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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 Dietary Supplementation of *Echinacea purpurea* on the Humoral Immune Response Against Newcastle Disease Vaccine in Broiler Chicks

Mansour Mayahi[1], Masghoud Reza Saify Abadshapory[2], Hossain Najafzadeh[3] and Marzieh Kefayat[4]
[1]Department of Avian Disease, [2]Department of Virology, [3]Department of Pharmacology,
Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran
[4]Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran

Abstract: In order to study evaluated effect of *Echinacea purpurea* on the humoral immune system against live vaccine of Newcastle disease, 120 day-old broiler chicks were divided randomly into 6 groups of 20 chicks each. Group 1, 2 chicks were kept as unvaccinated and vaccinated control chicks respectively and fed only basic diet. Group 3 and 4 chicks were fed basic diet supplement with 0.1 and 0.5% *Echinacea purpurea* respectively for 6 weeks. Group 5 and 6 chicks were fed basic diet supplemented with 0.1 and 0.5% Echinacea for first 2 weeks of age respectively. All group were vaccinated with live Newcastle (B strain) at 9 day of age by eye drop route. The antibody titer against Newcastle disease vaccine was measured by hemagglutination inhibition and ELISA tests. The result of present study showed that the *Echinacea purpurea* does not significantly increased specific antibody titer against vaccination with live Newcastle (B strain) vaccine. One time vaccination of birds with ND vaccine was not sufficient for protection of birds and revaccination is necessary. The correlation coefficient for ELISA and HI test was 91%.

Key words: *Echinacea purpurea*, humoral, Newcastle disease, chicks

INTRODUCTION

The Newcastle disease virus (NDV) is a RNA virus belonging to the genus Avulavirus of family paramyxoviridae (Mayo, 2002a,b). The first out breaks occurred in poultry in 1926, in Java, Indonesia and in Newcastle -upon- Tyne, England. The name of Newcastle disease was coined by Doyle (Alexander *et al*., 2004). The disease is economically important, since it causes high morbidity and mortality, reduces egg production, deteriorates egg quality and impairs live performance (Orsi *et al*., 2009). Isolates are classified into 1 of 3 virulence groups by chickens embryo and chicken inoculation as virulent (velogenic), moderately virulent (mesogenic) or low virulence (lentagenic). Lentogenic strains are used widely as live vaccines in healthy chickens (encyclopedia). The clinical sign seen in birds, infected with NDV vary widely and dependent on factors such as: the virus, the host species, age of host, infection with other organisms, environmental stress and immune status. The typical clinical signs are: a state of prostration and depression, greenish white diarrhea, head turned to one side, torticollis, paralysis of legs and wings. Other typical characteristics of the disease include rapid spread, death within 2-3 days (Beard and Hanson, 1984) and laboratory characterization (Beard and Hanson, 1984). Serodiagnosis for detection of NDV antibodies are based on the Haemagglutination Inhibition (HI) and Enzyme-Linked Immunosorbent Assay (ELISA) (Alders *et al*., 2001). On commercial farms, control measures should attempt to prevent viruses from infecting the flock. It is of paramount important that good hygiene and biosecurity aimed preventing the introduction of viruses. The middle virulent B1 and La Sota strains of NDV are currently the most widely used efficacious live-virus vaccines for prevention of Newcastle disease that are world wide (Alexander, 1997). These live-virus vaccines induced high levels of IgA, IgG and IgM antibodies in sera (Russel and Ezeilfka, 1995). The level of vaccine reaction is an important consideration for intensive commercial poultry and because HB1 has very wild vaccinal reaction. It has been widely used for initial vaccination of intensive poultry (Bell *et al*., 1990). Effort for ND prophylaxis in broiler in Iran are focused on the active immunization by the use of live lentogenic vaccines.

Echinacea purpurea is a kind of Asteraceae native perennial grown in north America normally used pharmacologically and for aesthetic enjoyment. This plant was used to treat trauma and alleviate symptoms of infection and inflammation. The *E. purpurea* has been proven to show good immunoregulation and anti inflammation effects (Zhai *et al*., 2007). There is several plant species used therapeutically including *Echinacea angustifolia*, *Echinacea pallida* and *Echinacea purpurea*. These species differ somewhat in their chemical make up, containing multiple substances, of which polysaccharides, caffeic acid derivatives (cichoric acid),

Corresponding Author: Marzieh Kefayat, Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran

alkamides and glycoproteins are the most important in activity. Cichoric acid is an appropriate marker of the quality of *E. purpurea* containing product, because it has immune stimulatory effect and it is susceptible degradation (Mancek and Kreft, 2005; Liu *et al*., 2006). *Echinacea purpurea* was effective treatment in human acute respiratory infection (Narimaniana *et al*., 2005) or canine upper respiratory tract infections (Reichling *et al*., 2003). The above mentioned studies indicated that *E. purpurea* may exert an immunostimulating effect in many aspects, enhancing cytokine production, stimulation macrophages and T lymphocytes and elevation NK cells (Currer and Miller, 2001). An animal study demonstrated *Echinacea*'s ability to enhance cellular immunity in leukemia, resulting in a suppressive effect on leukemic mice, resulting in a suppressive effect on leukemia, via increased production on endogenous inter from-gamma (Hayashi *et al*., 2001). Present study was designed to study the effect of *E. purpurea* on the humoral immune system against live vaccine of Newcastle disease.

MATERIALS AND METHODS

Animal and treatment: One-day-old 120 broiler chicks were randomly divided into 6 group of 20 chicks each. Group 1 and 2 chicks were kept as unvaccinated and vaccinated control group respectively and fed only basic diet. Group 3 and 4 chicks were fed basic diet supplemented with 0.1 and 0.5% *Echinacea purpurea* respectively for 6 weeks. Group 5 and 6 chicks were fed basic diet supplemented with 0.1 and 0.5% *E. purpurea* for first 2 weeks of life respectively. Chicks of group were vaccinated with live Newcastle (B strain) at 9 day of age by eye-drop route.

Sampling: Blood sample were collected from 10 chicks of each group via wing vein at 0, 8, 21, 42 days after vaccination. Sera sample were separated by centrifugation and keep in -20°C until used.

Haemagglutination inhibition test: The antibody titer against ND vaccine was measured by the standard HI method (Allan and Gough, 1974). The antigen used was reconstituted commercial NDV La Sota vaccine. For this purpose, a total of 5 ml of chicken blood was collected aseptically in a disposable syringe containing 1 ml of sodium citrate (4% solution) as an anticoagulant. The blood was centrifuged at 1500 rpm for 15 min and the plasma and buffy coat was pipetted off. After washing thrice with Phosphate Buffer Saline (PBS), 1% suspension in PBS was used in HI test. The test was performed as described by Allan and Gough (1974). Briefly, after making two fold serial dilution of test serum up to 10th well, 4 HA unit of Newcastle disease virus was added up to 11th well and kept at 25-30°C for 25-30 min. A 1% chicken RBCs suspension was added into each well. The samples showing peculiar central button shaped settling of RBCs were recorded as positive and the maximum dilution of each sample causing Haemagglutination inhibition was considered as the end point. The HI titer of each serum sample was expressed as reciprocal of the serum dilution.

Enzyme-Linked Immunosorbent Assay (ELISA): The ELISA kit used was basically developed by IDEXX company in USA. The diluted test sera (diluted in phosphate buffer at 1:500) were added into the appropriate wells, already coated with NDV and the plate was incubated at 37°C for 30 min. The contents of wells were aspirated and the plate was washed four times with the washing buffer (Phosphate buffered saline with TWEEN™ 20). 100 µl of conjugate reagent (Pre-diluted sheep anti-chicken immunoglobulin peroxidase-conjugated) was added to each well and the plate was again incubated at 37°C for 30 min. The plate was washed as above. 100 µl of prepared substrate reagent (OPD) was added to each well and the plate was incubated at room temperature for 10 min. 100 µl of stop solution was added. The micro titre plate reading was record by reading spectrophotometerically at 450 nm. Positive and negative sera were used as controls as instructed by the manufacturer.

Statistical analysis: All data were expressed as mean ± SE. The differences between groups were analyzed by one-way Analysis of Variance (ANOVA). All statistical analysis was carried out using SPSS 11.0 for Windows. The correlation coefficient for ELISA and HI tests was calculated statistics by ANOVA correlation test.

RESULTS

Antibody sera titers: The antibody titers against ND vaccine in broiler's sera were shown in Table 1. No significant difference in antibody titers was observed between vaccinated group (group 2) and group 3, 4, 5 and 6 in days of 0, 8, 21 and 42.

The significant difference in antibody titers was observed between unvaccinated (group 1) and vaccinated control group (group 2) after 21 days.

The correlation coefficient for ELISA and HI tests in group 1, 2, 3, 4, 5 and 6 were 90, 93, 91, 89, 90 and 93%.

DISCUSSION

Echinacea purpurea is one of immunomodulator drugs and increase various cytokines, Lymphocyte and phagocytosis activity (Bauer, 2002; Sasagawa *et al*., 2006). Nowadays this plant use for treating cold, influ, respiratory tract disease and sinusitis.

Berman (2003) reported that *E. purpurea* were effective for preventing and treating cold and other infection. Barbara *et al*. (2009) reported the highest NDV antibody titers were seen in the groups receiving fermented juice for 2 days.

Table 1: Specific antibody NDV in broiler chicks's sera by HI test and ELISA (Mean±SEM)

Time group	0		8		21		42	
	HI	ELISA	HI	ELISA	HI	ELISA	HI	ELISA
1	48/0±4/4	473±2167	35/0±7/1	59±439	0	0	0	0
2	48/0±4/4	473±2167	32/0±2	51±475	22/0±8/1	49±463	25/0±8/1	59±439
3	48/0±4/4	473±2167	3/0±½	71±512	22/0±8/1	43±430	28/0±6/1	71±382
4	48/0±4/4	473±2167	22/0±5/1	41±394	22/0±7/1	36±404	23/0±8/1	63±473
5	48/0±4/4	473±2167	28/0±2	54±482	26/0±8/1	33±416	28/0±7/1	71±404
6	48/0±4/4	473±2167	18/0±½	49±509	2	3±494	2/0±4/2	24±580

The specific antibody titer against NDV in unvaccinated chicks (group 1) was decreased and not detectable by HI and ELISA tests after 21 days.

No significant difference was found between ND specific antibody titers of group 3 and 4 chicks during this experiment ($p \leq 0.05$), and feeding of basic diet supplemented with 0.1 and 0.5% *E. purpurea* for 6 weeks had no effect on increasing antibody titers against NDV.

The antibody titers in group 6 chicks were higher than group 5 chicks in 8, 21, 42 days, but it's not significant ($p \leq 0.05$).

The best immune response were found in group 6 chicks that were taken 0.5% *E. purpurea* in basic diet for 2 weeks but this difference was not significant between group 6 and other groups.

This study suggested that the antibody titers for all groups were low during the experiments and one time vaccination of birds with NDV was not sufficient for protection of birds and revaccination is necessary.

The correlation coefficient for ELISA and HI tests in group 1, 2, 3, 4, 5 and 6 were 90, 93, 91, 89, 90 and 93% and this result is coincide withe the findings of other researchers. Maro de Sousa *et al.* (2000) reported that correlation coefficient for HI and ELISA tests to detect ND antibody titers in ostrich were 91 and 90%. Raj *et al.* (2004) also reported that the correlation for HI and ELISA tests in EDS disease antibody titer was 79.3%.

ELISA tests is sensitive, rapid and needs low serum and we can examine more serums in less time, while HI test needs less equipment and is cheaper than ELISA and have acceptable sensitive. So HI test is commended to diagnosis ND specific antibodies.

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