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ORIGINAL ARTICLE

Immunomodulatory Effect of Levamisole Hydrochloride and Mentofin® in Newcastle Disease Vaccinated Commercial Broilers

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SUMMARY

Newcastle disease (ND) outbreaks have been reported in vaccinated poultry flocks in Nigeria and this necessitated the need for administration of immunostimulants to improve immunity in such birds. The immunomodulatory effects of Mentofin® (a recent introduction into Nigeria) and Levamisole on antibody response of vaccinated chicks against ND were assessed. One hundred day-old chicks were randomly divided into five groups (A-E). Group A: unvaccinated; B, C, D and E were administered LaSota vaccine: at 14 and 42 days old. In addition, group C received Levamisole, group D received Mentofin® and group E, received a combination of Levamisole and Mentofin®orally for 3 days consecutively post-vaccinations. Blood samples were then collected from each group. ND virus antibody titers were determined using ELISA. Maternal antibody titre (76.55±2.35) at day-old declined at 56 day-old (3.83±0.3-7) in group A. At 14 days post primary vaccination, peak antibody titres in groups C, D and E (62.3±4.51, 60.2±3.84 and 64.9±5.58) were significantly higher than that of group B (42.3±4.28). Also, at 14 days post-secondary vaccination, peak titre in group E (77.9±3.14) was significantly higher than in groups A, B, C and D (3.83±0.37, 44.0±3.20, 71.1±3.48 and 70.3±3.25). This study shows that combined oral administration of Mentofin® and Levamisole significantly enhanced humoral response to live ND vaccination and their combination has a synergistic effect.

Key words: Levamisole, Mentofin®, Broiler, Antibody response, Newcastle disease vaccination.

INTRODUCTION

The challenges of food insecurity worldwide particularly in developing countries like Nigeria have continued to receive attention from governments and experts (Babatunde *et al.,* 2007). Studies have revealed that in spite of the numerous human and natural resources, Nigeria still remains among the least consumers of animal protein in Africa (Dalhatu and Ala, 2010).The protein intake of an average Nigerian is about 53.8% of standard recommendation with only 6.0 to 8.4 g/head/day protein of animal origin. Studies further revealed that North America, Western and Eastern countries consume 66,

39 and 33 grammes of animal protein per head per day, respectively. To increase protein intake in Nigeria, there is an urgent need to increase poultry production at both household and commercial holdings. Oluyemi and Roberts (1979) and Isika *et al.* (2006) postulated that poultry is strategic in addressing animal protein intake shortage in human nutrition because of its high fecundity, fast growth rate, short generation interval and unparalleled competence in nutrient transformation to high quality animal protein.

Poultry production has become a diverse industry with a variety of interests such as egg and broiler production, as well as hatchery and poultry equipment business (Oluyemi and Roberts, 2000). However, poultry farming in Nigeria has been prone to a heavy risk of increased disease incidences especially Newcastle disease causing high mortality, even when scheduled vaccination programmes are implemented.

Newcastle disease (ND) is a highly contagious viral disease of avian species especially the poultry species. The disease has deleterious effect on food security and source of livelihoods due to heavy mortality encountered in many countries (Amanfu, 2011). No treatment for (ND) exists yet and vaccination is the only major control of the disease (Fonseka, 1987). Despite vaccinations, 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 vaccination. Immune failure in chickens occurs quite often as a result of biological or chemical induced immune suppression, variation of avian pathogens and irregular use of vaccines (Xie and Song, 2005). Immuno-suppressed flocks may have high susceptibility to secondary infections and poor feed conversion ratio (FCR) and response to commonly used vaccines (Sharma *et al.,* 2000).

In poultry production, application of immunostimulants has been found to be essential in improving the immunity of birds. Some herbal products, whose modes of action are unknown, are effective immunopotentiating agents. Mentofin® is one of such products containing volatile oils with potent immunomodulatory effect on immune response of birds to vaccines (Awaad *et al.,* 2010). Also, it reduces the morbidity and specific lesions following infectious bronchitis virus (IBV) challenge infection (Barbour *et al.,* 2008) and have positive effect on weight gain and improves the FCR of broilers (Carli *et al.,* 2008). Furthermore, Levamisole a synthetic anthelmintic drug for animals (JECFA, 1991) has been reported to increase humoral antibody response against (ND) LaSota antigens in chickens (Cuesta *et al.,* 2002). It has also been reported that Levamisole injected with a DNA vaccine against the foot-and-mouth disease virus stimulated both humoral and cellular immune responses in conjunction with strong production of interferon (Jin *et al.,* 2004). Thus, this study was carried out to ascertain the immunomodulatory effect of Mentofin® (which is a recent introduction into Nigeria) and Levamisole, on broiler chicks vaccinated with (ND) vaccine and also to determine possible synergistic or otherwise effect of the combination of both mentofin® and Levamisole on the immune response of the vaccinated chicks.

MATERIALS AND METHODS

Experimental Chickens

One hundred (100) day old broiler chicks purchased from a commercial hatchery were reared in open-sided cages at the poultry house of the Department of Veterinary

Medicine, Faculty of Veterinary Medicine, University of Ibadan, Nigeria. The chicks originally received no Newcastle disease (ND) vaccinations from the hatchery as requested for the purpose of this study. The broiler chicks were divided into 5 groups (A, B, C, D and E) with 20 birds in each group. All groups were kept in separate cages with birds allowed free access to feed and clean water and reared under strict biosecurity conditions.

Vaccine antigen and vaccination

Newcastle disease vaccine (LaSota) was obtained from the National Veterinary Research Institute Zonal office, Akure, Ondo State, Nigeria. The vaccine was administered to groups B, C, D and E through drinking water at day 18 and 42 day old.

Experimental design

Broilers in group A were unvaccinated and were not treated with either Levamisole or Mentofin®. Groups B, C, D and E all received ND vaccine; in addition, group B received no treatment with Levamisole or Mentofin® while group C received Levamisole at 150mg/kg (Munir*et al.,* 2009), furthermore, group D received Mentofin® at 0.25ml/litre (Barbour *et al.,* 2008) while group E received a combination of Mentofin® (0.25ml/litre) and Levamisole (150mg/kg) all in drinking water for 3 consecutive days after each vaccination.

Evaluation of Newcastle disease antibodies

About 2 ml of blood was collected from all the Broilers in the five groups (A-E) via the jugular vein on days 1, 14, 25, 32, 39 and 56 of age. Sera were obtained from the clotted blood and were stored at -20°C until tested.

A commercial Enzyme linked immunosorbent assay (ELISA) kit (Affinitech, USA) was used to evaluate (ND) antibody titres in collected sera from the experimental birds. The ELISA procedure was carried out following the manufacturer's instructions.

Statistical analysis

Mean ± standard error of mean (SEM) of data obtained per group was determined using the statistical package Graph Pad Prism version 5.01 (San Diego, USA). Comparison between groups was carried out by one-way ANOVA and Tukey's multiple comparisons post-test for multiple comparisons at $P<0.05$.

RESULTS

The mean ND antibody titre at day 1 was 76.6±2.4 EU. The mean antibody titre at day 14 was 20.4±1.5 EU. This was done in other to determine the appropriate time to administer the first (primary) ND vaccine. The maternal antibody decreased from 20.3±1.8 EU to 3.8±0.4 EU in the unvaccinated (group A). The ND antibody titres at various post vaccination periods showed significant differences as shown in Table I.

DISCUSSION

Commercial broiler chickens have a limited natural resistance to infectious diseases due to compromised immune responses that in turn increase risks of disease in poultry (Balamurugan and Kataria, 2006). Naturally occurring or synthetic compounds capable of modulating immune responses (Kehrli and Roth, 1990) in order to confer greater protection against infectious agents have been of immense interest in human and veterinary medicine (Kehrli and Roth, 1990).

Newcastle disease (ND) has serious effect on food security and source of livelihoods because of heavy mortality encountered in many countries (Amanfu, 2011). In order to control ND, the use of vaccines is necessary and effective vaccination depends on some

TABLE I: Mean ND antibody titres at different post vaccination periods

Age (days)	Post vacc. period (days)	Group A	Group B	Group C	Group D	Group E
14	0	20.3±1.8	20.5±2.0	20.4±1.5	20.6±1.3	20.0±2.0
1st NCD Vaccination						
25	7	15.4±3.0	20.9±1.7	37.9±3.3	32.4±3.9	39.4±2.7
32	14	6.4±0.9	42.3±4.3	62.3±4.5	60.2±3.8	64.9±5.6
39	21	4.3±0.5	20.9±1.2	41.2±3.2	38.9±2.0	42.2±1.7
2nd NCD vaccination						
56	14	3.8±0.4	44.4±3.2	71.1±3.5	70.3±3.3	77.9±3.1

Values are expressed as mean ± SEM

critical factors including immune stimulators.

The ND antibody titre of the '1 day-old' chicks showed a uniformly high level of maternally derived antibody (MDA) with a mean of 76.6±2.4 EU, this shows effective vaccination of the breeder flock from which the chicks originated. This is similar to earlier reports of high MDA levels (Saeed *et al.,* 1988; Rahman *et al.,* 2002). However, the MDA dropped at day 14 (20.4±1.5 EU) which corroborates findings of Ibrahim *et al.* (1982) that showed that optimal titres of ND antibodies exists during the first two weeks of life in chicks. It should be noted that MDA or passive immunity is the device for the prevention of many diseases in newly hatched chicks. Thus, MDA could be considered as an effective means of protection of chicks against ND till two weeks of age (Islam *et al.,* 2003)

The findings of this study revealed that Mentofin® treated broilers (group D) showed significantly higher antibody response to ND (32.4±3.9, 60.2±3.8, 38.9±2.0 and 70.3±3.3) when compared to the positive control (group B) which received only ND vaccine (20.9±1.7, 42.3±4.3, 20.9±1.2 and 44.4±3.2). This might be due to the immunomodulatory effect of essential oils; Eucalyptus and Peppermint contained in Mentofin®, which has been reported to improve the ND virus antibody titres in poultry (Carli *et al.,* 2008) and enhanced the anti- NDV-HI antibodies in broilers (Awaad *et al.,* 2010).

The findings are similar to the reports of Rehman *et al.* (2013) who reported that Mentofin® treated broilers showed higher consistent antibody (anti-NDV HI antibody titre) response as compared to untreated broilers. Similarly, the Levamisole treated group C showed significantly higher antibody response (37.9±3.3, 62.3±4.5, 41.2±3.2 and 71.1±3.5) when compared to the (group B) positive control (20.9±1.7, 42.3±4.3, 20.9±1.2 and 44.4±3.2). This is in line with reports that Levamisole increases humoral antibody response against ND antigens in poultry (Cuesta *et al.,* 2002) and that chickens immunized with ND virus developed a significantly higher level of HI antibodies when treated with Levamisole than untreated ones (Kulkarni *et al.,*1973). More so, this study showed that Levamisole elicited higher ND virus antibody response than Mentofin®, though the difference is not statistically significant.

Furthermore, the ND antibody titre was higher in the Mentofin® + Levamisole group (77.9±3.1EU) than in the Levamisole (71.1±3.5EU) and Mentofin® (70.3±3.3EU) treated groups especially after the booster ND vaccination. This may suggest that the combination of Levamisole and Mentofin® has a synergistic effect on the immune response and health of the tested broilers because each compound would act at discrete levels of the immune system and

with differing mechanisms.

In conclusion, this study has provided experimental evidence that Levamisole and Mentofin® may be used as immunostimulants in broilers vaccinated with ND vaccine to prolong and maintain high antibody titres in such vaccinated birds. Further studies on cell mediated and humoral immune responses of birds to Levamisole and Mentofin® treatment over extended period, may provide evidence for the increased mobilization of immune cells and duration of immunity in birds treated with levamisole and Mentofin® before and after vaccination. Vaccination and immune stimulators are widespread areas in poultry industry and more investigation is needed based on other factors that affect immune responses.

REFERENCES

ALDOUS, E.W. and ALEXANDER, D.J. (2001): Detection and differentiation of Newcastle disease virus (avian paramyxovirus type 1) *Avian Pathology*;30: 117-128.

AMANFU, W. (2011): The challenges of transboundary animal diseases in Africa: diseases management options. *Journal of Commonwealth Veterinary Association*. 27(2): 102-107.

AWAAD, M.H.H., ABDEL-ALIM,G.A., SAYED,K.S.S., KAWKAB, AHMED,A., NADA,A.A., METWALLI,A.S.Z. and ALKHALAF, A.N. (2010):Immunostimulant effects of essential oils of peppermint and eucalyptus in chickens. *Pakistan Veterinary Journal*.30:(2):61-66.

BABATUNDE, R.O., OMOTOSHO, O.A.and SHOLOTAN, S.O. (2007): Socio-Economic characteristics and food security status of farming households in Kwara state, north central Nigeria. *Pakistan J. Nutr.* 6: 49-58

BALAMURUGAN, V. and KATARIA,J.M. (2006): Economically important non-oncogenic immunosuppressive viral diseases of chicken-current status. *Veterinary Research Communication*, 30(5):541-566.

BARBOUR, E.K., YAGHI,R.H., SHAIB, H.A., TAYEB, I.T. and SLEIMAN, F.T. (2008): Evaluation of an essential oil in treatment of immunosuppressed co-infected broilers. *American-Eurasian Journal of Sustainable Agriculture*, 2(3): 212-218.

CARLI, K.T., ONAT, K. and GUNAYDIN, E. (2008): Application of Mentofin® in broilers with clinical infectious Bursal disease to reduce *Escherichia coli* related problems after vaccination against Newcastle disease. Turkey *Journal of Veterinary and Animal Science*. 32(2):73-78.

CUESTA, A., ESTEBAN M. and MESEGUERJ. (2002):Levamisole is a potent enhancer of gilthead seabream natural cytotoxic activity. *Veterinary Immunology and Immunopathology*. 89(3-4): 169-174.

DALHATU, M. and ALA, A. L. (2010): Analysis of fish demand in Sokoto metropolis, Sokoto, Nigeria. *Nigerian Journal of Basic and Applied Science*, 18 (2):154-159.

FONSEKA, T.M. (1987): Sri Lanka: Poultry production. In: Newcastle disease in poultry. A new food pellet vaccine, J.W .Copland, by Ed., Australian center for International agriculture research (ACIAR), Canberra. Monograph No. 5: 100-104.

IBRAHIM, A.L., LAI, M.C. and AINI, I. (1982): Spray vaccination with an improved F Newcastle disease vaccine. A comparison of efficacy

with the B1 and LaSota vaccines. *British Veterinary Journal.* 139: 213-219.

ISIKA, M.A., AGIANG, E.A. and OKON, B.I. (2006): Dietary energy and crude protein requirements for chicks of Nigeria local fowl and crossbreeds. *International Journal of Poultry Science,* 5:271-274.

ISLAM, M.R., HUQUE, Q.M.E., GIASUDDIN, M.J. and RAHMAN, M. (2003): Assessment of maternally derived antibody of commercial flock against Newcastle disease. Proceedings of 3rd international poultry show and seminar, Bangladesh China friendship conference center, Dhaka, Bangladesh 28th February-2nd March.Pp.89-92.

JECFA, (1991): Joint FAO/WHO Expert Committee on Food Additives Levamisole. WHO Food Additive Series. 27:75–101.

JIN, H., LI, Y., MA, Z., ZHANG, F., XIE, Q., GU, D. and WANG B. (2004): Effect of chemical adjuvants on DNA vaccination. *Vaccine,* 22(21-22):2925-2935

KEHRLI, M.E. and ROTH, J.A. (1990): Chemically induced immunomodulation. In: domestic food animals". In: Advances in Veterinary Science and Comparative Medicine, Academic Press, USA. 38:103-119

KULKARNI, V., MULBAGAL, A., PARANJAPE, V., KHOT, J. and MANDA, A. (1973): Immunostimulating effect of tetramisole on antibody formation against Newcastle disease virus in chicks. *Indian Veterinary Journal,* 50:225-227.

MUNIR, K., MUNEER, M.A., TIWARI, A., MASAOUD E. and CHAUDHRYR.M. (2009): Effects of Salinomycin on cell-mediated immunity of broiler chickens against hydropericardium syndrome and Newcastle disease viruses. *Poultry Science,* 88: 86-91.

OLUYEMI J.A AND ROBERTS F.A. (1979): Poultry Production in Warm Climate. Macmillan Press Ltd. London, 197 p.

OLUYEMI J.A. and ROBERTS F.A. (2000): Poultry Production in Warm Wet Climate. (2ndEd.) Spectrum Books Ltd. Ibadan, Nigeria.

RAHMAN, M.M., BARI, A.S.M., GIASUDDIN, M.I., ALAM J. and SIL, G.C. (2002): Evaluation of maternal and humoral Immunity against Newcastle disease virus in chicken. *International Journal of Poultry Science.*1:161-163.

REHMAN, S.R., MUHAMMAD, K., YAQUB, T., KHAN, M., HANIF, S.K. and YASMEEN,R. (2013): Antimicrobial activity of Mentofin and its effect on antibody response of broilers to Newcastle disease virus vaccine. *J Anim Plant Sci.* 23(4): 1008-1011.

SAEED, Z.S., AHMAD, A., RIZVI, R.and AJMAL, M. (1988): Role of maternal antibody in determination of an effective Newcastle disease vaccination programme. *Pakistan Journal of Veterinary Research,* 1:18-21.

SHARMA, J.M. KIM, I.J. RAUTENSCHLEIN, S. and YEH, H.Y. (2000): Infectious Bursal Disease virus of chickens: pathogenesis and immunosuppression. Journal of Development and Comparative Immunology 24: 223-235.

Xie, Y. X. and Z. Y. Song (2005): Causes and control measures of ND immunization failure. *Livestock and Poultry Industry,* 178(2): 21.