P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

Research Article

Effect of Vitamin E and Selenium Supplementation on Antibody Titer against to Hemorrhagic Septicemia Vaccine in Buffalo Calves

Kashif Prince[1*], Muhammad Sarwar Khan[1], Muhammad Ijaz[1], Aftab Ahmad Anjum[2], Atif Prince[4] and Nisar Ahmad[3]

[1]Department of Clinical Medicine and Surgery; [2]Department of Microbiology; [3]Department of Parasitology, University of Veterinary and Animal Sciences Lahore, Pakistan; [4]Department of Zoology, University of Punjab, Lahore Pakistan
***Corresponding author:** kashif_prince@live.com

Article History: Received: August 28, 2016 Revised: September 18, 2016 Accepted: September 29, 2016

ABSTRACT

Vitamin E and selenium are considered as immune boosting agents. The present study was designed to evaluate effect of vitamin E and selenium on antibody titer against to hemorrhagic septicemia (HS) vaccine. Twenty unvaccinated buffalo calves (two to four month old) were randomly selected and divided into four groups (group A, B, C and D); and each group contain five calves. Group A was control; Group B was vaccinated against to HS vaccine, Group C was supported with vitamin E and selenium supplementation (VESS) and Group D was vaccinated against to HS along with VESS. Group C and D were supplement with VESS at day 0. After fifteen days, vaccine was administered to Group B and D. Three serum samples were collected at day 0, 30 and 45 of experimental trial from each buffalo calf. Samples were analyzed by complement fixation test to estimate antibody titer. One-way analysis of variance was performed to compare the treatment means and Tuckey's post hoc test was used to find difference between the treated groups. The level of significance was 0.05. Results showed that significantly higher antibody titer was developed in the group D (73.5±28.62) as compared to antibody titer of Group B (42.2±17.52) at day 45 of experimental trial. No development of antibody titer in Group A and C was observed at any stage. It was concluded that vitamin E and selenium significantly improve the antibody titer when it is given along with HS vaccine in buffalo calves.

Key words: Vitamin E, Selenium, Hemorrhagic Septicemia, Antibody titer, Buffalo calves

INTRODUCTION

The first reported study on *Pasteurella multocida* was performed by Louis Pasteur working on fowl cholera (Pasteur, 1880). Hemorrhagic septicemia (HS) is a disease caused by *P. multocida*, which is the normal commensal of respiratory tract of warm blood animals like ruminant and fowl, and it causes disease whenever immunity of the host is compromised due to any sort of stress e.g. work, disease or transport stress (Kumar *et al.*, 2004). *P. multocida* does not only cause HS in cattle and buffalo but also associated with versatile type of diseases like fowl cholera, atrophic rhinitis and snuffles in poultry, pigs and rabbits, respectively (Shivachandra *et al.*, 2011). It causes disease in a lot of avian and animals species even human can get infection (Christensen and Bisgaard, 2006). Mortality rate in HS is up to 100% in case of delay in treatment. *P. multocida* has two serotype B:2 and E:2 called as Asian and African serotypes (Biswas *et al.* , 2004). HS outbreaks in Asian countries are irrespective of the season and it can occur any time in the year but its occurrence is more common in rainy season (Kumar *et al.*, 2004). It is economically very important disease causing direct and indirect losses (Dziva *et al.*, 2008). Vaccines are used widely to prevent the disease all over the world against HS. Vaccine may be oil based or alum hydroxide based (Verma and Jaiswal, 1998).

Vitamin E and selenium supplementation alone or in combination have found to ameliorate antibody response against to *Escherichia coli* in pig, *Brucella* in cow, *chlamydia* in sheep, *taenia hydatigena* in dogs and *Leptospira interogans* in sheep, if it is administered parenterally (Larsen *et al.*, 1988; Nemec *et al.*, 1990; Giadinis *et al.*, 2000; Panousis *et al.*, 2001; Kandil and Abou-Zeina, 2005). Scares supply of selenium impairs neutrophil function and supplementation with selenium improves immunity even in stress (Spears and Weiss, 2008). It is still a mystery how selenium improves immunity of supplemented animal or what is mechanism of immune system impairment. But this could be due to

Cite This Article as: Prince K, MS Khan, M Ijaz, AA Anjum, A Prince and N Ahmad, 2017. Effect of Vitamin E and Selenium supplementation on antibody titer against to Hemorrhagic Septicemia vaccine in Buffalo Calves. Inter J Vet Sci, 6(1): 26-30. www.ijvets.com

modification in interaction of macrophages and lymphocytes. (Afzal *et al.,* 1984), or due to antioxidant properties in all cells including immune cells (Baalsrud and ØVernes, 1986; Reffett *et al.,* 1988). Vitamin E protect high free lipid containing membrane of lymphocytes (Nemec *et al.,* 1990).

Because there is an importance of the hemorrhagic septicemia and effect of vitamin E and selenium supplementation on humoral immune response in terms of antibody production, the presented study was designed to evaluate effect of selenium and vitamin E supplementation on the humoral immune response against to HS vaccine in buffalo calves.

MATERIALS AND METHODS

Grouping of the animals

Twenty healthy and unvaccinated buffalo calves (between 2 to 4 months old) were selected for this study. The calves were dewormed and randomly divided into four groups of 5 calves each. The groups were named A, B, C and D. Using Completely randomized design, treatments were assigned. Group A was control; Group B was vaccinated against to HS; Group C was supported with vitamin E and selenium supplementation; Group D was vaccinated against to HS along with vitamin E and selenium supplementation.

Treatment

At day 0 of study vitamin E and selenium supplementation was given to Group C and Group D. At day 15, Group B and D were vaccinated against to HS. No treatment was given to Group A. vitamin E and selenium supplementation was given in the form of intramuscular injection, one ml of which contains 25 mg tocopherol acetate and 2.2 mg sodium selenate. Oil based killed HS vaccine was used in this study.

Sample collection

Blood samples were collected three times, at day 0, 30 and 45 of the study from each animal. Blood was allowed to clot and serum was separated by centrifugation.

Antibody titration

Complement fixation test (CFT) was used by standard method to estimate the antibody level of each sample. Sheep red blood cell (RBC), antibodies against sheep RBC (amboceptors) and 3 hemolytic unit (3HU) complement were needed. Sheep for RBC, rabbits for amboceptors and guinea pig for complement were kept in department of microbiology, university of veterinary and animal sciences, Lahore, Pakistan. To raise amboceptors, total 6 injections were given at day 1^{st}, 3^{rd}, 5^{th}, 7^{th}, 9^{th}, 11^{th} with 0.1, 0.3, 0.5, 0.7, 0.9 and 1.0 ml of 5% sheep RBC were given to rabbit, respectively. After 21 days of last injection, 3ml of blood from rabbit was collected for serum (amboceptor) separation. Amboceptor was titrated with 1.5% RBC suspension and sub agglutination level was selected for the sensitization of RBC. Blood was taken directly from the heart of guinea pig and serum was used as a source of complement. Complement was titrated with sensitized sheep RBC and 3 hemolytic units (3HU) were made by diluting guinea pig serum in phosphate buffer saline (PBS) for CFT. Serum samples and amboceptors were heat in activated at 57^0C for 30 minutes before use. Antigen was prepared for CFT following Tanaka (1926) with little modification. *Pasteurella multocida* was grown on Mueller-Hinton broth and broth was centrifuged 3000 rpm and for 30 minutes and supernatant was discarded. The resulting pellet was washed three times with 30 mM tris-HCl, suspended in 0.5 ml 20% sucrose in 30 mM tris-HCl. Then 50 micro liter lysozyme (10mg/ml) was added and mixture was kept at freezing point for 30 minutes and sonicated at 200 watts for 10 minutes on ice. After sonication the solution was again centrifuged and clear supernatant was used as antigen. Before use antigen was titrated for anti-complementary activity with 3HU of complement. The highest dilution showing slight anti complementary activity was selected and half of its value was use for complement fixation test.

Statistical analysis

The results of the group A, B, C and D were compared at day 0, 30 and 45 of the presented study with one-way analysis of variance on IBM Statistical Package for the Social Sciences (SPSS) 20.0. LSD and Tuckey's post hoc test was used to compare the treatment groups level of significance was 0.05.

RESULTS

At day 0 there was no significant difference ($p>0.05$) between Groups A (1.3±0.55), B (1.3±0.55), C (1.1±0.89) and D (1.7±0.45) showing uniformity of the groups before the start of treatment trial. At day 30, highest antibody titer was found in group D (42.2±17.5) followed by B (32±17.52). No development of antibody titer was observed in Group A (1.5±0.55) and C (1.7±0.45). Similarly, at day 45 of study highest antibody titer was observed in Group D (73.5±28.62) followed by B (42.2±17.52) and no development of titer in Group A (1.7±0.45) and C (1.7±0.45). Titer development in Group B and D are higher as compared to day 30. Absence of antibody titer development in Group A shows that there is no involvement of field infection. Similarly, no development of antibody titer in Group C shows that vitamin E and selenium supplementation has not antigen to develop antibody titer. Group B and D shows significant ($P<0.05$) development of antibodies as compared to control showing vaccine used developed antibody titer. Group D has significantly higher antibody titer as compared to Group C confirming that vitamin E and selenium has improved antibody development at day 30 and 45 of presented study. Results show that vitamin E and selenium significantly improves ($P<0.05$) antibody production against HS vaccine. The results are shown in Fig 1.

DISCUSSION

Antibody response is dwindled in animals with selenium deficiency especially if it is associated with vitamin E deficiency (Dhur *et al.,* 1990). This condition is mainly associate with T cell dependent antigens (Chandra and Dayton, 1982). As we know B lymphocyte response

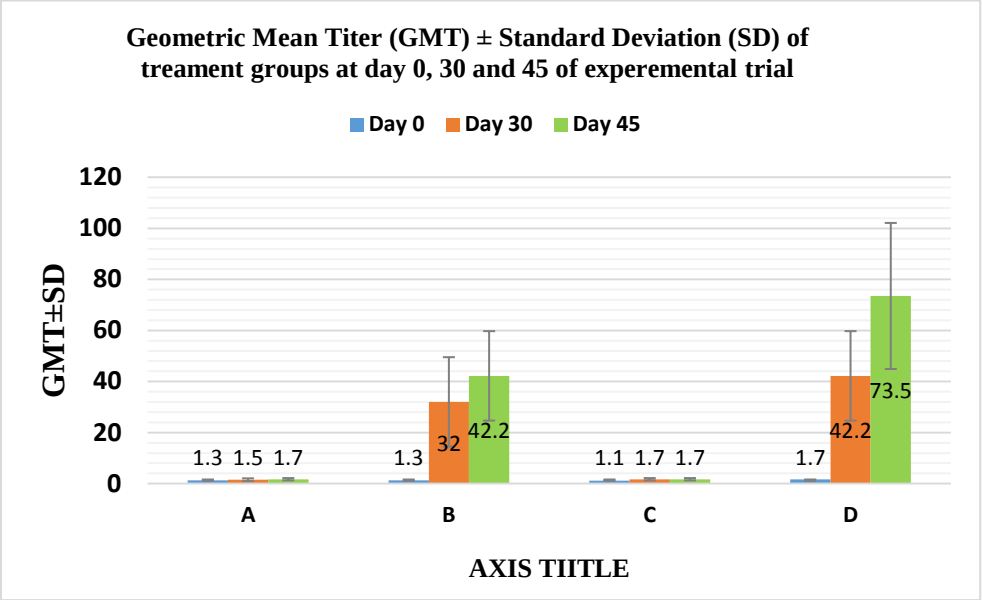

Fig. 1: Graph showing Geometric Mean Titer of Treatment Groups at day 0, 30 and 45 of study.

is dependent of T lymphocytes and macrophages, positive effect vitamin E and selenium on B lymphocyte is secondary to effect on T lymphocytes and macrophages (Burton *et al.,* 1977). Vitamin E and selenium supplementation improves immune response when given according to requirement. Excess or deficiency in in vitamin E or selenium is associated with impaired immune function this is confirmed in chicken (Marsh *et al.*, 1981), rat (Koller *et al.*, 1986) and mouse (Spallholz *et al.*, 1975).

Effectivity of selenium on immune system has been studied by many researchers in various animals such as lambs (Reffett *et al.*, 1988), piglets (Blodgett *et al.*, 1986), sheep (Larsen *et al.*, 1988) and cattle (Swecker *et al.*, 1989) and adult cattle (Swecker *et al.*, 1995). Similarly, vitamin E has also evaluated as immune boosting agent in calves (Cipriano *et al.*), chicks (Heinzerling *et al.*, 1974), pig (Ellis and Vorhies, 1976), sheep (Tengerdy *et al.*, 1983) and calves (Reddy *et al.*, 1986). Not only vitamin E and selenium is evaluated separately, but also in combination. The effect of Vitamin E and selenium on immunity in combination has been evaluated in chicks (Marsh *et al.*, 1981; Marsh *et al.*, 1986), pigs (Peplowski *et al.*, 1980), lamb (Reffett *et al.*, 1988), cattle (Droke and Loerch, 1989) and horse (Baalsrud and ØVernes, 1986) .Complement fixation test has been used for the detection and titration of antibodies against *Pasteurella multocida* by many researches in pigs and many other animals (Tanaka, 1926; Boulanger and Gwatkin, 1955).

Highest antibody titer was observed in Group D in which animals were supported with vitamin E and selenium supplementation along with HS vaccine. Antibody titer of Group B and D was higher at day 45 as compared to day 30 of the vaccination trial. At day fifteen, post vaccination, animals had not developed maximum antibody titer. Maximum antibody titer against HS vaccine is developed after 21 days post vaccination (Sabia and Hari, 2014). The results of presented study were in accordance with Droke and Loerch (1989), Panousis *et al.* (2001), Larsen *et al.* (1988), Knight and Tyznik (1990), Desowitz and Barnwell (1980) and Jelinek *et al.* (1988).

Droke and Loerch (1989) studied the effect of vitamin E and selenium supplementation on the *pastuella hemolytica* vaccine and found that highest antibody titer was observed in supplemented group.Panousis *et al.* (2001) conducted study to find the effect of vitamin E and selenium on *E.coli* in dairy cows and observed vitamin E and Selenium and combination or separately found to improve immune response. Larsen *et al.* (1988) evaluated the effect of vitamin E and selenium on the anti-tetanus toxoid, parainfluenza-3 virus and *Corynebacterium pseudotuberculosis* and highest level of IgG against the antigens was observed in selenium supplemented group. Jelinek *et al.* (1988) evaluated the vitamin E and selenium supplementation on *Brucella abortus* cells, rabbit red blood cells and *Corynebacterium pseudotuberculosis* toxoid as antigen and confirmed the results that vitamin E and selenium improves the immune response. Sheep red blood cells were use as antigen in Shetland ponies by Knight and Tyznik (1990) to evaluate effect of selenium on the anti-sheep red blood cell antibodies production and found selenium supplemented group had higher level of antibodies, Desowitz and Barnwell (1980) observed higher antibody response against killed *Plasmodium berghei* vaccine in Webster mice with selenium supplementation. Similar results were obtained by Sheffy and Schultz (1979).

Hayek *et al.* (1989), however, performed experimental trial on pigs to check effect of vitamin E and selenium on the immune response but no significant difference was found in IgG and IgA between supplemented and non-supplemented animals. Similarly, no effect of vitamin E and selenium supplementation was observed on total IgG in horses by Baalsrud and ØVernes (1986).

But the presented study proved that vitamin E and selenium supplementation improves the humoral immune response against HS vaccine in terms of antibodies development.

Conclusion

Vitamin E and selenium supplementation given 15 days before vaccination improve humoral immune response causing antibody production against to HS in the buffalo calves.

REFERENCES

Afzal M, RP Tengerdy, RP Ellis, CV Kimberling, CJ Morris, 1984. Protection of rams against epididymitis by a brucella ovis-vitamin e adjuvant vaccine. Vet Immunol Immunopathol, 7: 293-304.

Baalsrud KJ, G ØVernes, 1986. Influence of vitamin e and selenium supplement on antibody production in horses. Equine Vet J, 18: 472-474.

Biswas A, SB Shivachandra, MK Saxena, AA Kumar, VP Singh, SK Srivastava, 2004. Molecular variability among strains of pasteurella multocida isolated from an outbreak of haemorrhagic septicaemia in india. Vet Res Commun, 28: 287-298.

Blodgett DJ, GG Schurig, ET Kornegay, 1986. Immunomodulation in weanling swine with dietary selenium. Am J Vet Res, 47: 1517-1519.

Boulanger P, R Gwatkin, 1955. Complement-fixation tests of swine serum ii. In the demonstration of pasteurella multocida antibodies in experimentally produced rhinitis. Can J Comp Med Vet Sci, 19: 276-279.

Burton RM, PJ Higgins, KP McConnell, 1977. Reaction of selenium with immunoglobulin molecules. Biochim Biophys Acta, 493: 323-331.

Chandra RK, DH Dayton, 1982. Trace element regulation of immunity and infection. Nutrition Research, 2: 721-733.

Christensen H, M Bisgaard, 2006. The genus pasteurella. In The prokaryotes: Volume 6: Proteobacteria: Gamma subclass, M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer and E. Stackebrandt, eds. (New York, NY: Springer New York), pp. 1062-1090.

Cipriano JE, JL Morrill, NV Anderson, Effect of dietary vitamin e on immune responses of calves. J Dairy Sci, 65: 2357-2365.

Desowitz RS, JW Barnwell, 1980. Effect of selenium and dimethyl dioctadecyl ammonium bromide on the vaccine-induced immunity of swiss-webster mice against malaria (plasmodium berghei). Infect Immun, 27: 87-89.

Dhur A, P Galan, S Hercberg, 1990. Relationship between selenium, immunity and resistance against infection. Comparative Biochemistry and Physiology Part C: Comparative Pharmacology, 96: 271-280.

Droke EA, SC Loerch, 1989. Effects of parenteral selenium and vitamin e on performance, health and humoral immune response of steers new to the feedlot environment. J Anim Sci, 67: 1350-1359.

Dziva F, AP Muhairwa, M Bisgaard, H Christensen, 2008. Diagnostic and typing options for investigating diseases associated with pasteurella multocida. Vet Microbiol, 128: 1-22.

Ellis RP, MW Vorhies, 1976. Effect of supplemental dietary vitamin e on the serologic response of swine to an escherichia coli bacterin. J Am Vet Med Assoc, 168: 231-232.

Giadinis N, G Koptopoulos, N Roubles, V Siarkou, A Papasteriades, 2000. Selenium and vitamin e effect on antibody production of sheep vaccinated against enzootic abortion (chlamydia psittaci). Comp Immunol Microbiol Infect Dis, 23: 129-137.

Hayek MG, GE Mitchell, Jr., RJ Harmon, TS Stahly, GL Cromwell, RE Tucker, KB Barker, 1989. Porcine immunoglobulin transfer after prepartum treatment with selenium or vitamin e. J Anim Sci, 67: 1299-1306.

Heinzerling RH, CF Nockels, CL Quarles, RP Tengerdy, 1974. Protection of chicks against e. Coli infection by dietary supplementation with vitamin e. Proc Soc Exp Biol Med, 146: 279-283.

Jelinek PD, T Ellis, RH Wroth, SS Sutherland, HG Masters, DS Petterson, 1988. The effect of selenium supplementation on immunity, and the establishment of an experimental haemonchus contortus infection, in weaner merino sheep fed a low selenium diet. Aust Vet J, 65: 214-217.

Kandil OM, HA Abou-Zeina, 2005. Effect of parenteral vitamin e and selenium supplementation on immune status of dogs vaccinated with subunit and somatic antigens against taenia hydatigena. J Egypt Soc Parasitol, 35: 537-550.

Knight DA, WJ Tyznik, 1990. The effect of dietary selenium on humoral immunocompetence of ponies. J Anim Sci, 68: 1311-1317.

Koller LD, JH Exon, PA Talcott, CA Osborne, GM Henningsen, 1986. Immune responses in rats supplemented with selenium. Clin Exp Immunol, 63: 570-576.

Kumar AA, SB Shivachandra, A Biswas, VP Singh, VP Singh, SK Srivastava, 2004. Prevalent serotypes of pasteurella multocida isolated from different animal and avian species in india. Vet Res Commun, 28: 657-667.

Larsen HJ, K Moksnes, G Overnes, 1988. Influence of selenium on antibody production in sheep. Res Vet Sci, 45: 4-10.

Marsh JA, GF Combs, Jr., ME Whitacre, RR Dietert, 1986. Effect of selenium and vitamin e dietary deficiencies on chick lymphoid organ development. Proc Soc Exp Biol Med, 182: 425-436.

Marsh JA, RR Dietert, GF Combs, Jr., 1981. Influence of dietary selenium and vitamin e on the humoral immune response of the chick. Proc Soc Exp Biol Med, 166: 228-236.

Nemec M, M Hidiroglou, K Nielsen, J Proulx, 1990. Effect of vitamin e and selenium supplementation on some immune parameters following vaccination against brucellosis in cattle. J Anim Sci, 68: 4303-4309.

Panousis N, N Roubies, H Karatzias, S Frydas, A Papasteriadis, 2001. Effect of selenium and vitamin e on antibody production by dairy cows vaccinated against escherichia coli. Vet Rec, 149: 643-646.

Pasteur L, 1880. De l'attenuation du virus du cholera des poules. CR Academy of Science, 91: 673–680.

Peplowski MA, DC Mahan, FA Murray, AL Moxon, AH Cantor, KE Ekstrom, 1980. Effect of dietary and injectable vitamin e and selenium in weanling swine antigenically challenged with sheep red blood cells. J Anim Sci, 51: 344-351.

Reddy PG, JL Morrill, HC Minocha, MB Morrill, AD Dayton, RA Frey, 1986. Effect of supplemental vitamin e on the immune system of calves. J Dairy Sci, 69: 164-171.

Reffett JK, JW Spears, TT Brown, 1988. Effect of dietary selenium and vitamin e on the primary and secondary immune response in lambs challenged with parainfluenza3 virus1. J Anim Sci, 66: 1520-1528.

Reffett JK, JW Spears, TT Brown, Jr., 1988. Effect of dietary selenium and vitamin e on the primary and secondary immune response in lambs challenged with parainfluenza3 virus. J Anim Sci, 66: 1520-1528.

Sabia Q, MS Hari, 2014. Estimation of titers of antibody against pasteurella multocida in cattle vaccinated with haemorrhagic septicemia alum precipitated vaccine. Vet. World, 7: 224-228.

Sheffy BE, RD Schultz, 1979. Influence of vitamin e and selenium on immune response mechanisms. Fed Proc, 38: 2139-2143.

Shivachandra SB, KN Viswas, AA Kumar, 2011. A review of hemorrhagic septicemia in cattle and buffalo. Anim Health Res Rev, 12: 67-82.

Spallholz JE, JL Martin, ML Gerlach, RH Heinzerling, 1975. Injectable selenium: Effect on the primary response of mice. Proc Soc Exp Biol Med, 148: 37-40.

Spears JW, WP Weiss, 2008. Role of antioxidants and trace elements in health and immunity of transition dairy cows. Vet J, 176: 70-76.

Swecker WS, Jr., DE Eversole, CD Thatcher, DJ Blodgett, GG Schurig, JB Meldrum, 1989. Influence of supplemental selenium on humoral immune responses in weaned beef calves. Am J Vet Res, 50: 1760-1763.

Swecker WS, Jr., CD Thatcher, DE Eversole, DJ Blodgett, GG Schurig, 1995. Effect of selenium supplementation on colostral IgG concentration in cows grazing selenium-deficient pastures and on postsuckle serum IgG concentration in their calves. Am J Vet Res, 56: 450-453.

Tanaka A, 1926. A comparative study of pasteurella cultures from different animals. J. Infect. Dis., 38: 421-428.

Tengerdy RP, DL Meyer, LH Lauerman, DC Lueker, CF Nockels, 1983. Vitamin e-enhanced humoral antibody response to clostridium perfringens type d in sheep. Br Vet J, 139: 147-152.

Verma R, TN Jaiswal, 1998. Haemorrhagic septicaemia vaccines. Vaccine, 16: 1184-1192.