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PROTECTIVE EFFICACY OF HAEMORRAGIC SEPTICEAMIA VACCINE IN CATTLE.

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Haemorragic septicemia is contagious disease, important disease in Sri Lanka specially in dry zone. Mass vaccination is carried out for three decades countinuosly and the disease has been controlled successfully. Since the shelf life of mineral oil vaccine is limited the objective of the study was to compare the protection efficacy of "ISA Montanide 206 VG", adjuvant instead of mineral oil in local condition.

The minimum lethal dose of *Pasturella multocida* was determined by the calves and protection efficacy of the two vaccine were detected by the animal challenge experiment (n = 16).The antibody mediated immunity were monitored by indirect Haem agglutination test (IHA) both in disease free and endemic zone of the country. The lesion developments at the vaccinated site also were evaluated both at Bopaththalawa and Nikeweratiya.

The minimum lethal dose of *Pasturella multocida* type B as determined as 10^4 c.f.u./ ml subcutaneous route of administration and both adjuvant made vaccine given similar protection in potency test and IHA titters at two sites. In conclusion the ISA Montanide 206 VG was accepted as better adjuvant for HS killed vaccine in mass scale vaccination together with protection efficacy and proven extended shelf life.

Key words: HS, Cattle, vaccine, ISA Montanide 206 VG, Protection

Haemorragic septiceamia (HS) is fatal contagious disease in cattle and buffaloes, caused by gram negative bacteria called *Pasturella multocida* B:2(Sasaki M,1991.,Sawada T,1991.,De Alwis,1991.,De Alwis,1999). Many countries associate "HS" with sudden death whilst in some mild disease with high recovery rate was reported (Sasaki M, 1991). Meanwhile, it was considered as a common disease in Asia and Africa, specially where management practices and veterinary facilities were poorly developed (De Alwis, 1991). This is an important disease in Sri Lanka as well, specially in dry zone where the several epidemics reported (Hettiarachchi, 1991). However, HS has been controlled by active immunization of oil adjuvant vaccine for last 30 years. The vaccination is continued in dry zone, although no clinical case was reported for decade (Priyantha *et al*,2010). The vaccination is continued for two or three months completed before the North Eastern rain fall(De Alwis,1991). However, almost 2-3 months required to inclusive quality control, storage and distribution. Hence, current shelf life of the vaccine is regarded as limiting factor for this long process (Priyantha *et al*,2008). As an alternative, shelf life of the oil vaccine has been improved by incorporating ISA Montanide 206 VG, observed extended shelf life up to one year (Priyantha *et al*,2008). Simultaneously potency of the vaccine also vital, must be evaluated on natural host (De Alwis,1996). For that, Minimum Lethal Dose (MLD) of *Pasturella multocida* type B must be

calculated before the potency test since it is unknown in cattle.

The objective of the study was to determine the MLD of *Pasturella multocida* in cattle in order to carry out the challenge study using cattle. The second objective was to evaluate the protection efficacy of "Montanide ISA 206 VG" adjuvant made vaccine and comparison of humoral antibody response in field situation natural host.

MATERIALS AND METHODS

Animal trial one: determination of minimum lethal dose.

Sahival, almost one year (1.2 to 1.5) animals (n=10) were selected for animal trial and who were proven negative for HS by Indirect Haem Agglutination (IHA) test described the method in Wijewardana *et al*, (1998). All were managed intensively, not vaccinated for HS and separated into five groups when each consisted of two. After that, Eight hours incubated *Pasturella multocida("P 33")* in Caesin Sucrose Yeast broth were inoculated into the cattle subcutaneously as described in Priyantha *et al*,2009 (Table No:01). Clinical sings and mortality was observed for 120 hours and numbers of bacterial colonies were determined by the spread plate methods described in Quinn *et al*, 1994. The mean c.f.u. was taken as the number of colonies in the inoculation as described in table No:01.

Table No:01. Number of c.f.u. of *Pasturella multocida* in different challenged groups of calves.

Group No	Dilution($1x10^9$/ml) c.f.u.
1	10-3
2	10-4
3	10-5
4	10-6
5	10-7

Animal trial No:02

Sahiwal, HS free (n = 15) animals were selected for animal trial No: 02 and separated in to group representing 2 animals per each (Except in group 7 and one was died before the inoculation). Those were vaccinated as described Table No: 02 whilst control groups were not vaccinated. All were reared semi intensively in the Animal Research Farm at Gannoruwa , challenging was carried out exactly 365 days of post vaccination. The Mineral oil vaccine (MOV) was prepared by the method described by Peiris L.K.P.N. *et al*,(1991) and Montanide ISA 206 VG adjuvant based vaccine was produced by the Priyantha *et al*,(1998).Only different observed of two was the type of adjuvant and presences of Lanoline only in MOV, other parameters like dry weight of cells were similar (Priyantha *et al*,2009).

In Group 1 to 4, animals were challenged by ten times of minimum lethal dose of *Pasturella multocida* ("P 33") and animals in groups 6 to 8 were challenged with 100 times of minimum lethal dose. However, groups 5 and 7 remain unvaccinated.

Table No:02. Distribution of experiment groups of animal for two different vaccine .

Group	Adjuvant	Challenge dose per 1 ml.
1	206 VG	10^5
2	206 VG	10^5
3	Mineral oil	10^5
4	Mineral oil	10^5
5	Control	10^5
6	206VG	10^6
7	control	10^6
8	Mineral oil	10^6

Experiment No:03

Field trial was carried out in non vaccinated, HS free calves (4 to 6 months) in two farms of National Livestock Development Boards. Location were selected representing HS reported (Nikeweratiya) and not reported zones (Bopaththalawa). Serum samples were collected for one year at three months intervals. Friesian calves (n=40) were used at Bopaththalawa and Sahiwal calves (n=40) were used at Nikeweratiya farm,

mainly depend on the availability. Antibody titers of serum samples were determined by the IHA test by the method described in Wijewardna *et al*,(1998).
Meanwhile, post vaccination reaction at the site of inoculation of each vaccine was evaluated together with hygienic condition of rearing pens.

RESULTS

In experiment No: 01, the challenged animals in group 4 and 5 were survived; meanwhile all the animals in 1, 2, and 3 died within 72 hours after the inoculation. Meanwhile, no sign of clinical disease were shown among survived animals. Body temperature and general temperament were not affected. The respective organism, *Pasturella multocida* was isolated from heart blood and spleen of dead animals by conventional bacteriological methods described in Wijewardana *et al*,(1998). The observed minimum lethal dose of *Pasturella multocida* type B was 10^4 c.f.u./ ml and it was used for the second trial as10 times and 100 times of original value .

Table No: 03. The post vaccination reaction on site of vaccine administration.

Farm	Reaction at the site a week after vaccination of OAV	Reaction at the site a week after vaccination of ISA Motanide 206 VG	Abcess formed OAV	Abcess formed ISA 206 VG	Floor condition(Wet/Dry)
HS Free zone	4/20	5/20	0/20	1/20	Wet
HS endemic zone	1/20	1/20	0/20	0/20	Dry

Table No:04.IHA titter of the vaccinated calves at Bopaththalawa NLDB farm.(usually animals are not vaccinated for hemorrhagic septicemia in this region of the country :Upcountry.)

Adjuvant	Time duration after vaccination in month	Mean IHA titter	SD	Minimum	Maximum	Status
Mineral oil	3	96.5	187.68	10	640	HS free zone
	6	68	139.608	10	640	
	9	38	25.2305	10	80	
	12	95	70.4796	10	160	
ISA Montanide 206 VG	3	43	57.97	10	160	
	6	140.5	303.184	10	1280	
	9	38	27.06	10	80	
	12	95	134.81	10	640	

Table No: 05.IHA titter of the vaccinated calves at Nikeweratiya NLDB farm.(HS endemic zone :Dry zone, North Western province.)

Adjuvant	Time duration after vaccination in month	Mean IHA titter	SD	Minimum	Maximum	HS status
Mineral oil	3	43	39.6166	10	160	Endemic zone
	6	72	138.739	10	640	
	9	43	36.7299	10	320	
	12	55	78.5728	10	160	
ISA Montanide 206 VG	3	63.5	60.3837	10	160	
	6	92.5	153.756	10	640	
	9	39	26.1373	10	80	
	12	93	135.921	10	640	

Table No: 06. Comparison of OAV and ISA Montanide 206 VG by two sample t test of different groups at different zone separately.

Months after vaccination	Two samples "t" test at Bopaththalawa farm/ P value	Two samples "t" test at Nikeweratiya/P value
3	0.530	0.213
6	0.340	0.661
9	0.675	0.694
12	0.124	0.257

In experiment No: 2, the calves were challenged with relevant infective dose and observed for 72 hours. The causative bacterial agent, *Pasturella multocida*, was isolated from heart blood and spleen of dead calves. Animals in the control were died within 48 hours and all the vaccinated animals were survived with no sign of clinical disease for 120 hours after the inoculation.

Exactly one week after vaccination both sites were visited and post vaccination reaction at the injected sites were recorded (Table No:03) and evaluated against hygienic conditions observed (Wet or dry, dung contamination) in floor of the house on day of vaccination.

In the field trial, IHA test was carried out three month intervals both at Bopaththalawa and Nikeweratiya, summarized in the table No:4 and No:5 respectively. Each farm, IHA titers were compared statistically by Minitab 14.5 Software and summary was found in table No:5.

DISCUSSION

Vaccine is important tool in animal husbandry and have had great effect on mortality than any other measures (Plotkin S.A.,2009).There is long history of inactivated vaccine, began in the late 19th century with killed whole bacteria or virus, is still widely using in 21st century (Plotkin S.A.,2009). Similarly, the HS vaccine has bound a long history in VRI, upgrading is continued, in order to get longer immunity and shelf life.

According to the 1st experiment 10^3 number of c.f.u./ml was not enough to cause either clinical disease or mortality on susceptible host. Meanwhile, all inoculated animal of 10^4c.f.u./ml died with typical HS infection such as anorexia, fever, dyspnea, salivation and recumbence (Horadagoda *et al*,,1991) by 10 fold

concentration. Hence, the MLD of HS was 10^4 c.f.u./ml in route of sub cutanious and the value was directly used in second experiment. However, MLD can be varied with other routes of challenge like intramuscular or intraperitoneal and (Horadagoda *et al*,1991) another natural host such as buffaloes, since buffaloes are more susceptible for HS infection than cattle (Horadagoda *et al*,1991).

In experiment No: 02, all animals were survived against 10 times and 100 times of

lethal dose challenged in both mineral oil and ISA Montanide 206 VG adjuvant made vaccines. Both were proven sufficient protection against 100 times lethal dose of *Pasturella multocida* B:2 one year after each vaccination. The purpose of incorporating an adjuvant into vaccine is to induce immune response to poorly immunogenic protein in bacteria bacterial composition remain unchanged ((Plotkin S.A.,2009)).However, There were no significant differences observed on potency among two adjuvants used in this trial.

Immunity to HS appears to be antibody mediated (Johnson *et al*,1991),comparison of humeral immunity executed at two different location representing both HS endemic and HS free zone of the country. According to the statistical interpretation carried out , there as no significant difference observed on antibody formation between the mineral oil and Montanide 206 VG at any stage from three month to 12 months post vaccination (Table No:06). Meanwhile, it was not altered in the endemic zone as well as the free zone (Table No: 04.,05.,06). At the same time, no significant differencet as observed in distribution of IHA titres of each vaccines at any stage of post vaccination, both vaccine responded similarly on antibody mediated immune system of vaccinated calves. Similar result was observed in another study (Priyantha *et al*, 2009) in which no difference as observed in four different adjuvants (Mineral oil, ISA Montanide 50 V2,ISA Montanide 206 VG and ISA Montanide 70 VG) made HS vaccine on antibody formation.

Minimum reaction at site of application is an encouraging factor during mass vaccination in order to minimize public resistance. The reaction at the site had been studied with both adjuvants and post vaccinated lesions mainly by invasion of secondary bacteria which found in feaces mainly her. In Bopaththalawa farm floor was damp and floor contamination by feaces was common (Table No:03). Since Nikeweratiye is in dry zone damp floor was uncommon. Contamination may lead to abscess at the site of vaccination. It is assumed as the reason that no abscess formed in the Nikeweratiya although few reported in Bapaththalawa. Usually Gluteal group of muscles are used for the vaccination in adults in Sri Lanka and contamination is considered comparatively lower than hamstring group. However, Hamstring muscle were utilized in this trial since calves were used. Hamstring was better place due to the musculature during early time of the age.

CONCLUSION

In conclusion, Mineral oil and ISA Motanide 206 VG based vaccine gave similar level of protection both cell mediated and humeral immunity concerned. In field condition both adjuvants showed similar type of antibody response in tow different geographical location in the country. However, shelf life of ISA Montanide 206 VG was observed almost one year, concluded as better adjuvant for the Haemorragic septiceamia oil adjuvant in the country.

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