SHORT COMMUNICATION *Pakistan Vet. J., 25(3): 2005*

POTENCY AND SAFETY TESTING OF HAEMORRHAGIC SEPTICAEMIA OIL ADJUVANTED VACCINE IN SWISS ALBINO MICE

A. R. Bhatti, K. Tasneem, Z. J. Gill and A. Raza

Veterinary Research Institute, Zarar Shaheed Road, Lahore Cant, Pakistan

ABSTRACT

The present study was conducted to evaluate the potency and safety of haemorrhagic septicaemia oil adjuvant vaccine in Swiss albino mice via subcutaneous route of inoculation. The vaccine was proved successful as it gave 70 to 100% protection in Swiss albino mice.

Key words: Vaccine, haemorrhagic septicaemia, potency, mice.

INTRODUCTION

Haemorrhagic septicaemia (HS) is one of the most fatal infectious diseases of buffaloes and cattle in Asia caused by *Pasteurella multocida* serotype B: 2 (Carter, 1955; Heddleston *et al*., 1972). This disease is kept under control mainly by the use of an alum-precipitated vaccine, which has been replaced in many countries by the haemorrhagic septicaemia oil adjuvanted vaccine (HSOAV). The latter can protect cattle and buffaloes from the disease more effectively than alum precipitated vaccine up to one year (Neramitmansook, 1993).

It has been recommended that potency of HSOAV should be carried out in cattle and buffaloes (Anonymous, 2004). However, it is not always possible economically to check each and every batch of vaccine in cattle and buffaloes. Therefore, laboratory animals like rabbit and mice can be used for potency and safety testing of HS vaccines. In the present study, Swiss albino mice were used for potency and safety testing of HSOAV (Yadev and Ahooja, 1983). The mouse provides a good model for HS infection as it manifests septicaemic form of disease similar to that observed in the natural hosts (Ose and Muenster, 1968; Dawkins *et al*., 1990; Tabatabaei *et al*., 2002).

MATERIALS AND METHODS

Experimental mice

For potency testing, protocols of Ose and Muenster (1968) and OIE Terrestrial Manual (Anonymous, 2004) were followed. A total of 160 Swiss albino mice, about 5-6 weeks old and weighing approximately 25-30 gms were used. These mice were divided into four groups A, B, C and D, with 40 mice in each group. Mice of groups A, B and C were vaccinated with three different batches of HSOAV prepared at Veterinary Research Institute Lahore. Group D animals were kept as unvaccinated control.

Vaccination

Three batches of HSOAV prepared according to the standard protocols (Anonymous, 2004) were evaluated for potency and safety. All the experimental mice in groups A, B and C were vaccinated with respective batch @ 0.25 ml (0.37 mg dry bacterial weight approximately) subcutaneously. A booster dose of 0.25 ml/mice subcutaneously was given after 15 days (Gupta and Sareen 1976; Tasneem, 1993).

Lethal dose calculation

Pasteurella multocida serotype B: 2 was cultured in tryptose phosphate broth and LD_{50} was calculated according to Reed and Muench (1938). LD_{50} of challenge was about $10^{-5.5}$, 100 LD_{50} was $10^{-4.5}$ and 1000 LD_{50}.was $10^{-3.5}$

Challenge

Fourteen days after booster dose each group was further divided into two subgroups (A_1, A_2, B_1, B_2, C_1 C_2 and D_1, D_2). Mice of each subgroup were challenged with 100 LD_{50} and 1000 LD_{50} of challenge inoculums @ 0.25 ml/mice subcutaneously and were kept under observation for seven days.

RESULTS AND DISCUSSION

The results obtained are summarized in Table 1. Mice of Group A_1 vaccinated with batch No.1 showed 100% protection with 100 LD_{50} and Group A_2 mice showed 90% protection with 1000 LD_{50}. Mice of groups B_1 and B_2 which were vaccinated with batch No. 2 showed 90% protection with100 LD_{50} and 75% with

Table 1: Protection levels of three batches of haemorrhagic septicaemia vaccine in mice

Batch No.	Group	Subgroups	Challenge dose	Mice			%age protection
				Inoculated	Survived	Died	
1	A	A_1	100 LD_{50}	20	20	0	100
		A_2	1000 LD_{50}	20	18	2	90
2	B	B_1	100 LD_{50}	20	18	2	90
		B_2	1000 LD_{50}	20	15	5	75
3	C	C_1	100 LD_{50}	20	18	2	90
		C_2	1000 LD_{50}	20	16	4	80
4	D	D_1	100 LD_{50}	20	0	20	0
		D_2	1000 LD_{50}	20	0	20	0

1000 LD_{50}. Similarly, groups C_1 and C_2 which were vaccinated with batch No. 3 showed 90% protection with100 LD_{50} and 80% with 1000 LD_{50}. All mice of group D (D_1, D_2), which were kept as unvaccinated control, died after challenge with 100 LD_{50} and 1000 LD_{50} of challenge culture.

In this study, albino mice were used for potency and safety testing of HSOAV. A similar study was conducted by Tasneem (1993), who evaluated potency and safety of HSOAV by intraperitoneal route of inoculation and observed high protection. In this study, subcutaneous route was adopted for vaccination of mice instead of deep intramuscular or intra peritoneal inoculation. This route was proved to be safe without any adverse local reaction at the site of injection as was observed in the case of large animals (Anonymous, 2004). Time interval of booster and challenge were increased so that vaccine can work properly, as in oil adjuvant vaccine antigen is slowly released from depot. The results showed that HSOAV gave good protection level up to 90%, which was higher than recommended level to pass a batch of vaccine to be used in field (Ose and Muenster, 1968). The results obtained in the present study coincide with those of Ose and Muenster (1968) and Yadev and Ahooj (1983) that vaccine was found safe and potent if it gave 2 log unit protection in laboratory animals.

These results indicate that it is possible to use mice successfully for safety and potency testing of HSOAV via subcutaneous route. There was no death after vaccination and secondly there was no untoward reaction in mice up to seven days.

REFERENCES

Anonymous, 2004. OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Office International Des Epizooties, Paris, France.1: 537-548

Carter, G. R., 1955. A haemagglutination test for the identification of serological types. Amer. J. Vet. Res., 16: 481-484.

Dawkins, R. H., R. B. Johnson, T. L. Spencer and B. Adher, 1990. *Pasteurella multocida* infection in mice with reference to haemorrhagic septicaemia in cattle and buffalo. Immunol. Cell Biol., 68: 57-61.

Gupta, B. K. and R. L. Sareen, 1976. Evaluation of haemorrhagic septicaemia oil adjuvant vaccine by mouse protection test. Indian Vet. J., 53: 489-492.

Heddleston, K. L, J. E. Gallagher and P. A. Rebers, 1972. Fowl cholera: gel diffusion precipitin test for serotyping *Pasteurella multocida* from avian species. Avian Dis., 41: 265-274.

Neramitmansook, P., 1993. Country Report Thialand. In: "B. E. Patten T. L. Spencer, R. B. Johnson, D. Hoffmann and L. Lehane (eds) Pasteurellosis in Production Animals. An International Workshop, Bali, Indonesia, No.43: 234-237.

Ose, E. E. and O. A. Muenster, 1968. A method of evaluating vaccines containing *Pasteurella multocida*. Amer. J. Vet. Res., 29: 1863-1866

Reed, L. V. and H. Muench, 1938. A simple method for estimating fifty percent end point. Amer. J. Hyg., 27: 493.

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. Infec. Immu., 70: 3355-3362.

Tasneem, K., 1993. Studies on HS oil adjuvant vaccine prepared by dense culture of *Pasteurella multocida* Robert type 1 on improved culture media, MSc Thesis, Univ. Agri., Faisalabad, Pakistan.

Yadev, M. S. and M. L. Ahooja, 1983. Immunity trials in mice, rabbit and calves with oil adjuvant and multi emulsion oil adjuvant vaccines against haemorrhagic septicaemia. Indian J. Anim. Sci., 53: 705-708.