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Full Length Research Paper

Effect of vitamin AD_3E supplementation for haemorrhagic septicaemia vaccine in laboratory mice

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Haemorrhagic septicemia is a fatal disease in tropical countries and annual vaccination is carried out in endemic areas. It is a killed vaccine with mineral oil as the adjuvant. The protection level of animal against infectious agent can be improved by the maintenance of proper nutrient status and vitamin supplementation. This method is followed by many vaccines both in animal and human medicine to enhance both cell mediated and humoral immunity. The objective of this study was to evaluate the logarithmic protection values of haemorrhagic septicaemia (HS) vaccine by vitamin AD3E supplementation in laboratory mice model. Active mouse protection test was performed by Reed and Muench's method to calculate the logarithmic protection value in HS vaccine using mice model. The logarithmic protection value of non-supplemented group was 6.33 and supplemented groups were 6.73 and 6.75; and were found to be significant in treatment. It was concluded that the logarithmic protection level of vaccine can be significantly improved by the addition of vitamin AD3E in mice and presumption may be the same with livestock in field situation.

Key words: Haemorrhagic septicaemia (HS), immunity, vitamin AD_3E.

INTRODUCTION

Haemorrhagic septicaemia (HS) is caused by infection with *Pasteurella multocida* serotype B: 2, a fatal systemic disease of cattle and buffaloes in countries of South and South East Asia (Hodgson et al., 2005). In Sri Lanka, the highest incidence of the disease was in the low country dry zone and out breaks occurred regularly (De Alwis et al., 1980). However, no clinical cases was reported for the last 9 years, though, it is still considered as the reporting disease in Veterinary Reporting System in the Country (Priyantha et al., 2009).

Managing the epidemic of HS is considered as a milestone of local animal husbandry and annual vaccination is carried out in endemic areas of the country as the main prevention strategy. A single dose of oil adjuvant vaccine is given to calves at 4 to 5 months of developed solid immunity waned gradually thereafter. Older animals, those over four years have apparently acquired a high level of immunity presumably due to the repeated annual vaccination (De Alwis et al., 1980).

The potency of HS vaccine, routinely test after the production has been tested in laboratory animals such as rabbits and mice (Alwis, 1992; De Alwis et al., 1976) due to economical reasons. The active mouse protection test was originally used by Ose and Muenster (1968) for evaluation of non-HS type *Pasteurella* bacterins and is been adopted by many workers for evaluating HS vaccines as well (De Alwis et al., 1980; Arawwawela et al., 1981). In adopting the active mouse protection test by Ose and Muenster (1968) for HS vaccine, a minimum of 4 to 5 logarithmic units in active mouse protection test (AMPT) was recommended and with minimum antigen content of 1.5 mg of dry bacterial mass per dose (Alwis et al., 1992). Nutritional status of recipient has still not been evaluated as a parameter for success of mass vaccination, although it has been identified as a vital tool for inducing immunity against a disease (Reddy et al., 1987). Apart from the total nutrition, some vitamins has been recognized as having unique influence on immunity during vaccination, affecting both humoral and cell mediated response (Reddy et al., 1987). This immunostimulatory effect reported is proven in vitamin A, E and D in livestock (Reddy et al., 1985).

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Vitamin E is a lipid soluble antioxidant present in cellular membranes that protect the cell from free radical by preventing lipid peroxidation (Chew et al., 1995; Tengerdy et al., 1983). It was proven by the different studies that the effect of enhancing antibody response in calves is by oral supplementation (Chew et al., 1995). Dairy cows injected with 1000 mg dl- alpha- tocopheryl acetate prepartum reported greater bactericidal activity at calving although; phagocytosis was not affected (Hogan et al., 1993). The positive benefit has been also proven in experimental animal by supplementary vitamin E that age dependent deterioration of the immune system can be altered (Oskar et al., 2001). The same effect was also reported with vaccine in human antibodies titer against hepatitis B vaccine and this significantly increased in subjects receiving supplemental vitamin E (Oskar et al., 2001). Furthermore, it was demonstrated that alpha tocopherol intake was negatively correlated with rates of clinical mastitis (Afzal et al., 1984).

Vitamin A was also considered as a potent antioxidant that received a significant effect on immunity, though disease etiology (Chew et al., 1995; Smith et al., 2005) was found as a modulating agent on cellular and non-cellular host defense system in animals (Chew et al., 1995). Beta carotene is caused by an induced lymphocyte proliferation and blastogenesis in cattles and pigs. It was also perceived causing increased helper/inducer T lymphocyte, peripheral monocytes, interleukin 2 receptors, transferring receptors, natural killer cells, cytotoxicity and tumor necrosis factors (Chew et al., 1995; Merker, 1985). A similar result was noticed in Holstein cows by higher phagocytic activity of netrophils, higher bactericidal activity during peripartum and lower intra-mammary infection during the lactation. However, the study further concluded that lower concentration of Vitamin A associated with sub-optimal host defense mechanism lowered the somatic cell counts in milk (Chew et al., 1995). On the other hand, carotinoids may modulate immune function by deactivating reactive chemical species such as free radicals, singlet oxygen and photochemical sensitizers (Chew et al., 1995).

The main function of Vitamin D is to regulate calcium homeostasis, bone function and resumption (Catorna et al., 2004). Meanwhile, it has been demonstrated that the effect of immune response is basically on peripheral mononuclear cells which acts as immune regulators in animal physiology (Catorna et al., 2004). Significant effect was examined on helper T cell which activates stronger cell mediated immunity against an infectious disease in human (Cartona et al., 2004).

Objective

The objective of the study was to evaluate logarithmic protection values of the haemorrhagic septicaemia killed vaccine in laboratory mice model by vitamin AD_3E parental supplementation. The finding of this study provided information on the effect of vitamin supplementation on vaccination efficiency in animals.

MATERIALS AND METHODS

Calculation of logarithmic protection value

The logarithmic protection level calculation was done by the active mice protection test as described by Reed and Muench's method in Cruickshank (1970) and same method was described in Priyantha et al. (2009) for HS killed vaccine.

Active mouse protection test

One hundred mice were vaccinated with the oil adjuvant vaccine that was described in Reed and Muench's method. Each mouse was vaccinated with 0.25 ml per animals subcutaneously and booster vaccination was given 14 days after the first vaccination. On day 21, the mice were divided into 10 groups each of which has 10 mice, and each group was challenged with 10 fold dilution of a 6 to 8 h CSY broth of a field strain, by the intra-peritoneal rout. Simultaneously, 100 unvaccinated control mice were also subjected to challenge with the same dilution. All mice were observed for five consecutive days for mortality.

Survivors in each group were noted and the LD_{50} value for vaccinated and unvaccinated mice is calculated as shown in Table 1 using the formula:

$$\text{Proportionate distance} = \frac{\text{Mortality above 50\% - 50}}{\text{Mortality above 50\% - mortality below 50\%}}$$

Negative logarithmic of LD_{50} titer = Negative logarithm dilution above 50% mortality + proportionate distance.

$$= \frac{70 - 50}{70 - 45.45}$$

$$= 0.81$$

$$= -1 + ^{0.81}$$

$$= 10^{-1.81}$$

LD_{50} titre = $10^{-1.81}$

Study design

The group one was neither vaccinated with HS vaccine nor Vitamin AD_3E supplemented parenteraly. These groups of mice were used as control in the calculation of logarithmic protection values for each group of mice according to the prescribed method by Reed and Muench (Cruickshank, 1970). The group two mice were vaccinated subcutaneously and mice were challenged by ten fold dilution of a 6 to 8 h CSY broth of *P. multocida* field strain by the intra-peritoneal route. However, group two was not treated with parental injection of vitamin AD_3E.

Group three was vaccinated as same with previous methodology described in the Reed and Muench's. Vitamin AD_3E injection was given following days before the fist vaccination days of 1, 3, 5, 7, 9, 11 and 13 day as 0.1 ml doses intramuscularly. It means that one

Table 1. Calculation method of LD_{50} in mice.

Challenge group			Accumulated values			
Dilution of challenge inoculation	No. of mice that died	No. of that mice survived	Died (D)	Survived (S)	Mortality ratio	Percent ($\frac{D}{D+S} \times 100$)
10^{-1}	4	6	14	6	14/20	70
10^{-2}	4	6	10	12	10/22	45.45
10^{-3}	4	6	6	18	6/24	25
10^{-4}	2	8	2	26	2/28	7.14
10^{-5}	0	10	0	36	0/36	0
10^{-6}	0	10	0	46	0/46	0
10^{-7}	0	10	0	56	0/56	0
10^{-8}	0	10	0	66	0/66	0
10^{-9}	0	10	0	76	0/76	0
10^{-10}	0	10	0	86	0/86	0

Table 2. Logarithmic protection values and LD_{50} of each group of mice after challenge with *Pasturella mutocida* broth cultures.

Group	LD_{50} value	Logarithmic protection value
Non vaccinated	9.49	
Vaccinated/No vitamin AD_3E treatment	3.16	6.33
Vaccinated/with vitamin AD_3E treatment-1	2.76	6.73
Vaccinated/with vitamin AD_3E treatment-2	2.74	6.75

dose of vitamin was given 14 days before the vaccination.

In group four, treatment were similar as group 3 and used as the replication. Vitamin AD_3E injection was given on day 1, 3, 5, 7, 9, 11 and 13 day as 0.1 ml doses before the 1st dose of HS vaccination. DUFAVIT AD_3E was used as the source of the vitamin supplementation available in the local market currently. The composition is as follows:

Contains per 1 ml solution
80000 iu Retinyl palmitate (A)
40000 iu Cholecalciferol (D_3)
20 mg Alpha tocopherol acetate (E)

The challenged culture, *P. multocida* "P33"was used and 1 ml of each broth containing 1.9×10^9 organisms on agar medium for 24 h after incubation at 37°C.

RESULTS

The calculated LD_{50} of the non-vaccinated mice was 9.49. It was 3.16 in the vaccinated without vitamin treatment and 2.76 and 2.74 unvaccinated with parental injection of vitamin AD_3E, respectively. Logarithmic protection values of the each vaccinated group were calculated and it was 6.33, 6.73 and 6.75 in vitamin supplementation as shown in Table 2.

Statistical analysis

The result was analyzed statistically by one way Analysis of Variance (ANOVA) using Minitab 14 statistical software to confirm whether the difference between two groups of vaccinated with vitamin AD_3E and vaccinated without vitamin AD_3E. The P value was 0.027 at 95% confidence intervals.

DISCUSSION

In this study, all vaccinated groups were shown over 4 logarithmic protection value considered as recommended for HS killed vaccine (Vipulasiri et al., 1982; Priyantha et al., 2009). However, statistically significant logarithmic values were observed in both replications (P value, 0.027) as compared to the non supplemented group. It was assumed that the synergistic effect rather than the individual role played by each vitamin differently in both mediated cells and humoral immunity may reveal the total effect of protection.

Vaccination supplementation with vitamin is not a new practice and has been proven by the number of researches in the world. The most of research was done with human vaccine such as tetanus toxoid and a six fold high titer with vitamin E was observed (Meydani et al., 1997). Same study further concluded that there was 65% improvement in delayed type hypersensitivity as well. Human vaccine supplementation with vitamin A also is widely practiced with BCG or Polio vaccine (Chew et al., 1995) and it is followed in Sri Lanka presently.

Mass scale vaccination was carried out for HS in dry zone of the country where the most of the cattles and

buffaloes are found. Those animals were reared there extensively and potential immune status was not evaluated prior to vaccination. Immune supplementation was not widely practiced in the local animal husbandry such as vitamin and mineral. Since vaccination is carried out annually in Sri Lanka, simultaneous supplementation of vitamin A, D and E will enhance the protection efficacy in local herds. This practice can be applicable to the epidemic or outbreaks situation where the protection level is critical. It may be valid for other disease like black quarter, brucella and foot and mouth disease where vaccination is carried out annually.

The study is being suggestive that vaccination efficacy can be improved by supplementation, especially vitamins which have effect on immune system such as vitamin A, E and D. Synergistic effect of vitamin AD_3E was proven affirmative as it responds to HS vaccine in mice. The natural host may respond similarly in field level. It was also suggested that body score of an animal can be considered as parameter for nutritional status of an animal, need to evaluate with in future. In contrast, the choice of animals in this study was restricted to mice only due to the practical difficulties and economic losses by using livestock.

Conclusion

The logarithmic protection values of HS vaccine had significant effect on Vitamin AD_3E supplementation in laboratory mice, due to the fact that it provided better protection than the others. Same presumption can be applied to natural host. It was also concluded that vitamin AD_3E supplementation provided better health in the prevention of infectious disease in livestock al local provision.

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