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

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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 interrogans 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

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modifications 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; Refsett 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 septicaemia 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 tocotherol 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 1st, 3rd, 5th, 7th, 9th and 11th 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°C 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±17.52) 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 A (1.5±0.55) and C (1.7±0.45). 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 (Dhut et al., 1990). This condition is mainly associate with T cell dependent antigens (Chandra and Dayton, 1982). As we know B lymphocyte response...
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., chick (Heinzlerling 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, 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


