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CURCUMA LONGA FOR PROTECTING CHICKS AGAINST NEWCASTLE DISEASE VIRUS INFECTION AND IMMUNOSUPPRESSIVE EFFECT OF MAREK'S DISEASE VIRAL VACCINE

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Madbouly*, H.M.; Saif**, M.A. and Hussein*, A.S.

ABSTRACT

A total of 300 one day old Hubbard chicks were divided into 6 groups (G1-G6: 50 chicks/each) The G1(control neither vaccinated nor treated), G2 vaccinated with NDV, G3 (vaccinated with MDV Rispen strain), G4 (vaccinated with MDV and NDV) , Group 5 vaccinated with MDV vaccine and treated with Curcuma Longa , and G 6 vaccinated with MDV, NDV and treated with Curcuma Longa . Chicks vaccinated with NDV vaccine received Hitciner B-1 strain at 7th day of age then boosted with LaSota strain at 21st day of age in drinking water, while groups vaccinated with MDV vaccine (0.2ml/chick) at one day of age by S/C injection. Serum samples at 10, 14, 17, 21, 28, 35, and 42 of age for HI test against NDV. Heparinized blood samples at 10, 14, 17, 21, days of age for phagocytic activity of macrophages. All groups were challenged with vvNDV for detecting the protection percent. From this study it could be concluded that MDV vaccine has an immunosuppressive effect on chicks and this could be antagonized by immunostimulant as curcuma longa The surprising immuno-stimulatory effect of curcuma is in the induction of protection level 80% in treated but not NDV vaccinated group which equivalent to that group vaccinated with NDV vaccine only and not treated. From the obtained results we recommend the use of curcuma longa powder in poultry rations for enhancing the immune response against either field infection or vaccination.

Keywords: Marek's disease, New Castle disease, immune, Curcuma.

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* Virology Department, Fac.Vet.Med., Beni-Suef University.
**Animal Health Research Institute, Kafr El-sheikh regional lab.
(Mohamed_saif85@hotmail.com).

INTRODUCTION

Birds facing during their life many stress factors among them vaccination which is considered the most important one of them. Immunostimulants are used to counteract the effect of such immunosuppressive factors and to potentiate the immune response of poultry for the applied vaccines **(Abd El-Fatah *et al.*, 1999 and Madbouly *et al.*, 1999)**. MDV is known since long time as immunosuppressive agent and this virus immunosuppression interfere with the immune response against microbial agents infection and vaccines and the degree of immunosuppression be associated with the severity of the disease **(Purchase *et al.*, 1968; Payne, 1970; Sharma, 1987 ; Rivas and Fabricant, 1988 and Heidari *et al.*, 2010)**. Field as well as vaccinal virus strains of MD has gross changes in both bursa of Fabricious and thymus glands of chickens with drastic reduction in packed cell volum and hematopoiesis which results in immunosuppression **(Jakowski *et al.*, 1969; Sharma, 1978; Purchase and Sharma, 1974 and Jakowski *et al.*, 1970)**. Curcumin (diferuloylmethane) is an orange-yellow component of turmeric (Curcuma longa), a spice often found in curry powder. Traditionally known for its an anti-inflammatory effects, curcumin has been shown in the last two decades to be a potent immuno-modulatory agent that can modulate the activation of T cells, B cells, macrophages, neutrophils, natural killer cells, and dendritic cells. Curcumin can also down regulate the expression of various pro-inflammatory cytokines including TNF, IL-1, IL-2, IL-6, IL-8, IL-12, and chemokines, most likely through inactivation of the transcription factor NF-kappaB. Interestingly, however, curcumin at low doses can also enhance antibody responses. This suggests that curcumin's reported beneficial effects in arthritis, allergy, asthma, atherosclerosis, heart disease, Alzheimer's disease, diabetes, and cancer might be due in part to its ability to modulate the immune system. Together, these findings warrant further consideration of curcumin as a therapy for immune disorders **Bright (2007) , Jagetia and Aggarwal (2007) and Sikora *et al.* (2010)**. Modern science has revealed that curcumin mediates its effects by modulation of several important molecular targets, including transcription factors (e.g.,

NF-kappaB, AP-1, Egr-1, beta-catenin, and PPAR-gamma), enzymes (e.g., COX2, 5-LOX, iNOS, and hemeoxygenase-1), cell cycle proteins (e.g., cyclin D1 and p21), cytokines (e.g., TNF, IL-1, IL-6, and chemokines), receptors (e.g., EGFR and HER2), and cell surface adhesion molecules. Because it can modulate the expression of these targets, curcumin is now being used to treat cancer, arthritis, diabetes, Crohn's disease, cardiovascular diseases, osteoporosis, Alzheimer's disease, psoriasis, and other pathologies. (**Aggarwal *et al.*, 2005**.).

The laboratory studies have identified a number of different molecules involved in inflammation that are inhibited by curcumin including phospholipase, lipooxygenase, cyclooxygenase 2, leukotrienes, thromboxane, prostaglandins, nitric oxide, collagenase, elastase, hyaluronidase, monocyte chemoattractant protein-1 (MCP-1), interferon-inducible protein, tumor necrosis factor (TNF), and interleukin-12 (IL-12). Curcumin has been demonstrated to be safe in six human trials (human trials using 1125-2500 mg of curcumin per day and up to 8000 mg of curcumin per day for 3 months found no toxicity from Curcumin). (**Chainani-Wu, 2003**).Curcumin significantly reduced Coxsackievirus RNA expression, protein synthesis, and virus titer and protected cells from virus-induced cytopathic effect and apoptosis (**Si *et al.*, 2007**)

Mazumber *et al.* (1995) demonstrated that curcumin has an antiviral activity, being a HIV-1 integrase inhibitor (IC_{50} = 40 μM) and suggested that curcumin analogs may be developed as anti-AIDS drugs. Data showed that curcumin inhibited the replication of HIV-1 integrase protein. **Eigner and Scholz (1999)** reported that curcumin was claimed for anti-HIV-1 and HIV-2 activities in a recent patent application.

The aim of the present study is to clarify the non-specific immuno-stimulatory effect of Curcuma longa against NDV infection and immunosuppressive effect of MDV

MATERIALS & METHODS

MATERIALS

Chicks:

A total of 500 one day old Hubbard chicks were fed balanced ration and reared in good hygienic measures and divided into 10 groups (G1-

G10 50 chicks/each) The G1(control neither vaccinated nor treated), G2 vaccinated with NDV, G3 (vaccinated with MDV Rispen strain), G4 (vaccinated with MDV and NDV) , Group 5 vaccinated with MDV vaccine and treated with Curcuma Longa, and G 6 vaccinated with MDV, NDV and treated with Curcuma Longa. Chicks vaccinated with NDV vaccine received Hitciner B-1 strain at 7th day of age then boosted with LaSota strain at 21st day of age in drinking water (Both vaccines purchased from Serum and vaccine research Institute , Abbassia, Egypt) , while groups vaccinated with MDV(TAD Marek Vac Forte) vaccine received only one dose (0.2ml/chick) at one day of age by S/C injection. Serum samples of all chicks were collected at 10,14, 17, 21, 28, 35, and 42 of age for determining the antibodies against NDV using HI test. Heparinized blood samples were collected at 10, 14, 17, 21, days of age for detecting the phagocytic activity of macrophages. All groups were challenged with vvNDV (Serum and vaccine research and production, Abbassia, Egypt) for detecting the protection percent.

Curcuma longa:
Curcuma longa was purchased as a powder from popular supper market for buying aromatic and medicinal herbal plants and used as feed additives in a percentage of 1%

Blood samples:
Two groups of blood samples were collected, from each chick by wing vein puncture, in sterile plastic centrifuge tube with heparin (20 IU/ml) for macrophage cells separation (10, 14, 17, 21, days of age for detecting the phagocytic activity of macrophages) or without heparin for serum separation (10, 14, 17, 21, 28, 35, and 42 of age for determining the antibodies against NDV using HI test).

Roswer Park Memorial Institute (RPMI-1640) medium:
RPMI-1640 medium was purchased from GibcoBRI Cat No. 51800-019, Lot No, 3072701, used in phagocytic activity assay.

Ficol hypaque:
This medium was used for the separation of mononuclear leukocyte cells from peripheral blood, obtained from Biochrom AG Cat No. L 6113 Lot No. 729B, stored at +2-+25°C.

Culture medium for C. albicans:
Sabouraud dextrose agar medium containing chloramphenicol 40 g/ml was kindly supplied from Dept. of Mycology, Animal Health

Research Institute, Dokki, Egypt. And used for cultivation of Candida albicans.

Fetal calf serum (F.C.S.):

Biochrom AG, Cat No. S 0113, Lot No. 224 B inactivated at 56°C for 30 min. and preserved at -20°C. This serum was added to the medium at a final concentration of 20%.).

METHODS

Haemagglutination-inhibition (HI) test:

HI test was done according to ***Majiyagbe and Hitchner (1977).***

Challenge test:

The chickens were challenged intramuscularly with 0.2 ml suspension containing 10^6 NDV/chicken (Velogenic strain).

Phagocytic activity and percentage of chicken peripheral monocyte using *C. albicans*: according to **Richardson and Smith (1981)**, and **Barry and John (1988)** as modified by **El-Enbaway (1990), and Saif (2004)**. The phagocytic activity was calculated according to the following equations:

$$\text{Percentage of phagocytosis} = \frac{\text{No. of ingesting phagocytes X 100}}{\text{Total No. phagocytes including non ingesting cells}}$$

$$\text{Phagocytic index} = \frac{\text{Total No. phagocytes with more than 3 blastospores}}{\text{Total No. phagocytes ingesting blastospores}}$$

RESULTS & DISCUSSION

Some immunosuppressive agents like NDV and MDV play an important role in exposing chickens to contact dangerous viral or bacterial diseases even if their agents are of low virulence. Newcastle disease virus inducing fatal disease in young chicks and respiratory, nervous disorders besides decreasing in egg production in adults (**Aldous and Alexander, 2001; Office Internationale des Epizooties, 2001 and Ali *et al.*, 2004**). To modulate the immunosuppressive effect of these agents, some immunostimmulants either natural or synthetic were used. In this study curcuma longa powder is used as feed additives for studying its effects against the immunosuppression of Marek's disease virus vaccine and infection with very virulent Newcastle disease virus. To achieve the main goal of this study three hundreds young one day old Hubbered chicks were divided into 6 groups (G1-G6, 50 chicks/each). The G1(control neither vaccinated nor treated), G2 (vaccinated with NDV), G 3 vaccinated with MDV Rispen strain .Group 5 vaccinated with MDV Rispen strain and

treated with Curcuma longa powder. Groups 4 vaccinated with MDV and NDV while group 6 (vaccinated with MDV and NDV) and treated with Curcuma longa powder. All these 6 groups were challenged with vvNDV at 42nd day of age.

In this study natural immunostimmulants as Curcuma Longa powder, was purchased from popular supper market for aromatic and medicinal herbal plants and used as feed additives in a percentage of 1% and used as an immunostimulant in chicken.

Groups 3-6 were vaccinated with MDV vaccine Respin strain as one dose 0.2ml/chick S/C in the first day of life while groups 2,4 and 6 received two doses of NDV vaccine at 7 (Hitchner B1 by eye drop inoculation) and 14 day of life (LaSota by drinking water) . Groups 5& 6 fed on ration containing Curcuma longa powder as 1%. Group 1 was left unvaccinated and untreated group. The HI antibody titers that determined in all groups vaccinated with NDV vaccines showed gradual increase and reached the peak at the third week of age. On comparing these groups according to their treatment, the obtained results reaveled that group 2 that only received the NDV vaccine showed the highest HI antibody titer at 21st day of age then declined at day 28th and elevated again from day 35th of age while group 4 that vaccinated with MDV and NDV showed decrease in HI antibody titer from the 21st day of age onward tell the end of the experiment and this declare the immunosuppressive effect of MDV vaccine. The highest HI antibody at 21st day of age 819.2 was detected in group 6 that vaccinated with MDV &NDV and treated with Curcuma longa and this denotes to the immuno-stimulatory effect of Curcuma longa **(Table1).**

Regarding the phagocytic activity of macrophages in these groups, group 1 (not MDV vaccinated) showed higher percentage of phagocytosis (34 & 24%) than group 3 (only MDV vaccinated) (24 & 17%) at 10th & 14th day of age. While, group 3 showed lower percentage of phagocytosis (24, 17, 55 & 49) other than group 5 (MDV vaccinated & Curcuma treated) (44, 58, 75 & 71) at 10, 14, 17 & 21 days post vaccination **(Figure 1).**

However ,the phagocytic indexes in vaccinated & treated groups were higher than group 4 (vaccinated but not treated with any Curcuma longa). Group 1 (not MDV vaccinated) and group 3 (only MDV vaccinated) showed lower index (0.324 and 0.333, respectively) than groups 5 that showed 0.523 (MDV vaccinated and treated with Curcuma) at 10th day of age. The same picture was found at 14th, 17th & 21st day of age. Group 6 (MDV & NDV vaccinated and Curcuma treated) showed higher index (0.567 & 0.544) at 17th and 21st day of age than the other groups **(Figure 2).**

On the protection level against experimental infection with vvNDV, Group (5) treated with Curcuma and vaccinated with MDV vaccine but not with NDV vaccine and challenged with vvNDV showed 80 % protection. Group (6) treated with Curcuma and vaccinated with both MDV & NDV vaccines and challenged with vvNDV showed 100 % protection. While group 2 (only NDV vaccinated) showed 80 % protection, but some birds showed severe symptoms of ND in group 4 then survived and this may be attributed to the immunosuppressive effect of MDV vaccine **(Figures 3 and 4).**

The surprising immunostimulatory effect of Curcuma in induction of protection level (80%) (in treated but not NDV vaccinated group) equivalent to that group vaccinated with NDV vaccine only (but not treated) needs further studies for confirming these obtained results. The main effect of protecting these chicks from infection with vvNDV may be attributed to the anti inflammatory effect of Curcuma longa. Different molecules involved in inflammation that are inhibited by curcumin including phospholipase, lipooxygenase, cyclooxygenase 2, leukotrienes, thromboxane, prostaglandins, nitric oxide, collagenase, elastase, hyaluronidase, monocyte chemoattractant protein-1 (MCP-1), interferon-inducible protein, tumor necrosis factor (TNF), and interleukin-12 (IL-12). Curcumin has been demonstrated to be safe in six human trials (human trials using 1125-2500 mg of curcumin per day and up to 8000 mg of curcumin per day for 3 months found no toxicity from Curcumin). **(Chainani-Wu, 2003)** Curcumin's reported beneficial effects in arthritis,

allergy, asthma, atherosclerosis, heart disease, Alzheimer's disease, diabetes, and cancer might be due in part to its ability to modulate the immune system **(Natarajan and Bright, 2002; Aggarwal *et al.*, 2003; Chan *et al.*, 2003 ; Chendil, 2004; Adams *et al.*, 2005 ; Fang and Holmgren, 2005 and Furness *et al.*, 2005).** Together, these findings warrant further consideration of curcumin as a therapy for immune disorders **Jagetia_and Aggarwal (2007).** Modern science has revealed that curcumin mediates its effects by modulation of several important molecular targets, including transcription factors (e.g., NF-kappaB, AP-1, Egr-1, beta-catenin, and PPAR-gamma), enzymes (e.g., COX2, 5-LOX, iNOS, and hemeoxygenase-1), cell cycle proteins (e.g., cyclin D1 and p21), cytokines (e.g., TNF, IL-1, IL-6, and chemokines), receptors (e.g., EGFR and HER2), and cell surface adhesion molecules. Because it can modulate the expression of these targets, curcumin is now being used to treat cancer, arthritis, diabetes, Crohn's disease, cardiovascular diseases, osteoporosis, Alzheimer's disease, psoriasis, and other pathologies (**Aggarwal *et al.*, 2005**).

On the other hand Curcuma longa may be involved in retarding the replication pathway of NDV by preventing its entry to the host cells, replication of viral nucleic acid and /or releasing of the progeny virus particles from the infected cells. A third explanation in our opinion may be to the synergistic effect of the curcuma longa as anti inflammatory and antiviral. The antiviral effect of the curcuma longa was existed in different studies contributing to different viruses. Curcumin significantly reduced Coxsackievirus RNA expression, protein synthesis, and virus titer and protected cells from virus-induced cytopathic effect and apoptosis **Si *et al.* (2007) and Mazumber *et al.* (1995)** demonstrated that curcumin has an antiviral activity, being a HIV-1 integrase inhibitor (IC_{50} = 40 μM) and suggested that curcumin analogs may be developed as anti-Aids drugs. Data showed that curcumin inhibited the replication of HIV-1 integrase protein. **Eigner and Scholz (1999)** reported that curcumin was claimed for anti-HIV-1 and HIV-2 activities in a recent patent application.

From this study it could be concluded that MDV vaccine has an immunosuppressive effect on chicks and this could be antagonized by immunostimulants as Curcuma longa.

From these results we recommend the use of Curcuma powder in poultry ration for enhancing the immune response against either field infection or vaccination.

Table 1. Mean heamagglutination Inhibition antibody titer against NDV vaccines.

	Mean HI titer / days of age						
Groups*	***10th day***	***14th day***	***17th day***	***21st day***	***28th day***	***35th day***	***42nd day***
2	256	204.8	430.4	716.8	179.2	230.4	204.8
4	460.8	409.6	430.4	409.6	204.8	204.8	153.6
6	409.6	204.8	716.8	819.2	358.4	179.2	409.6

*G2: NDV vaccine only. G4: NDV vaccine + MDV vaccine.
G6: NDV vaccine + MDV vaccine + Curcuma.

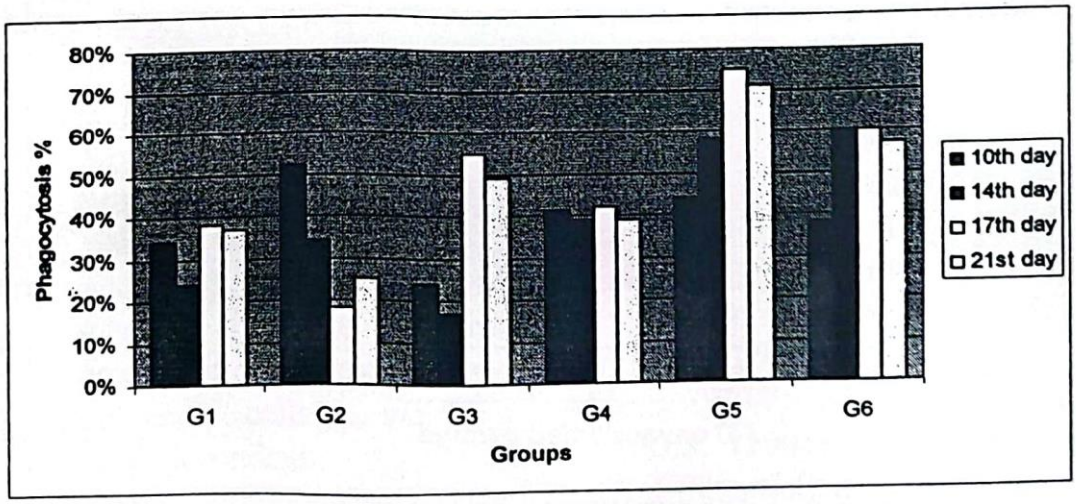

Figure 1. Phagocytosis % for all groups.

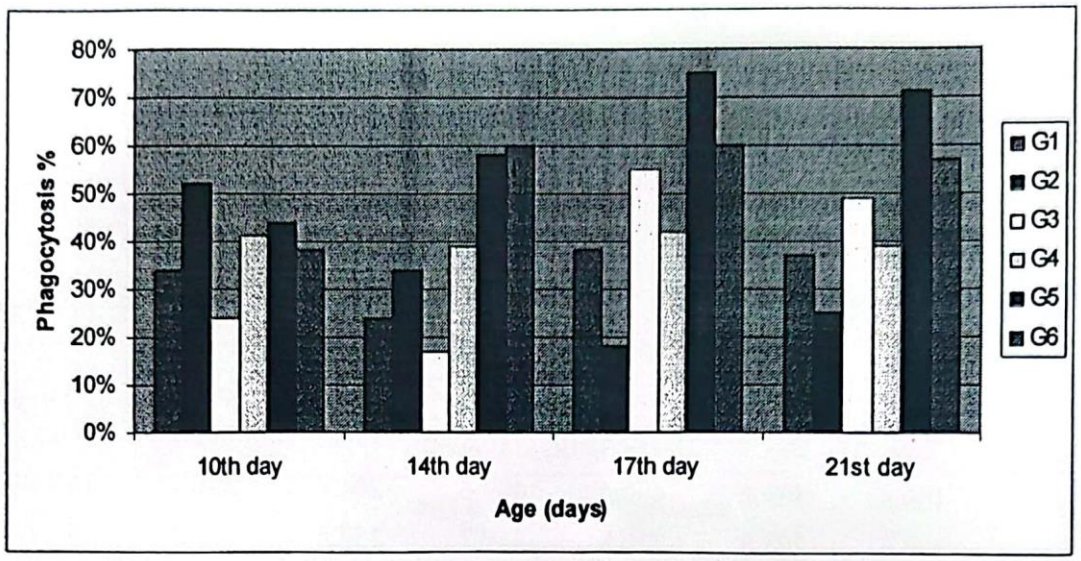

Figure 2. Phagocytosis Index for all periods.

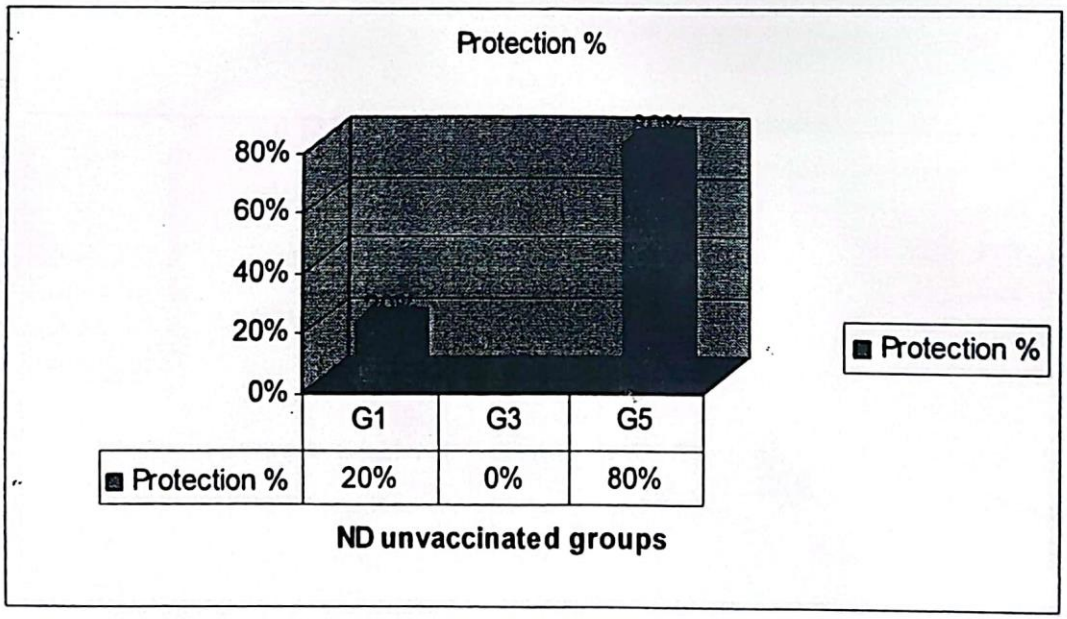

Figure 3. Protection level against experimental infection with vvNDV in unvaccinated groups.

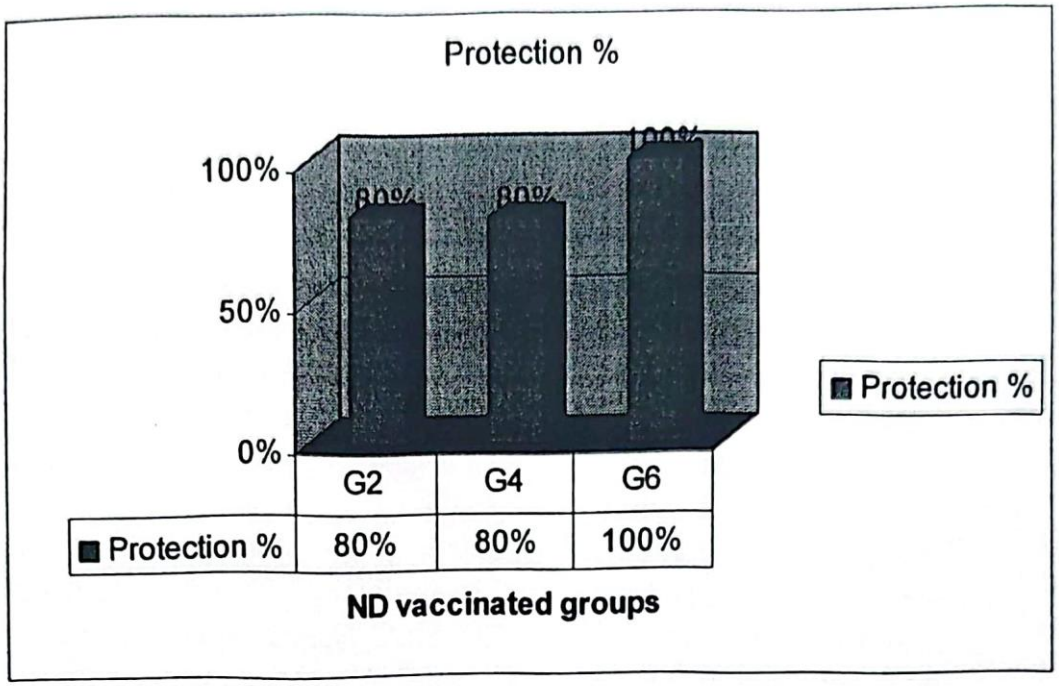

Figure 4. Protection level against experimental infection with vvNDV in vaccinated groups.

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