INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2006/08–5–645–647
http://www.fspublishers.org

Immune Response of Buffaloe Calves to Haemorrhagic Septicemia Oil Adjuvant and Alum Precipitated Vaccine

K.T. JAFFRI[1], Z.J. GILL, A.R. BHATTI AND A. RAZA
Veterinary Research Institute, Lahore–Pakistan
[1]Corresponding author's e-mail: asfarasool@yahoo.com

ABSTRACT

Twenty-four buffalo calves of approximately same age (six month to one year of age) were used to monitor the immune response of haemorrhagic septicemia oil adjuvant (HSOAV) and alum precipitated (HSAPV) vaccines. Twenty-four calves after de-worming were divided in three groups, i.e. A, B and C, comprising of eight animals each. Group A was vaccinated with HSAPV @ 5 mL S/C and then it was sub-divided into two groups AI and AII (four animals each), AI group received a booster shot of vaccine @ 5 mL S/C after 30 days. Group B having eight animals was vaccinated with HSOAV @ 5 mL intramuscularly and it was sub-divided into two groups BI and BII. BI group received a booster shot of vaccine at the same dose rate after 90 days of 1st injection. Group C having eight animals was kept as un-vaccinated control. All animals were bled prior to vaccination and then every 30 days post vaccination and serum samples were subjected to Indirect Haemagglutination Test (IHA) and Passive Mouse Protection Test (PMPT) for one year. IHA titer and PMPT test results showed that APV gave protective antibody titre up to 120 days and 60 days (IHA 1:64) and PMPT gave 20% and 0% protection with and without booster. The animal vaccinated with OAV showed protective antibody titres up to 300 days and up to 210 days (IHA 1:64) and PMPT gave 60% and 50% protection with and without booster against challenge (LD_{50}- 10^{-6} dilution). This result approves the hypothesis that HSOAV gave long lasting immunity and booster dose of vaccine was necessary for strong immunity for longer duration.

Key Words: Buffalo; Calves; IHA test; PMP test; Haemorrhagic septicaemia

INTRODUCTION

Haemorrhagic Septicemia (HS) caused by *Pasteurella multocida* is an important disease of cattle and buffalo, which causes heavy economic losses (De Alwis, 1992). Due to the sudden onset, brief duration and fatal nature of the disease, treatment in most cases is not possible and the only satisfactory and practical method of control and prevention is by carrying out timely vaccination of all healthy and in contact animals and adopting hygienic measures.

During the past 2 or 3 decades, HS bactrin was used for vaccination but the period of immunity conferred by this is very short and in many places vaccination have to be repeated two or three times a year as animals were reported to be succumbed to the disease after a short period of vaccination. Therefore, bactrin was switched over to adjuvanted vaccine.

At present, alum precipitated vaccine is used as mass scale vaccine in Pakistan. Immunity induced by this vaccine lasts for 3 - 4 months only, which reflects an un-protective state of the vaccinated animals between two vaccinations, scheduled (Vancheswar *et al.*, 1955; Israil & Qauder, 1960). Alum precipitated vaccine is now being slowly switched over to oil adjuvanted vaccine, which gives long-term immunity up to one year (Bain & Jones, 1955; Anonymous, 2004).

In this study, immune response of buffalo calves to oil adjuvanted and alum precipitated vaccines prepared according to the OIE manual (Anonymous, 2004) was studied and was compared through Indirect Haemaglutination test (IHA) and Passive Mouse Protection test (PMPT).

MATERIALS AND METHODS

Twenty-four buffalo calves were procured from Bahadarnagar farm Okara. All these calves were approximately of same age (six month to one year of age) and were sero-negative to HS. After de-worming of all animals with Oxfendazole (Oxafax), all these calves were divided into three equal groups A, B and C.

Group A was vaccinated with alum-precipitated vaccine (HSAPV)@ 5 cc/subcutaneously and then it was further divided into two groups, AI and AII (four animals in each). Group AI was boosted up with HSAPV @ 5 cc S/C after 30 days. Group B having eight animals was vaccinated with oil adjuvanted vaccine (HSOAV) @ 5 cc deep intramuscularly and then it was divided into two groups BI and BII (four animals in each). B1 was given a booster dose of HSOAV @ 5 cc deep intramuscularly after 90 days and Group C was kept as un-vaccinated control. AII animals were bled prior to vaccination and after every 30 days post vaccination and serum samples were subjected to IHA as described earlier (Carter, 1955; Wijewardana, 1986) and

Table II. Geometric mean titre of the serum against *Pasteurella multocida* in group A vaccinated with ALUM precipitated vaccine and group B vaccinated with oil adjuvanted vaccine

Post Vaccinal days	Groups				
	Vaccinated with HS APV		Vaccinated with HS OAV		Control
	A1 (booster)	A2	B1 (booster)	B2	C
0	4.47	4.75	4.75	4.75	0
30	22.6	45.25	29.77	32	0
60	90.5	53.81	128	64	0
90	53.8	32	152.21	107.63	0
120	64	19.02	430.53	152.21	0
150	25.39	20.15	256	90.50	0
180	12.69	5.03	152.21	53.81	0
210	6.34	6.34	90.50	53.81	0
240	4	4	76.10	38.05	0
270	6.34	4	76.10	22.62	0
300	2.51	1.58	45.25	16	0
330	0	0	26.90	11.37	0
360	0	0	19.02	4	0
390	0	0	8	2.82	0
420	0	0	4	2.82	0
460	0	0	0	2	0
490	0	0	0	0	0

*GMT calculated by Log_{10} method.

Passive Mouse Protection Test (PMPT) to measure the immune response of the calves to both of the vaccines (Bain *et al*., 1982; Gomis *et al*., 1988/89).

IHA test. The test was performed as described by Carter (1955) using fresh sheep RBC as by Wijewardana *et al*. (1986) using 96 well IHA plate on all serum samples.

Pathogenicity test. LD_{50} of *Pasteurella multocida* (local vaccine Strain) was determined by using Reed and Meunch (1938) method to challenge the mice.

Passive mouse protection test (PMPT). PMPT was done with sera of vaccinated animals to protect mice using 10 mice per sample inoculated @ 0.25 cc/mice-subcutaneously and expressed protection as the percentage surviving out of ten after challenge with 100 LD_{50} ($10^{-6.5}$) *P. multocida* organisms (Bain *et al*., 1982; Gomis *et al*., 1989; Chandrasekaran *et al*., 1993).

RESULTS

Two experimental animals (one from group A1 & one from A11), vaccinated with alum precipitated vaccine died due to some extraneous reasons, the rest of the experimental animals along with control, were bled for serum collection and IHA test and Mouse Protection Test were performed.

The results of IHA test tabulated in Table I, indicate that HSOAV gave protective antibody titre (IHA titre 1:64) up to 300 days after booster shot and up to 180 days with out booster and antibodies could be detectable in serum of the vaccinated animals up to 420 days (IHA titre 1:4) with comparison to HSAPV, which gave protective antibody titre (1:64) up to 150 days with booster and up to 60 days with out booster. Antibodies were detectable in the serum of the vaccinated animals up to 330 days (IHA titre 1:4) with booster.

Mouse were used for potency testing as also indicated in British Veterinary Codex (1953) and as used by Tabatabaei *et al*. (2002). PMPT was done using serum of the vaccinated animals 60 days post vaccine and then it was done at one month intervals to judge the protection offered by vaccine. PMPT results are shown in Table III. Results showed that Animal Serum vaccinated with HSOAV gave prolonged and strong protection against the challenge dose as compared to the Alum precipitated vaccine.

Table I. Serum antibody titres against *Pasteurella multocida* in group A (vaccinated with HS ALUM precipitated vaccine) and group B (vaccinated with HS oil adjuvanted vaccine)

	Groups	No. of animal	Antibody Titres (DAYS)																
	Group A_1		0	30	60	90	120	150	180	210	240	270	300	330	360	390	420	460	490
Vaccinated With Hs Alum Precipitated Vaccine		1	1:4	1:64	1:128	1:8	1:64	Died	-	-	-	-	-	-	-	-	-	-	-
	(Booster)	2	1:4	1:32	1:128	1:64	1:32	1:8	1:4	1:8	1:4	1:16	1:4	0	0	0	0	0	0
		3	1:8	1:16	1:64	1:128	1:128	1:32	1:16	1:4	1:4	1:4	0	0	0	0	0	0	0
		4	1:4	1:8	1:64	1:128	1:64	1:64	1:32	1:8	1:4	1:4	1:4	0	0	0	0	0	0
	Group A_2	5	1:4	1:64	1:64	1:32	1:32	1:16	1:14	1:4	1:16	1:4	1:4	0	0	0	0	0	0
		6	1:4	1:64	1:64	1:32	1:16	Died	-	-	-	-	-	-	-	-	-	-	-
		7	1:4	64	1:32	1:32	1:16	1:16	1:4	1:4	1:4	1:4	1:4	0	0	0	0	0	0
		8	1:8	1:16	1:64	1:32	1:16	1:32	1:16	1:8	1:4	1:4	1:4	1:4	0	0	0	0	0
Vaccinated With hs oil Adjuvanted Vaccine	Group B_1	9	1:4	1:64	1:128	1:128	1:512	1:256	1:256	1:128	1:64	1:64	1:64	1:32	1:16	1:16	1:4	1:4	0
	(Booster)	10	1:8	1:64	1:128	1:256	1:512	1:512	1:128	1:128	1:128	1:128	1:64	1:32	1:32	1:16	1:8	1:4	0
		11	1:4	1:32	1:128	1:128	1:256	1:512	1:512	1:256	1:128	1:64	1:128	1:128	1:64	1:32	1:32	1:4	0
		12	1:4	1:64	1:128	1:128	1:512	1:256	1:256	1:128	1:64	1:64	1:64	1:32	1:16	1:16	1:4	1:4	0
	Group B_2	13	1:4	1:32	1:64	1:64	1:128	1:32	1:64	1:32	1:64	1:16	1:4	1:4	1:4	0	0	0	0
		14	1:4	1:64	1:64	1:128	1:128	1:128	1:64	1:64	1:32	1:32	1:32	1:16	1:4	1:4	1:4	0	0
		15	1:4	1:16	1:64	1:128	1:256	1:128	1:32	1:64	1:32	1:16	1:16	1:16	1:4	1:4	1:4	1:4	0
		16	1:8	1:32	1:64	1:128	1:128	1:128	1:64	1:64	1:32	1:32	1:32	1:16	1:4	1:4	1:4	1:4	0
	Group C (Control)	17 – 24	Sero Negative																

Table III. Mouse protection test

	Groups	No.of animal	Antibody Titres (DAYS)																
			0	30	60	90	120	150	180	210	240	270	300	330	360	390	420	460	490
Vaccinated with HS ALUM Precipitated Vaccine	Group A_1	1	-	-	10%	20%	10%	Died	-	-	-	-	-	-	-	-	-	-	-
	(Booster)	2	-	-	10%	20%	10%	20%	20%	20%	10%	20%	0%	0%	0%	0%	0%	0%	0%
		3	-	-	10%	10%	10%	20%	20%	20%	10%	10%	0%	0%	0%	0%	0%	0%	0%
		4	-	-	10%	20%	10%	20%	20%	20%	10%	20%	0%	0%	0%	0%	0%	0%	0%
	Group A_2	5	-	-	20%	10%	10%	20%	30%	30%	10%	10%	10%	0%	0%	0%	0%	0%	0%
		6	-	-	10%	20%	20%	Died	--	--	--	--	--	--	--	--	--	--	--
		7	-	-	10%	10%	10%	20%	20%	10%	10%	10%	10%	0%	0%	0%	0%	0%	0%
		8	-	-	10%	10%	10%	20%	20%	10%	20%	10%	0%	0%	0%	0%	0%	0%	0%
Vaccinated with HS oil Adjuvanted Vaccine	Group B_1	9	-	-	20%	20%	30%	60%	50%	50%	60%	50%	60%	50%	10%	10%	0%	0%	0%
	(Booster)	10	-	-	20%	20%	30%	60%	50%	60%	60%	60%	60%	60%	40%	40%	0%	0%	0%
		11	-	-	20%	20%	30%	20%	60%	60%	30%	50%	50%	30%	10%	10%	0%	0%	0%
		12	-	-	20%	20%	30%	60%	50%	60%	30%	30%	30%	40%	40%	40%	0%	0%	0%
	Group B_2	13	-	-	10%	20%	30%	40%	50%	40%	50%	50%	30%	20%	10%	0%	0%	0%	0%
		14	-	-	20%	10%	20%	40%	40%	40%	40%	40%	40%	30%	10%	10%	0%	0%	0%
		15	-	-	10%	10%	20%	40%	40%	40%	40%	10%	20%	30%	10%	10%	0%	0%	0%
		16	-	-	10%	20%	30%	40%	50%	40%	30%	50%	30%	10%	10%	0%	0%	0%	0%
	Group C (Control)	17 – 24	Sero Negative																

*Ten mice/serum sample; **Challenge $100LD_{50}$

DISCUSSION

This research work was designed to evaluate and compare HS Alum precipitated vaccine and oil adjuvant vaccine. The mineral oil and lanolin added vaccine (OAV) has a better and stronger immunogenic activity than the Alum precipitated bacterin, owing to its retention in the tissue as a depot for longer period thus, providing a prolonged antigenic stimulus for antibody formation (Vancheswara *et al*., 1955; Gomis *et al*., 1988 - 89). Our results are in complete agreement with these scientists.

Another objective of this study was to know or to rather confirm the importance and the need of booster dose of vaccine and the results shown in the Table I and II clearly indicate that a booster dose is necessary for prolonged and strong immune response of vaccine especially in the case of killed antigen vaccines, because the immune response, which follows a single inoculation of a dead antigen usually falls to a negligible level within a few weeks. It can be rapidly revived at this time by secondary or booster dose, usually of about the same quantity of antigen as given in the primary inoculation.

IHA test and Passive Mouse Protection Test (PMPT) were used to calculate the potency of both the vaccines (Nagarajan *et al*., 1972; Gupta & Sareen, 1975; Chandrasekaran & Yeap, 1978). PMP Test has been described as satisfactory for measuring immunity in either vaccinated or naturally immune animals and survival of any mice in this test group identifies an immune serum, provided that all of an equal number of control mice die (Bain & Jones, 1955; Brain, 1963; Thomas, 1970).

REFERENCES

Anonymous, 2004. OIE Manual of Diagnostic tests and vaccines for Terrestrial Animals. Office International Epizooties, Paris, France, 1: 537–48

Bain, R.V.S. and Jones, R.F., 1955. Studies on haemorrhagic septicaemia of cattle production of an oil adjuvant vaccine. *British Vet. J.*, 111: 30–4

Bain, R.V.S., 1963. Haemorrhagic septicaemia. FAO Agricultural studies No. 62. FAO, Rome

Bain, R.V.S., M.C.L. De Alwis, G.R. Carter and B.K. Gupta, 1982. *Haemorrhagic Septicaemia*. FAO Animal Production and Health paper No. 33, FAO Rome

Carter, G.R., 1955. A haemagglutination test for identification of serological types. *American J. Vet. Res.*, 16: 481–4

Chandrasekaran, S. and P.C. Yeap, 1978. Safety and potency testing of haemorrhagic septicaemia oil adjuvant vaccine by the mouse protection test. *Kajian Veterinar*, 10: 28–34

Chandrasekaran, S., L. Kennett, P.C. Yearp, N. Muniandy, B. Rani and T.K.S. Mukkur, 1993. Relationship between active protection in buffalo vaccinated against haemorrhagic septicaemia and passive mouse protection and serological tests. Pasteurellosis in production animals. An International workshop held at Bali, Indonesia, 10–13, August 1992. *ACIAR Proceeding No.*, 43: 215–8

De Alwis, M.C.L., 1992. Pasteurellosis in Production Animals. ACIAR Proceeding No. 43, Australia

Gomis, A.A.U., M.C.L. De Alwis, S. Radhakrishnan and S.A. Thalagoda, 1988/1989. Further studies on stability and potency of the haemorrhagic septicaemia oil adjuvant vaccine. *Sri Lanka Vet. J.*, 36: 9–20

Gupta, M.L. and R.L. Sareen, 1976. Evaluation of haemorrhagic Septicaemia Oil adjuvant vaccine by mouse protection test. *India Vet.*, 53: 489–92

Nagarajan, V., S. Sunderam and K. Ramani, 1972. Evaluation of the potency of haemorrhagic septicaemia alum precipitated vaccine by mouse protection test. *Indian Vet. J.*, 49: 1080–3

Israil, M. and M.A. Qauder, 1960. Haemorrhagic Septicaemia oil adjuvant and Alum precipitated vaccine. *Vet. Bult. Abst.*, 37

Reed, L.V. and H. Muench, 1938. A simple method for estimating fifty % end point. *Amaricon J. Hug*., 27: 483–97

Tabatabaei, M., Z. Liu, A. Finucane, R. Parton and J. Coote, 2002. Protective immunity conferred by attenuated aero A derivatives of *Pasteurella multocida* B:2 strain in a mouse model of Haemorrhagic septicaemia. *Infection and Immunity*, 70: 3355–62

Thomas, J., 1970. Studies on HS Oil adjuvant vaccine III Serological Studies. *Kajian Veterinarise Vet. Bult.*, 1972. Abst. 1718

Vancheswara, L.S.K.S., K. Gopal and R. Ramani, 1955. The effects of adjuvant upon the immunizing value of formalin killed Pasteurella boviseptica. *Indian Vet. J.*, 31: 379–91

Wijewardana, T.G., M.C.L. De Alwis and H.L.G. Bastiiansz, 1986. Cultural, biochemical and pathogenicity studies on Pasteurella multocida isolated from carrier animals and from out breaks of haemorrhagic septicaemia. *Sri Lanka Vet. J.*, 34: 43–57

(Received 16 February 2006; Accepted 16 June 2006)