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Trials for improving the efficiency of inactivated Newcastle disease virus vaccine by using propioni-bacterium and lipopolysaccharide (IM-104) as immunostimulant

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SUMMARY

The ability to improve the efficacy of an inactivated Newcastle disease vaccine by addition of immunomodulator as propioni-bacterium and lipopolysaccharide (IM-104) was studied. Chickens were divided into three groups, one received inactivated Newcastle vaccine containing the IM-104, second group received vaccine alone and third group of chickens was left without vaccination as control. Blood samples were collected 3 days post vaccination, and weekly till four weeks for estimation of the effect of (IM-104) on T-lymphocyte transformation (cellular immune response). Sero-conversion for sera at weekly intervals was done to evaluate the immune response using HI test and ELISA. Challenge test was carried out on 3rd and 6th week post vaccination for evaluation of protective efficacy of these vaccines. The results obtained revealed that (IM-104) that contain 2 extraction of E. coli lipopolysaccharide and propioni-bacterium plays roles in stimulating both T-lymphocyte and macrophage to release different types of interleukin and consequently released immunoglobulins. Also, stimulate non-specific resistance to viral infection. The results obtained by challenge test run in parallel with that of serological test.

INTRODUCTION

Viral diseases of poultry constitute one of the most major problems facing the rapidly expanding poultry industry in Egypt causing considerable economic losses due to serious mortality associated with different infectious viruses.

Newcastle disease (ND) is one of the main poultry pathogens severely endangering out the poultry industry. ND causes great economic losses due to high rate of mortality, reduction of meat and drop in egg production (Biswal and Morrill, 1954).

Recently, non-specific immunostimulant are gaining increasing attention in recent years to counteract the effect of environmental immuno-suppression and potentiating the immune response to applied vaccines.

For improvement of the effectiveness of inactivated Newcastle disease vaccine some substances were added as immunostimulant. The administration of the immunostimulant (IM-104) containing lipopoly-saccharide (LPS) has a significant effect in argumenting both humoral and cell mediated immune response (Omara et al., 2000).

So, the present work was designed to study the levels of induced immunity of vaccinated chickens in the presence and absence of IM-104 (Inmunair).

MATERIAL AND METHODS

Strains:

1. Virus strain:

LaSota strain was supplied by Central Veterinary Lab., Weybridge, England.

2. Virulent strain:

A velogenic viscerotropic Newcastle disease virus local strain identified by Reda and Sheble (1976) was used for challenge.

Inmunair 17.5:

Composition:

Inactivated cells of propioni-bacterium acnes	0.17 mg
Lipopolysaccharide from E coli	0.05 mg
Thiomersal	0.10 mg
Excipient q.s.	1 ml

Sale Agent:

Laboratorios Caller, S.A. C/Barcelone's 26 (Pla del Ramassa), Spain.

Experimental chicks:

One hundred and fifty Hubbard chicks (United Company for Poultry Production) were reared under complete hygienic measures. They were divided into three groups as follows:

Group (1): 50 birds were vaccinated with inactivated ND oil emulsion vaccine in addition to immunostimulant (Inmunair).

Group (2): 50 birds were vaccinated with inactivated ND oil emulsion vaccine alone.

Group (3): 50 birds were kept as control non-vaccinated.

Each bird of vaccinated groups received 0.5 ml of its appropriate vaccine injected I/M. Blood and serum samples were obtained from all vaccinated and non-vaccinated groups at weekly intervals to

evaluate both cellular and humeral immune response.

Preparation and inactivation of Newcastle disease virus:

LaSota strain virus was propagated in ECE according to Allan et al. (1973). Allantoic fluid was harvested and titrated according to standard method described in FAO Publication (1978). The titre was 10^{11} EID_{50}/ml. Inactivation of NDV was carried out using formalin 0.1 % at final concentration for 18 hours. Completion of inactivation for NDV must be ensure. This accomplished by inoculation of formalized inactivated virus into 9 day old specific pathogen free (SPF) ECE. Three successive blind passages were done before the batch of the prepared vaccine was considered safe.

Preparation of oil emulsion inactivated ND vaccine:

It was done according to Thayer et al. (1983). The immunostimulant was added to the inactivated vaccine while it was stirred and the mixture emulsified for 10 minutes.

The prepared oil emulsion inactivated NDV vaccines were tested for contaminants according to United State Code of Federal Regulation (1987).

Evaluation of cell mediated immunity:

It was estimated by blood lymphocyte blastogenesis (Lucy, 1974 and 1977) and identified by Charles et al. (1978). Evaluation of the test using tetrazolium calorimetric assay according to Mosmann (1983). Results of test were expressed as delta optical density where:

ΔOD = (ΔOD of PHA – ΔOD of media) – (ΔOD of cell – ΔOD of media)

Evaluation of humoral immunity (HIT):

It was used for estimation of the haemagglutinating antibodies against NDV. It was done according to Majujabe and Hitchner (1977).

Challenge test:

Twenty birds from each group were challenged three weeks and six weeks post vaccination. Velogenic Viscerotropic Newcastle disease virus was inoculated intramuscularly with 0.5 ml containing 10^6 EID_{50} and kept under observation for 15 days. Dead birds and those showing symptoms through the period of observation were kept for PM examination.

RESULTS AND DISCUSSION

The poultry industry has expanded and integrated dramatically in last years. Thus, poultry are considered without doubt the most appropriate source of protein supply of high nutritive value for human beings. This is due to efficient cost of production and its short life cycle. No vaccine should be expected to protect all recipients completely against a disease, even in ideal conditions. Nowadays, a number of immunostimulant became available to be used for improvement of the effectiveness of vaccine as IM-104. The

present study was aimed to improve the immune response of inactivated ND vaccine.

Regarding quality control of the prepared vaccine, it was found that it was free from foreign aerobic and anaerobic bacteria, fungi and mycoplasma.

For evaluation of immune response of chicken vaccinated with prepared vaccines, both cellular and humoral immunity were estimated. In attempt to measure the antigenic response of peripheral blood T-lymphocytes of vaccinated chickens lymphocyte blastogenesis test was carried out. Table (1) revealed that ascending increase was noticed in lymphocyte transformation expressed as delta optical density (Δ OD), where it is 0.125 and 0.123 in 2nd week post vaccination for first and 2nd groups respectively and reached 0.129 and 0.113 in third week post vaccination for groups 1 and 2 respectively. This increase was more detectable and appears to be significant in chickens received immunostimulant (IM-104) with the vaccines. Discussing these results, it was found that Iovane et al. (1998) explained the effect of lipopolysaccharide as it stimulate the production of variety of humoral factors as IL-1, alpha IL-6, INF-alpha, INF-gamma. Also Maslong et al. (1999) confirm the role of gram negative bacteria and components of the outer membrane (lipopolysaccharide LPS) in enhancing humoral and cell mediated immune response.

Humoral immune response against NDV was estimated by HI test as shown in table (2). The results revealed that the antibody titres of chickens vaccinated with Inmunair exhibited the highest HI antibody titre where it reached 10^{11} in 7 weeks post vaccination comparing with the other groups. These results are in accordance with those of Tizzard (2000) who stated that propioni bacterium acnes, posses adjuvant activity which enhance antibody formation against viral infection.

From data shown in tables (3, 4), it can be cleared that the chickens vaccinated with NDV with inmunair gave protection percent 100%, 95% three weeks and six weeks post vaccination respectively, while the other group gave protection percent 95%, 90%. The non-vaccinated group gave no protection when challenged (0 %). Carter and Wagner (1984) reported that propioni bacterium acnes stimulate non-specific resistance to viral infection.

The conclusion of these findings was the improvement of the efficacy of vaccine by addition of inmunair as it shares in augmentation of both cellular and humoral immunity of chickens and increase protection of chickens to virulent infection.

Table (1): Evaluation of cell mediated immune response of vaccinated groups of chickens by lymphocyte transformation expressed by Delta optical density

Groups	Weeks Post Vaccination				
	3rd day	1 week	2 weeks	3 weeks	4 weeks
1	0.034	0.104	0.125	0.129	0.133
2	0.089	0.099	0.123	0.113	0.117
3	0.009	0.030	0.010	0.012	0.014

Group (1): Vaccinated with inactivated ND oil emulsion vaccine with immunostimulant.
Group (2): Vaccinated with inactivated ND oil emulsion vaccine alone.
Group (3): Control unvaccinated.

Table (2): Haemagglutination inhibition (HI) antibody titres of chicken vaccinated with ND vaccines with addition of immunostimulant and without immunostimulant

Groups	Weeks Post Vaccination											
	1	2	3	4	5	6	7	8	9	10	11	12
1	2	4	6	7	8.5	9	11	10	10.5	10	9	8
2	2	4	5	5	7	8	8.5	8.5	8	7	6	6
3	0	0	0	0	0	0	0	0	0	0	0	0

Group (1): Vaccinated with inactivated ND oil emulsion vaccine with immunostimulant.
Group (2): Vaccinated with inactivated ND oil emulsion vaccine alone.
Group (3): Control unvaccinated.

Table (3): Immunization efficacy of chickens vaccinated with inactivated ND oil emulsion vaccine with and without addition of immunostimulant three weeks post vaccination

Groups	No. of chickens	No. of survived chickens	No. of dead chickens	HI titre * mean	Protection %
1	20	20	0	13	100 %
2	20	19	1	11	95 %
3	20	0	20	0	Zero

Group (1): Vaccinated with inactivated ND oil emulsion vaccine with immunostimulant.
Group (2): Vaccinated with inactivated ND oil emulsion vaccine alone.
Group (3): Control unvaccinated.

Table (4): Immunization efficacy of chickens vaccinated with inactivated ND oil emulsion vaccine with and without addition of immunostimulant six weeks post vaccination

Groups	No. of chickens	No. of survived chickens	No. of dead chickens	Protection %
1	20	19	1	95 %
2	20	18	2	90 %
3	20	0	20	Zero

Challenge dose 0.5 ml of 10^6 EID_{50}/ml I/M

$$\text{Protection \%} = \frac{\text{No. of survivors}}{\text{Total No. of challenge birds}} \times 100$$

Group (1): Vaccinated with inactivated ND oil emulsion vaccine with immunostimulant.
Group (2): Vaccinated with inactivated ND oil emulsion vaccine alone.
Group (3): Control unvaccinated.

REFERENCES

Allan, W.H.; Lancaster, J.E. and Toth, (1973): The production and use of Newcastle disease vaccine. Food and Agriculture Organization, p. 53, Rome, Italy.

Biswal, G. and Morrill, C.C. (1954): Newcastle disease virus. Poult. Sci., 33: 880-897.

Carter, G.R. and Wagner, E.C. (1984): Pasteurella multocida and Pasteurella haemolytica. Adv. Vet. Sci., 11: 321-379.

Charles, R.C.; Rubi, A.; Henry, R. and bose, J.R. (1978): Suppression of the mitogen stimulated blastogenic response during reticuloendo-theliosis virus induced tumorigenesis.

FAO, Animal Production and Health (1978): Newcastle disease vaccine, their production and use. Series No. 10.

Iovane, G.; Paginine, P.; Galdiero, M.; Cipollaro Del'Ero, G.; Vitiello, M.; D'Isanto, M. Morcatitia (1998): Role of Pasteurella multocida povin o cytokine expression and release by murine splenocytes. Vet. Immunol. Immunopathol, 66 (3-4): 391-404.

Lucy, F.L. (1974): In-vitro assay of mitogen stimulation of peripheral lymphocytes. Avian Dis., 18: 602-608.

Lucy, F.L. (1977): Chicken lymphocyte stimulation by mitogen microassay with whole blood cultures. Avian Dis., 22: 296-307.

Majujabe, K.A. and Hitchner, S.B. (1977): Antibody response to strain combination of Newcastle disease virus as measured by haemagglutination inhibition test. Avian Dis., 21: 576-584.

Maslong, I.S.; Motobu, M.; Hayashida, N.; Yoshihara, K.; Morozumi, T.; Matsumura, M. and Hirota, Y. (1999): Effect of the lipopolysaccharides protein complex and crude capsular antigens of Pasteurella multocida serotype A on antibody responses and delayed type hypersensitivity in the chicken. J. Vet. Med. Sci., 61 (5): 565-567.

Mosmann, J. (1983): Rapid calorimetric assay for cellular growth and cytotoxicity assays. J. Immunol. Methods, 55: 55.

Omara, M.E.; Abdel Ghani, A.I.; Rawia, H. Sadek, and Safia T. Kandil (2002): Autogenous vaccination of meat type vaccines under Egyptian conditions for

controlling of vaccine Pasteurellosis using propioni - bacterium and lipopolysaccharide as immunostimulant. 3rd Sci. Con. Rabbit Prod. Hot Clim, 8-11 Oct., 449-459.

Reda, I.M. and Sheble, A. (1976): Isolation and characterization of local viscrotropic Newcastle disease virus. Unpublished data.

Thayer, C.S.; Edison and Kelvin, S.H. (1983): Multivalent inactivated virus oil emulsion vaccines in broiler breeder chickens. Newcastle disease virus and infectious bursal disease virus bivalent vaccines. Poult. Sci., 62: 1978-1983.

Tizzard, I.R. (2000): "Veterinary Immunology. An Introduction. 6th ed., W.B., Saunders Company, Pennsylvania, USA.

United State.Code of Federal Regulations (1987): Animal and Animal Products, 1987. Published b the Office of the Federal Register National Archives and Records Administration.

محاولات لتحسين كفاءة لقاح النيوكاسل المثبط وذلك باستخدام مادة البروبيونوباكتريم واللليبوبولى سكاريد كمادة محفزة

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هذه الدراسة تمت لتحسين كفاءة لقاح النيوكاسل المثبط وذلك بإضافة بعض المحسنات المناعية مثل البروبيونوباكتريم واللليبوبولى سكاريد. وتم تقسيم عدد 150 دجاجة الى 3 مجموعات، 50 دجاجة لكل مجموعة. تم تحصين المجموعة الأولى بلقاح النيوكاسل المثبط الزيتى مع إضافة مستخلص بروبيونوباكتريم واللليبوبولى سكاريد، أما المجموعة الثانية تم تحصينها بلقاح النيوكاسل الزيتى المثبط بمفرده والمجموعة الثالثة تركت كضابط للتجربة. تم تجميع عينات دم بعد 3 أيام من الحقن ولمدة أربعة أسابيع وذلك لقياس معدل تحور الخلايا الليمفاوية (المناعة الخلوية). تم تجميع عينات سيرم اسبوعياً وذلك لقياس المناعة الخلطية بالاختبارات السيرولوجية المختلفة. كما تم عمل اختبار التحدى بعد 3 أسابيع وستة أسابيع من الحقن.

أوضحت النتائج أن المحفز المناعى (IM-104) يلعب دوراً هاماً فى تحفيز كلا من الخلايا الليمفاوية (T-cells) والخلايا البلعمية وتم بذلك خروج انواع مختلفة من الأنترلوكين الذى ساعد على تكوين الأجسام المضادة المختلفة. ايضاً يزيد من مقاومة الطيور ضد الاصابة بالفيروسات وهذه النتائج أكدها اختبار التحدى.