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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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Effect of Levamisole as an Immunomodulator in Cockerels Vaccinated with Newcastle Disease Vaccine

M.E. Sanda[1]*, B.M. Anene[2] and A. Owoade[3]
[1]Department of Animal Production, Kogi State University, Anyigba, Nigeria
[2]Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria
[3]Faculty of Veterinary Medicine, University of Ibadan, Nigeria

Abstract: The efficacy of Levamisole Hydrochloride-a standard immunomodulator was tested in cockerel chicks. Forty day old chicks were allocated into two groups A and B. Group A received Levamisole at the recommended dosage of 10.05mg/20 birds for 3 days prior to the NDV vaccinations (B1, LaSota and Komarov). Group B received all the afore mentioned vaccinations but was not treated with Levamisole. Sera samples were analyzed by Heamagglutination Inhibition (HI) tests. The data obtained were analyzed statistically using paired t-test. High antibody titers against NDV were observed in both groups. There was no significant immunostimulation by Levamisole in this study.

Key words: Newcastle disease, levamisole, vaccination, immunomodulators

INTRODUCTION

Newcastle disease (ND) is an important viral disease of local, exotic and wild birds. It causes high morbidity and mortality reducing the productivity of the affected birds. (Spradbrow, 1990; Nguyen, 1992). It has been identified as one of the poultry diseases responsible for drop in egg production (Farooq *et al*., 2001) and eggshell defects.

The disease is also of public health importance in that there are reports of human infections such as eye infections like unilateral or bilateral reddening, excessive lacrimation, edema of the eye lids, conjunctivitis and subconjuctival hemorrhage (Chang, 1981).

No treatment for ND exists yet and vaccination is the only major control of the disease (Fonseka, 1987). Vaccination is either by using live vaccines such as NDV intraocular, LaSota and Komarov produced by the National Veterinary Research Institute, Vom, Nigeria or inactivated (killed) vaccines.

Despite the vaccinations, against ND, there are reports worldwide of birds dying or still showing clinical infections (Aldous and Alexander, 2001). This could probably be due to improper handling of vaccines, wrong timing of vaccinations or interference of vaccine antigen with the maternal antibody (Rahman *et al*., 2002) or the inability of the birds to maintain the immunity after the vaccination.

Levamisole is an optic isomer of the phenylimidothiazole salts of tetramisole. It has been shown to have a high level of anthelminthic activity against many parasitic nematodes (Janssen, 1976). It has also been found to possess immunostimulating effects (Renoux and Renoux, 1971).

An attempt has been made in this study to boost immunity and enhance the level of protection conferred on ND vaccinated birds by the administration of Levamisole before the vaccination.

MATERIALS AND METHODS

Experimental chicken: Forty day-old Harco cockerels were obtained for this study and raised in metal cages. The birds were grouped into A, B and C with 20 birds in each group. The chicks originally received no vaccinations from the hatchery as requested.

Housing: The experiment was carried out in the Experimental Animal House of the Department of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria.

Vaccine antigen: The vaccine antigen used was obtained from the National Veterinary Research Institute sub station at Oji, Enugu State, Nigeria. Levamisole was obtained as a 1.5% solution from Animal Health Division, Sam Pharmaceutical Ltd., Ilorin, Kwara State, Nigeria.

The birds were randomly divided into 2 groups of 20 chicks each. The experimental groups were designated A and B. Group A received Levamisole at 10:05mg/20 birds in drinking water for 3 consecutive days before each vaccination. Group B received only the vaccinations with no prior treatment with Levamisole. The vaccination schedule is as shown in Table 1.

Sera collection: The birds were bled via jugular vein on days 7, 14, 21, 28, 35, 42, 49, 56, 63 and 70. sera were obtained from the clotted blood after slanting the bijou bottle containing the blood and allowing it to stay over night. The samples were then clarified by centrifugation at 3000rpm for 5 minutes. Sera samples were stored at -20°C.

Table 1: Vaccination schedule

Day of Life	Group A	Group B
8	NDVB1	NDVB1
22	NDV LaSota	NDV LaSota
43	NDV Komorov	NDV Komorov

Table 2: Newcastle Disease-Mean HI Titers

Time (Days)	Group A	Group B	Level of Significance
7	1024.0 (420.6)	1356.8 (427.6)	NS
14	409.6 (62.7)	460.8 (149.3)	NS
21	108.8 (39.9)	166.4 (38.4)	NS
28	10547.2 (6290.7)	716.8 (125.4)	NS
35	326.4 (180.5)	134.4 (35.6)	NS
42	646.4 (397.6)	22.4 (3.9)	NS
49	1177.6 (36.2)	1280.0 (343.5)	NS
56	422.4 (166.4)	435.2 (159.8)	NS
63	230.4 (82.4)	198.4 (87.2)	NS
70	185.6 (90.7)	153.6 (91.3)	NS

*Group A, Levamisole + ND vaccination; Group B, No treatment + ND vaccination; ( ) Standard Error of Mean; NS, Not Significant

Red blood cell indicator: Blood was collected from ND antibody free chickens and 0.6% red blood cell suspension was prepared using Phosphate buffered saline (PBS) as described by Wosu (1984).

Hi titration: Haemagglutination inhibition technique was used for the detection of antibody level against ND as described by Orajaka *et al.* (1999).

Sera double dilution of each test serum was made in "U" bottomed micro titer plates. Equal volume (0.03ml) of four Haemugglutinating Units (4HAU) of NDV LaSota antigen was then added to each well. This was then incubated at room temperature for 45 minutes before same volume (0.03ml) of the 0.6% chicken red blood cell was added to each well.

Red blood cell (RBC) control was also included in the protocol by adding 0.03 ml of the 0.6% chicken RBC to wells containing only 0.03ml of Phosphate Buffered Saline (PBS) with pH of 7.2. The whole set up was incubated at room temperature until the RBC in RBC control wells settled. The HI titers were read as the reciprocal of the highest dilution of the sera which inhibited haemagglutination (HA) of chicken RBC.

Analysis of data: The data was analyzed using paired t-test by computerized statistical programme (SPSS version 13.0).

RESULTS AND DISCUSSION

The ND-haemagglutination Inhibition (HI) titer of the birds are shown in Table 2. The ND-HI titer before vaccination (Day 7) showed that the chicks possessed a uniformly high level of maternal derived antibody (MDA) titer in all groups ranging from a mean of 7.20-9.40. This shows the breeders vaccination was effective in the farm from which the chicks originated. High level MDA was also reported by Saeed *et al.* (1998) and Rahman *et al.* (2002).

The chicks possessed uniformly high antibody titers ranging from a mean of 1024-1356.8 on day 7 of life prior to the vaccinations. This indicates a high Maternally Derived Antibody (MDA) in both groups revealing effective breeder vaccinations against ND. However, the antibody level dropped after the primary vaccination to a mean value of 409.6 in group A and 460.8 in Group B. This could be due to MDA interference with the introduced vaccine antigen. Rahman *et al.*, 2002 observed a decrease in antibody level of chicks after primary vaccination and explained that it could be due to the use of either low quality vaccine, failure of maintenance of cold chain or interference of vaccine antigen with the maternal antibody. Awang *et al.*, 1992 reported that maternal antibody neutralizes the introduced vaccine antigen rendering the vaccine ineffective.

Futhermore, an increase in antibody level was noticed on Day 28 after the secondary vaccination that took place on Day 22. Similar increase was observed on Day 49, a week after the chicks had received the tertiary vaccination. This shows that ND vaccinations (secondary and tertiary) were effective in this study. However, the similarity in HI titers of Levamisole treated Group A and untreated Group B seems to indicate that levamisole as used in this study did not have any appreciable effect on the humoral immunity of ND vaccination. Brunner and Muscoplat (1980) and BIOBRAN (2005) indicated that immunomodulators do not exert their effect on normal cells. This may be the case in this study since the birds were kept under standard management conditions and therefore in very good health condition which leaves all the body cells normal through out the study period.

Conclusion: Levamisole is not an efficient immunomodulator for enhancement of response to Newcastle disease vaccine by chicken.

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