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Efficacy of Oil Based Haemorrhagic Septicaemia Vaccine: A Field Trial

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ABSTRACT

An oil adjuvanted vaccine prepared from *Pasteurella multocida* serotype 6:B was evaluated under field conditions for induction of immune response in cattle and buffaloes and its efficacy was compared with conventionally used Alum precipitated vaccine (APV). A total of 2703 cattle and buffaloes of various age groups (i.e., Adults, Heifers and Calves) were vaccinated with oil adjuvant vaccine (OAV). Alum precipitated vaccine was administered in 813 animals. Two hundred serum samples from vaccinated and 50 from un-vaccinated control animals were collected at regular intervals up to 360 days. Sera were tested for the presence of antibodies against *P. multocida* by indirect enzyme linked immunosorbant Assay (I-ELISA). Comparatively higher antibody titers were observed in animals of all age groups vaccinated with oil adjuvant vaccine throughout the trial period; while, the titres in animals vaccinated with APV declined after 3 months following vaccination and reached at minimal level 180 days post vaccination. The results justified the replacement of APV with the newly developed oil adjuvant vaccine for the control of haemorrhagic septicemia in animals.

Key Words: Oil based; Haemorrhagic septicaemia; Vaccine; Field trial

INTRODUCTION

Haemorrhagic septicaemia (H.S.) is the most common contagious bacterial infection of cattle and buffaloes in Pakistan. It is a highly fatal disease. Effective antibiotics for the treatment of H.S. are available, but due to short incubation period, mortality rate is reported above 70%. Economic losses have exceeded more than 1.887 billion rupees per annum due to this disease in the country (Chaudhary & Khan, 1978). Vaccination is the most accepted method for the control of the disease. At present, alum precipitated bacterin is being used for the control of H.S. in Pakistan but it gives immunity for a shorter duration and its protective efficacy is only 60% (FAO, 1991).

During the past several decades, significant advancement has been made in adjuvant technology and researchers have used various adjuvants to improve immunogenecity of different vaccines. At our institute, we have utilized oil as an adjuvant to enhance the immune response against H.S. This experiment was conducted to evaluate the immunogenicity and constraints in the application of newly developed HS vaccine under field conditions in cattle and buffaloes.

MATERIALS AND METHODS

Seed culture. *Pasteurella multocida (*type 6:B) used for the production of haemorrhagic septicaemia (H.S.) vaccine in Pakistan was utilized (courtesy Dr. Iftikhar Ahmed Butt Veterinary Research Institute, Lahore) for the production of experimental vaccine.

Experimental Vaccines

Alum Precipitated Vaccine (APV). Commercially available H.S. vaccine prepared by Veterinary Research Institute (VRI), Lahore was used in this field trial. It was formalinized alum precipitated bacterin of *P. multocida* type 6:B containing $2x10^9$ cells/mL. The vaccine was administered to the animals following manufacturer's instructions i.e. subcutaneous route @ 5 mL/600 lb body weight twice a year.

Preparation of oil-adjuvanted vaccine

(i) Revival of pathogenicity of *P. multocida.* *Pasteurella multocida* (Freeze-dried) was reconstituted in normal saline solution and 0.2 ml of this preparation was injected subcutaneously into each of two mice. Both mice died after 24 hours. The organism was re-isolated from heart blood on tryptose agar and utilized for the production of oil-adjuvant vaccine.

(ii) Oil adjuvant vaccine (OAV). *P. multocida* type 6:B was grown in brain heart infusion broth (Difco Labs, Detroit) at 37°C for 24 h in a shaking water bath @ 40 rpm. The broth culture was formalinized (0.5%) and used in vaccine preparation. The bacterial suspension was concentrated so that it contained the same number of organisms per dose ($1x10^{10}$/3 mL) as alum precipitated bacterin.

The vaccine was prepared by mixing mineral oil (SEPPIC, France), lanolin and bacterial suspension in the ratio of 45:5:50, respectively. Mineral oil and lanolin were mixed at low speed (13000 rpm) in a Hamilton Beach Drink

Mixer (Hamilton Beach Div., Scovill, NC, US). Bacterial suspension was gradually added over a period of five minutes. Mixing was carried out at high speed (18000 rpm) for 15 min. The preparation was kept overnight at room temperature and re-emulsified for 15 min the following day. This vaccine was stored at 4°C until used in the experimentation.

Location of the trial. The trial was carried out in five villages of district Sheikhupura (Hidiala Virkan, Kot Pindi Das, Khan Pur, Feroze Walla and Qila Sattar Shah) located in an HS enzootic area. Most of the animals (cattle & buffaloes) in this area were vaccinated particularly during rainy season i.e. July and August.

Animals. A total of 2703 cattle and buffaloes of various age groups were vaccinated against HS using OAV. Alum precipitated vaccine was administered in 813 animals as shown in Table I. Two hundred and fifty animals in various villages were kept as control. Two hundred vaccinated and 50 unvaccinated animals were selected randomly from three villages. These animals had minimal titer to *P. multocida* as determined by indirect Enzyme Linked Immunosorbant Assay (ELISA). Ten-mL blood was collected from each animal at days 60, 120, 180, 240, 300 and 360 post vaccination. Serum was separated and stored until analyzed by indirect ELISA.

Enzyme-linked immunosorbent assay. Test serum samples were titred for antibodies to *P. multocida* by indirect ELISA (Muneer *et al*., 1993). Briefly, 96-well flat-bottom micro-titer plates were sensitized by adding 100 µL *P. multocida* antigen (containing 1.5 µg protein per well as determined by Lowery's method) in each well and incubated at room temperature for 18 h. The plates were washed three times with wash solution (PBS containing 0.05% Tween 20). Test serum (100 µL diluted 1:50 in wash solution) was added in duplicate wells and plates were incubated at 37°C for 2 h. After three washings, 100 µL of rabbit anti-bovine IgG (1:5000) conjugated to horse reddish peroxidase was added in each well and plates were again incubated at 37°C for 2 h. The plates were washed three times; 100 µL substrate (O-phenylenediamine dihydrochloride mixed with 0.01% hydrogen peroxide) was added in each well and plates kept in a dark place for 20 min. Colour development was stopped by adding 50 µL 2N sulfuric acid in each well. The optical density was measured by an ELISA reader at 492 nm. Appropriate controls were also run on each plate.

Table I. Animals and vaccines used in H.S field trials in different villages of district Sheikhupura

Village	HS OAV		HS APV	
	Cattle	Buffaloes	Cattle	Buffaloes
Kot Pindi Das	192	325	45	167
Qila Sattar Shah	164	263	-	-
Vandiala Nasir	132	443	-	-
Hadiala Virkan	213	494	-	-
Khanpur	165	312	267	334
Total	866	1837	312	501
	2703		813	

RESULTS AND DISCUSSION

To determine the immune response in different species and age groups the animals were divided into three categories i.e. (1) adult cattle, and buffaloes (2) Young animals 1 to 3 years old (3) Young calves less than one year old. The mean ELISA titres of adult buffaloes and cattle are depicted in Fig. 1. Throughout the study period of one year, the mean titres of the group of animals vaccinated with OAV remained substantially higher than those of both the groups vaccinated with APV and the non-vaccinated control group. The antibody titres of the group vaccinated with APV started declining after three months following vaccination and reached at minimum levels after 180 days. The non-vaccinated control group also showed rise in antibody level at day 60 and 240.

The mean ELISA titres of young animals are shown in Fig. 2. The antibody titres of all three groups of animals showed similar trend as of adult buffaloes and cattle.

The mean ELISA titers of calves are depicted in Fig 3. An increase in the antibody level was observed in both OAV and APV.

A comparatively higher antibody titer upto 360 days was observed in experimental animals vaccinated with oil adjuvant vaccine throughout the trial. Similar trend has also been reported by Shah *et al.* (1997), Muneer and Afzal (1989, 1993), Mittal *et al*. (1979). In Pakistan, outbreaks of HS are usually reported during rainy season (July-August) but sporadic cases are seen throughout the year. Use of such a vaccine that can protect animals against HS for at least a year would help reduce economic losses incurred due to this disease.

A sudden rise in antibody titer was observed in almost all experimental animals about seven months post vaccination. Six buffalo calves (vaccinated against HS using APV and four unvaccinated calves) in village (Khanpur) died and the disease was confirmed later on as HS. This infection in the area may have boosted the immunity in animals resulting rise in anti body level of animals in control group. This natural infection also determined the superiority of OAV over APV as there was no mortality in animals vaccinated with OAV.

In recent years, ELISA has proved a useful technology for measuring the interaction of antigen and antibody. Various infections including *P. multocida* have been diagnosed and monitored by ELISA methods (Dawkins *et al*., 1990; Borkowska *et al*., 1998). For the evaluation of immune response by each vaccine, indirect ELISA was developed and utilized in our studies. Use of ELISA in such experiments was highly desirable due to convenience to process a large number of samples within a limited time.

Advancement in adjuvant technology has opened new

Fig. 1. Mean and standard deviation antibody titres against oil adjuvant (OAV) and alum precipitated (APV) Heamorrhagic septicemia vaccines in adult cattle and buffaloes

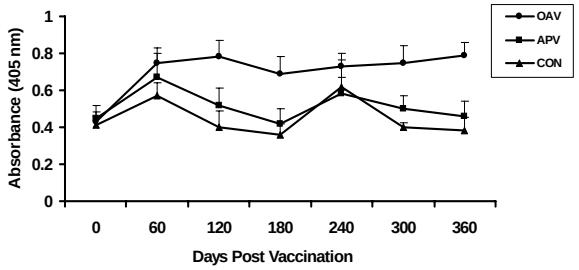


Fig. 2. Mean and standard deviation antibody titres against oil adjuvant (OAV) and alum precipitated (APV) vaccines in young animals (1-3 years old)

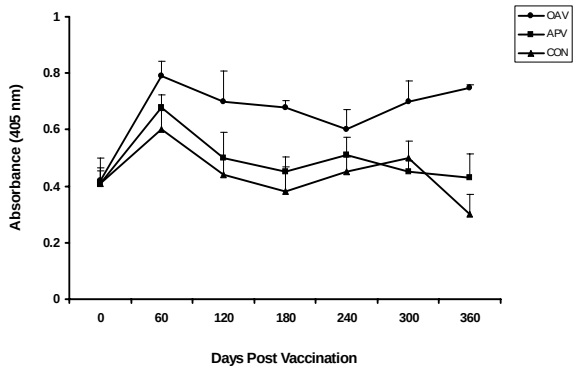
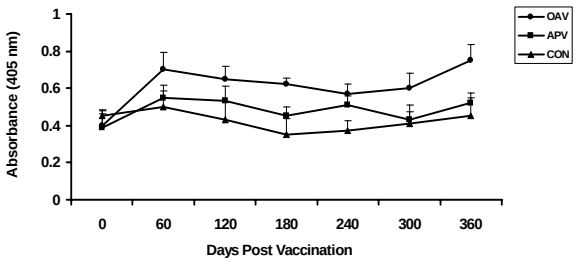


Fig. 3. Mean and standard deviation antibody titres against oil adjuvant (OAV) and alum precipitated (APV) Heamorrhagic septicemia vaccines in calves



avenues and conventional vaccines are being formulated with more immunogenic compositions. The use of oil as an adjuvant in vaccines dates back to 1916 when a lipo-vaccine was produced against *Salmonella typhimurium* by emulsifying a suspension of organism in liquid paraffin using lanolin as a stabilizing agent (cited by Bain *et al*., 1982). The limiting factors in widespread use of this technology were high viscosity causing problems for slow inoculation under field conditions, poor stability and adverse reactions at the sites of inoculation. However, induction of high and prolonged antibody titer by oil adjuvant vaccines has inspired the scientific community. Therefore, several scientists have utilized this approach by different methods and combinations. For instance, Muneer *et al.* (1993), Reshindra *et al.* (1997), Shah *et al.* (1997, 2001) and Rashid (2003) used different adjuvants and emulsifiers to improve its quality. They observed that the experimental oil adjuvant vaccine was superior to broth bacterin in providing protection against experimental HS in young buffalo calves beyond 250 days.

A highly potent vaccine with reasonable shelf life (one year at room temperature) and long duration of protective immunity and also economically viable are considered main features of a good quality vaccine. In present experimentation, the results justified the replacement of APV with the newly developed oil adjuvant vaccine to protect animals against HS in Pakistan.

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