COMPARATIVE IMMUNE RESPONSE OF BROILER CHICKS TO NEWCASTLE DISEASE VACCINE (LASOTA STRAIN)

M. Shuaib, M. Ashfaque, Sajjad-ur-Rahman, M.K. Mansoor and I. Yousaf [1].
Department of Veterinary Microbiology, [1] Department of Clinical Medicine and Surgery, Faculty of Veterinary Science, University of Agriculture, Faisalabad 38040, Pakistan

ABSTRACT

An experimental study was designed to asses the humoral and cell mediated immune response in the broiler chicks double vaccinated against Newcastle disease (ND) using LaSota strain of ND virus vaccine. Double vaccination 7 days following 1st vaccination gave haemagglutination inhibition (HI) titers ranging from 1:16 to 1:128, that was significantly higher than the HI antibody titer recorded after single vaccination. Similarly, macrophage migration inhibition (MMI) activity ranged from 28.57 to 40.86%, with mean activity of 36.07%. No correlation was found between HI titer and MMI test.

Key words: Haemagglutination, Newcastle disease vaccine, macrophage migration, broiler chicks.

INTRODUCTION

Pakistan poultry industry is still suffering losses of million of rupees due to Newcastle disease. Biosecurity and vaccination are two important measures to address the problem. Vaccination protects the birds by producing protective antibody titer and cell mediated immune response. Both these responses are essential for complete protection against infections (Chandraseker *et al.*, 1989). Cell mediated immunity (CMI) is produced when infection causing microorganisms enter and grow within tissue cells e.g. Newcastle disease (Duguid *et al.*, 1978). Impairment of cell mediated immunity results in increased susceptibility to virus infections (Lodmell *et al.*, 1973). Similarly, humoral immunity responds to any infection by producing antibodies against that specific infection so the vaccination would be successful only when it produces CMI and humoral immunity to a level, which is protective against the specific disease. To assess whether a vaccine has been successful in producing a desired immune response, different serological tests are used e.g. HI, IHA and MMI test. In this study HI and MMI tests were used to measure the humoral and cell mediated immune response in chicks receiving two shorts of Newcastle (LaSota strain) disease (ND) vaccine with an interval of 7 days.

MATERIALS AND METHODS

Experimental birds

Three groups of broiler chicks i.e. A, B and C containing 10 birds each were formed and provided optimal with monumental conditions. The group A was kept as unvaccinated control, while the birds of group B were primed with commercially available ND vaccine (LaSota strain) through drinking water at 14 days of age and then boosted at 21 days of age. Birds of group C were also vaccinated with commercially available ND vaccine (LaSota) through drinking water at the age of 14 days. Blood samples were collected aseptically at fourteen days post boosting. Serum was separated and stored at –20 C for HI antibody titration, while the plasma along with buffy coat was treated with LaSota strain of ND virus and stored for MMI factor determination.

Macrophage migration inhibition test

To determine MMI factor, Harrington *et al.* (1973) method was used with slight modification, as suggested by Shuaib (1993). For collection of chicken peritoneal macrophages, Sephadax (G 50) stimulation method was used, as described by Qureshi and Miller (1991). Macrophage- inhibition test was performed in 24 well disposable polystyrene titration plates using agarose droplet method. Vaccinated samples were subjected to macrophage migration measurements, and percentages were calculated in relation to the control using following formula:

$$\text{Macrophage migration (\%)} = \frac{\text{Migration distance in the presence of antigen}}{\text{Migration distance in the control}} \times 100$$

Similarly, percentage macrophage migration inhibition was determined as follows:

Macrophage migration inhibition (%)
= 100 - %age macrophage migration

Haemagglutination inhibition test

The serum samples were inactivated by heat treatment at 56 ^{0}C for 30 minutes in a water bath. These inactivated serum samples were used for HI antibody titration (Allan *et al.*, 1978). Geometric mean HI antibody titer of all the sera were determined, as described by Burgh (1978).

RESULTS AND DISCUSSION

The HI antibody response in double vaccinated group (B) birds ranged from 1:16 to 1:128, where 10% samples showed HI antibody titer of 1:16, 20% samples showed a titre 1:32%, 50% samples showed 1:64 and 20% samples showed a titre of 1:128. The overall HI antibody titer (GMT) against LaSota strain of NDV in this group was 55.692.

The HI antibody response in single vaccinated group (C) birds ranged from 1:8 to 1:32, where 40% samples showed HI antibody titer of 1:8, 40% samples showed a titre of 1:16 and 20% samples showed the titre of 1:32. The birds in this group showed an overall HI antibody titer with GMT of 13.931.

The unvaccinated controlled group (A) showed an overall antibody titer with GMT of 3.99. The HI antibody response in this group ranged from 1:2 to 1:8, where 40% samples showed 1:2 HI antibody titer, 20% samples showed 1:4 HI antibody titer and 40% samples showed 1:8 HI antibody titer.

The results of this study show that booster vaccination of NDV results in higher antibody titer than single vaccination. These results are in agreement with those reported by Matuka *et al.* (1980), who reported a higher antibody titer in birds previously vaccinated with Mukhteswar or LaSota and revaccinated with LaSota strain of NDV. Similarly, Giamborne (1981) reported the greatest serologic response and best resistance to clinical ND with low level of maternal immunity at the time of 1st vaccination at one day of age and a booster vaccination at 20th day of age. The present study concludes that the 2nd vaccination is necessary to attain the higher protection against ND.

The MIF activity of the control group samples showed an average migration of macrophages as 410.23µ. In contrast to this, the double vaccinated group (B) showed that macrophages migration inhibition activity ranged from 28.57% to 42.86% with the average of 36.07% (Table 1). Further, it was found that maximum MIF activity of 42.86% in this group was recorded in 40% samples, followed by 35.7% in 20% samples, 32.13% in 10% samples and 28.5% in 30% samples. In group C with single vaccination maximum MIF activity of 35.7% was recorded in 40% samples, followed by 28.57% in 30% samples, 21.43% in 20% samples and 14.29% in 10% samples (Table 2). The percent macrophage migration inhibition of the chicks observed after double vaccination was significantly higher than that recorded after single dose of vaccination. These results are in line with Onaga and Ishii (1980), who reported a high leucocytes migration inhibition factor in thrice-inoculated chicks with *Eimeria tenella* even three weeks after last inoculation. The results are also in agreement with those reported by Konopa (1984), who studied cell mediated immunity in experimental listeriosis in different animals and reported that strongest and most durable cell mediated immune response was induced by subcutaneous injection of immunogenic strain followed after 14 days

Table 1. Macrophage migration inhibition in the double vaccinated broiler chicks with NDV (Lasota strain, group B)

Ocular reading (mm)	Experimental migration (µ)	Inhibition of migration (µ)	MIF-activity (%)
8	234.4	175.83	42.86
10	293.0	117.23	28.57
8	234.4	175.83	42.86
8	234.4	175.83	42.86
9	263.7	146.53	35.70
9.5	278.4	131.83	32.13
10	293.0	117.23	28.57
10	293.0	117.23	28.57
8	234.4	175.83	42.86
9	263.7	146.53	35.70
Average	262.24	147.99	36.07

Table 2. Macrophage migration inhibition after single vaccination with NDV (LaSota strain) at the age of 14 days in broiler chicks (group C)

Ocular reading (mm)	Experimental migration (μ)	Inhibition of migration (μ)	MIF-activity (%)
9	263.7	146.53	35.70
11	322.3	87.93	21.43
9	263.7	146.53	35.70
9	263.7	146.53	35.70
10	293.0	117.23	28.50
10	293.0	117.23	28.50
12	351.6	58.63	14.29
11	322.3	87.93	21.43
9	263.7	146.53	35.70
10	293.0	117.23	28.50
Average	293.0	117.23	28.57

Macrophage migration in control =410.23μ

by oral infection with a virulent strain. Suzuki *et al.* (1987) also reported an enhanced macrophage migration inhibition induced by sensitized T cells from mice treated with toxoplasma lysat antigen.

REFERENCES

Allan, W.H., J.E. Lancerter and B. Toth, 1978. Newcastle disease vaccines, their production and use. Food and Agriculture Organization of United Nations, Room pp. 51-62.

Burgh, M. A., 1978. Simple method for recording and analyzing serological data. Avian Dis., 2: 362-365.

Chandrasekar, S., R. A. Venkatesan, V. D. Padmanaban and P. R. Masiilamony, 1989. Nature of protective immunity responses in chicken against Ranikhet disease. Indian Vet. J., 66: 801-806

Duguid, J.P., B.P. Marmion and R.H.A. Swain, 1978. Medical Microbiology, Vol. 1, 13th Edit. ELBS, London p. 144.

Giamborne, J.J., 1981. Laboratory evaluation of the immune response of the young broiler chicken vaccinated against Newcastle disease under field condition. Poult. Sci., 60: 1204-1208.

Harrington, J. R., T. John and P. Statstny, 1973. Macrophage migration from an agarose dropt: Development of micro method for assay of delayed hypersensitivity. J. Immunol., 110: 752-759.

Konopa, M., 1984. Cell mediated immunity in experimental listeriosis in animals. Wetrynaria, 41: 17-32. (Vide Vet. Bull. Abst ., 56 (2): 756; 1986).

Lodmell, D.L., A. Ninex, K. Hayashi and A. L. Notkins, 1973. Prevention of cell to cell transmission of Herpes simplex virus by leukocytes. J. Exp. Med., 113: 706-720.

Mutuka, O., P. Snezana, K. Salahovic and S. Mladel, 1980. Double application of Lasota Newcastle disease vaccine by spray and in the drinking water to broiler chicks. Veterinaria Yogoslavia, 29: 356-360.

Onaga, H and T. Ishii, 1980. Leucocyte migration inhibition in chicken immunized with *Eimeria tenella*. Japnese J. Vet. Sci., 42: 345-351.

Qureshi, M. A. and L. Miller, 1991. Comparison of macrophage function in several commercial broiler lines. Poult. Sci., 70: 2094-2101.

Shuaib, M., 1993. Cell mediated immune response to Newcastle disease vaccine (LaSota strain) determined by macrophage migration inhibition in chicks. MSc Thesis. Dep. Vet. Micro. Univ. Agri. Faisalabad.

Suzuki, N., K. Kikushima, T. Miyagami, I. Igarashi, H. Sakurai, A. Saito and H. Osaki, 1987. Madulator effect of Toxoplasma lysate antigen in mice experimentally infected with *Plasmodium berghei*, Zentralblblatt-fur-Bakteriologie, Mikrobiologie-und-Hygiene, 264: 422-434. (Vide Protozoological Abst., 12: 149; 1988).