Wayamba Journal of Animal Science - ISSN :2012-578X; P20-P22, 2009
First Submitted December 22, 2009; Number 1261469195

Evaluation of shelf life of three Haemorragic Septiceamia vaccines made of improved adjuvant with present oil adjuvant vaccine in Sri Lanka.

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Haemorragic Septiceamia (HS) is an acute, fatal disease affecting cattle and Buffaloes in tropical countries in Asia and Africa. The causative agent has been identified as specific serotype of 6: B of Pasteurella multocida. This is an economically important disease due to the high mortality especially in young animals. Killed vaccine is practiced in Sri Lanka annually as a mass-scale vaccination program before the onset of rainy season. Mineral oil is used as the adjuvant of current vaccine that has given success so far. Shelf life of current HS vaccine is 6 months at 40C temperature and time duration is not enough for quality testing, transportation and vaccination in field. To overcome that, new vaccines were made and tested with improved adjuvant of ISA 50V2, ISA 206VG, ISA 70VG with mice model. Viscosity of each vaccine also was evaluated since it was important during administration. Both ISA 50V2, ISA 206VG adjuvant made vaccine were given better logarithmic protection level after one year period of storage and rest were not. Viscosity of the ISA 2 206 VG adjuvant also was satisfied and shown 12 months of extended self life, proven as better adjuvant for Haemorragic Septicemia vaccine in Sri Lanka.

Key words: Haemorragic Septiceamia, Killed vaccine, shelf life, Sri Lanka

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Haemorragic Septiceamia (HS) is an acute, fatal, septicaemic disease affecting in cattle and water buffaloes by specific serotype of the bacterium called Pasteurella multocida, which causes a variety of disease syndrome in species of animals (De Alwis, 1991). This is a common disease in wet tropical countries in Asia and Africa ,The specific sero type has been identified as 6: B (Namioka -Carter)((De Alwis,1986,De Alwis et al,1996)).HS is an economically important disease in Asia due to the loss of milk producers and drought animals in rice field (De Alwis, 1999).HS is suspected to have occurred in Sri Lanka since early part of the century, first confirmed outbreak reported in year 1955/1956(Hettiarrachchi, 1991).Most of the HS cases in Sri Lanka reported from NCP and entire outbreaks were confined to dry zone, low country intermediate and mid country intermediate zone until 1985(Hettiarrachchi.R, 1991). An oil adjuvant vaccine (OAV) for HS was first developed in 1950s.It was consist of water in oil emulsion, where the aqueous phase was consist of dense broth culture and oil phase was light mineral oil (De Alwis, 1999).Single dose of OAV was given to calves 4-5 months of age. The vaccine gave complete protection for 6 months and partial protection for 9 months(Hettiarachchi.R, 1991, De Alwis et al, 1996).The annual mass3 scale vaccination for HS was commenced in 1984, and drastic reduction cattle mortality reported from 1986 -1990 (Hettiarrachchi, 1991). However, Preventive vaccination for HS is the one of the most satisfactory method in Sri Lanka, resulted single clinical case was not reported for last 7 years in the country. In contrast, shelf life of OAV was detected as 6 months at 40C and the potency can be varied with storage temperature, not recommend for period of more than one month at room temperature (Vipulasiri et al, 1982).

Mass-scale vaccination program are carried out over three months from June to September before the main rainy season in Dry zone (De Alwis, 1991) and peak incidence of HS was reported from October-November and to lesser peak in January- February (Hettiarachchi R., 1991).Haemorragic septiceamia in Sri Lanka is produced by the Veterinary Research Institute and distribution as well as vaccination is done by the Department of Animal Production & Health (DAPH). Since shelf life of OAV is limited extended shelf life of the vaccine is preferred in the field. Low viscosity is extra benefit of during mass vaccination. Vaccine is injected into hamstring or gluteal muscles as an intramuscular injection during mass scale vaccination campaign. Restraining of individual animals is difficult where in the dry zone especially with water buffaloes that live in jungle for part of the year as free. However, Viscosity of vaccine is one of the criteria which need to be considered in such field situation.

The objective of this study was to determine and compare the new vaccine to maintain better shelf life for at least one year under refrigeration temperature .However viscosity of such vaccine also were evaluated comparatively.

MATERIALS AND METHODS

Vaccine

Malaysian strain, C 82 was used as seed strain, stored in frozen stage in citrated bovine calf blood. The organism was plated out on Tryptose agar with 0.3% yeast extract and 5% bovine blood. Hereafter, batch culture was grown in a simple medium formulated by Arawwawela et al,1981 in a special fermentor (Sartorious 90L).The final products was harvested at 12 hours, inactivated with 0.5% formalin(36-40% formaldehyde solution) and emulsified with equal volume of bacterine and mineral oil (Lanka white oil,BP,432. CPC) accompanied with 4% lanoline (L.K.P.N.Peiris, 1991). However, Rest of the vaccine mineral oil and lanoline were replaced by ISA 50V2, ISA 206VG adjuvants. The harvest is matched against a standard suspension which contains 1.5mg dry bacteria per ml. Thus a 3 ml dose will contain 1.5 ml of adjuvant and 2.25mg of dry bacteria (L.K.P.N.Peiris et al, 1991). In OAV vaccine lanoline were used as against emulsified but not with rest of vaccine. All vaccines were formulated prescribed by De Alwis for OAV, proportionately 50% of dense bacterine mixed with 50% of adjuvants, in order to maintain the correct weight of dry bacteria in a unique volume of vaccine. The proportion was differed in 70VG adjuvant 70% of volume contain adjuvant and rest was filled by inactivated dense bacterine. The composition was decided according to the manufacture guidance.

The vaccine is formulated as one ml of vaccine contained 0.75mg of dry bacteria in all products except in ISA 70VG adjuvant made vaccine, which it was 0.45mg of dry weight. The vaccines were stored at 4°C, constantly maintained through the experiment.

Potency test

The standard Potency test was performed after 2, 5, 9 and 12 months in storage of each vaccine at 40C.Safety of the each vaccine was assessed by inoculation 0.5 ml of vaccine subcutaneously in laboratory mice (International cancer research /ICR strain) mice were observered for 7 days for mortality. Mean while, vaccine of 0.1 ml was grown on blood agar,

MacConkey agar and Sabouroid agar and observed for any growth at 370C for 48 hours. Potency of each vaccine was carried out by the method of active mouse protection test described by the Karber (R.Cruickshanketal et al, 1970). For each adjuvant made vaccines 100 mice (ICR) were vaccinated on Day 0 and Day 14 with 0.25ml of each vaccine by the subcutaneous route. On day 21, mice were divided into 10 groups.Each group was challenged with a ten fold dilution of 8 hours broth culture of Pasturella multocida field strain "33"(Tryptose broth with 0.3% yeast extract) intraperitoneally ranging from 10-1 to 10-10. The number of Viable Pasturella multocida bacterial count has been written in table No: 02 and generally it was within the range of 1-3 x109 count per ml. The similar challenge was made on 100 of unvaccinated mice, using the same volume of inoculums. The mice mortality was observed for maximum 72 hours and recorded.

Viscosity of vaccine

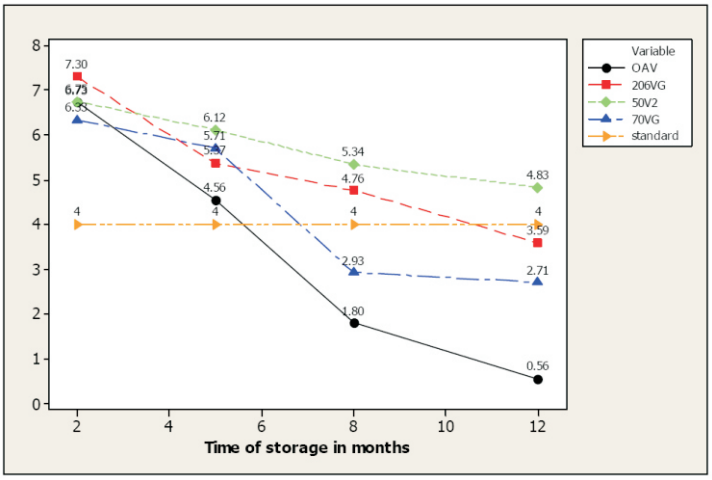
The viscosity of the vaccine was calculated at room temperature (250C) after 12 months of storage at 40C. Viscosity of the each product was measured by centipoises(cP) unit and viscosity of distilled water was considered as 1 during the calculation In this trial, 500 µl volume of each vaccine were used in a Brookfield digital viscometer (DV-II +with a CP - 40 spindle) (www.electronicsurplus).The viscosity is measured as the resistance or torque generated by the liquid on the rotating spindle. Spindle speeds of 12 rpm to 0.3 rpm were used depending on the product. Viscosity was described in cP unit and distilled water was considered as 1cP unit ((www. electronicsurplus , Brookfield-digital-viscometer).

RESULTS AND DISCUSSION

According to the Graph No : 01, All four vaccines were given 6 log unit protection after 2 months of storage at 40C temperature. Acceptable level of logarithmic Protection was further persisted for 5 months, maintained over 4 log unit by all vaccines. However, new vaccine had been given 5 log unit of log protection or above for 5 months storage, while OAV observed almost 4 log unit. It was recommended by previous studies that 4 log units were the acceptable level logarithmic protection level for HS in cattle and water buffaloes (Vipualsiri, 1982.) According to that, Only 206 VG maintained the 4.83 log unit even after 12 months of storage,which was in the protective range. Log unit protection level of the 50V2, 70VG and OAV vaccine were not sufficient for 12 months. However, OAV is the current successful vaccine in Sri Lanka maintained the accepted level of logarithmic protection and reduced drastically from 6 months. In ISA 70VG, protection level had been reduced after 8 months of storage and maintained up to 12 months at same temperature. It can be lower dry weight of bacteria in the vaccine compare to rest. In previous studies, it has been recommended that a minimum of 2 mg bacteria dry weight per animals is required for effectively immunizing cattle by a killed vaccine (De Alwis, 1999).

Both 206VG and 50V2 was shown accepted log unit of protection for 8 months period. However, protection log unit of 70VG and mineral oil were not in accepted range after 12 months of storage at 40C temperature.

The Highest viscosity of vaccine was observed in 50V2 adjuvant than other three (Table No: 01). The 50V2 was approximately two times thicker than current OAV used in the field. In 206VG and 70VG, vaccines were comparatively low in viscosity than OAV. However, the lowest viscosity was reported with 70VG adjuvant although potency was not satisfactory after the 8 months of storage.



Graph No: 1. Logarithmic protection of different adjuvant made vaccines against time of storage at refrigerator temperature after the production of each vaccine.

Table No: 1. viscosity of each vaccine with different adjuvant used.

Type of adjuvant	Viscosity(cP)
ISA 50V2	62.6
OAV	38.8
ISA 206VG	25.5
ISA 70VG	22.5

Table 02: Total bacterial counts of inoculated broth after 8 hours incubation at 370C

Month of storage	Total Bacterial counts of broth after 8 hours incubation at 37^0C per ml
2	$1.2 x10^9$
5	$1.1 x 10^9$
8	$1.8 x 10^9$
12	$1.9 X10^9$

CONCLUSION

In this study highest logarithmic protection level for 12 months storage was observed with 206VG adjuvant at 40C temperature. The same adjuvant was shown acceptable level of protection 6 months more than the current OAV, also be replaced mineral oil and lanoline from chemical composition as adjuvant and emulsifying agent respectively. Since the viscosity of the 206VG also met as satisfied, 206VG was concluded that the potential adjuvant for HS vaccine, which having extended shelf life for 12 months at 40C temperature.

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