunity is an important factor but play a minor role in chicken vaccinated with ND vaccine (11,12,25)

In conclusion, the results of this study support the concept that humeral immunity to NDV is a key component in the protection against ND. Therefore, vaccination programs should be directed toward eliciting and maintaining high antibody level to NDV in flocks of birds.

#### Acknowledgment

This study was supported by the College of Veterinary Medicine, University of Mosul, also greet full thanks to Dr. M. Y. Al-attar and Dr. S. Y. Al-baroody, Department of Microbiology.

#### References

- Chansiripornchai N and Sasipreeyajan J. Efficacy of live B1 or ulster 2C Newcastle disease vaccines simultaneously vaccinated with inactivated oil adjuvant vaccine for protection of Newcastle disease virus in broiler chickens. *Acta Veterinaria Scandinavica*. 2006;48(2): 1-4.
- Abbas A, Muneer MA, Ahmed MD, Khan MA, Youns M and Khan I. Comparative efficacy of five different brands of commercial Newcastle disease Lasota virus vaccines in broilers. *Pakistan Vet J*. 2006;26(2):55-58.
- Rahman MM, Bar ASM, Giasuddin M, Islam MR, Alam J, Sil GC, Rahman MM. Evaluation of maternal and humeral immunity against Newcastle disease virus in chicken. *Interna J Poul Sci*. 2002;1(5):161-163.
- Lancaster JE. The control of Newcastle disease. *Worlds Poul Sci J*. 1981;37: 84-96.
- Chandana Manna, Manna SK, Das R, Batabyd K and Roy RN. Development of in ovo vaccine against Newcastle disease of birds. *Current Sci*. 1993; 9:1305-1309.
- Jennifer LG, Edmund DB, Ellen DK. Immune function across generations: Integrating mechanism and evolutionary process in maternal antibody transmission. *Proc R Soc Lond*. 13.2003; 270:2309-2319.
- Hamal KR, Burgess SC., Pevzner I.Y. and Erf GF. Maternal antibody transfer from dams to their egg yolks, egg white and chicks in meat lines of chickens. *Poult. Sci*. 2006; 85: 1364-1372.
- Giambrone JJ, Ronald PC. Vaccination of day-old broiler chicks against Newcastle disease and infectious bursal disease using commercial live and or inactivated vaccines. *Avian Dis*. 1986; 30(3): 557-561.
- Awang IPR., Wan WS. And Abdurazak J. Detection of maternal antibodies against Newcastle disease virus in chicks using an indirect immunoperoxidate test. *J Vet Malaysia*. 1992;4: 19-23.

10. Sarker N., Tsudzuk M., Nishibori M., Yasue H. and Yamamoto Y. Cell mediated and humoral immunity and phagocytic ability in chicken lines divergently selected for serum immunoglobulin M and G levels. *Poult Sci.* 2000;79:1705-1709.
11. Reynolds DL and Maraqa AD. Protective immunity against Newcastle disease: The role of cell mediated immunity. *Avian Dis.* 2000;44: 145-154.
12. Al-Shahery MN, Al-Zubedy AZ, Al-Baroodi SY. Evaluation of cell mediated immune response in chickens vaccinated with Newcastle disease virus. *Iraqi J Vet Sci.* 2008;22(1):21-24.
13. Cardoso WMA., Filho A., Romao JM., Salles PPR and Camara. Interference of infectious bursal disease virus on antibody production against Newcastle disease and infectious bronchitis virus. *Brazilian J Poult Sci.* 2006;8(3):177-182.
14. Kulikova L, Jurajda Vandjuranova R. Effects of infectious bursal disease vaccination strains on the immune system of leghorn chicken. *ACTA. Vet BRNO.* 2004;73:205-209.
15. Tran Quang VU, Lohr JE, Kynle MN, Lessin KH, Baumann MPO. Antibody levels against Newcastle disease virus infectious bursal disease virus and avian influenza virus in rural chickens in Viet Nam *Int J Poult Sc.* 2002; (5): 127-132.
16. Pawan KA. And Reynold DL. Evaluation of cell mediated immune response of chickens vaccinated with Newcastle disease virus as determined by the under agarose leukocyte migration inhibition technique. *Avian disease.* 1991;35: 360-364.
17. Osman E and Ucan US. Evaluation of three different vaccination regimes against Newcastle disease in central Anatolia. *Turk J. Vet. Anim. Sc.* 2003;27: 1065-1069.
18. Marangons and Busani L. The use of vaccination in poultry production *Rev. Sc., Tech. off. Int. Epiz.* 2006;26(1): 265-274.
19. Alexander DJ. Newcastle disease. In *diseases of poultry* (Y.M. Saif, ed.), 11th Ed. Iowa State University Press. Ames. 2003;64-87.
20. Giambrone J.J. and Closser J. Effect of breeder vaccination on immunization of progeny against Newcastle disease 1990; 34: 114-119.
21. Bermudez AJ and Stewart Brown B. Disease prevention and diagnosis. In *diseases of poultry* (Y.M. Saif, ed.), 11th Ed. Iowa State University Press, Ames. 2003;17-54.
22. Dashab Gh, Sadeghi Gh and M. Performance and humoral immune response to Newcastle disease in two strains of broiler chickens. *Journal of animal and veterinary advances.* 2007;6(3): 451-453.
23. Beard CW. And Brugh A. Immunity to Newcastle disease. *Am. J. Vet. Res.* 1975: 509-512.
24. Andereason C. Band Latimer KS. Separation of avian heterophils from blood using Ficoll-Hypaque discontinuous gradients. *Avian diseases.* 1989;33: 163-167.
25. Alexander AJ. And Manvell DJ. Experimental assessment of the pathogenicity of the Newcastle disease viruses outbreaks in Great Britain in 1997 for chickens and turkeys and the protection afforded by vaccination. *Avian Pathol.* 1999;28: 501-511.