

## PREPARATION AND EVALUATION OF ALUM PRECIPITATED AND OIL-BASED HAEMORRHAGIC SEPTICAEMIA VACCINES

M.A. Tariq, K. Muhammad<sup>1</sup>, P. Das<sup>1</sup>, and M. A. Sheikh<sup>1</sup>

Avicenna Laboratory, Bhikhi, Sheikhpura-Faisalabad Road, Sheikhpura, <sup>1</sup>Microbiology Section, College of Veterinary Sciences, Lahore, Pakistan

### ABSTRACT

*Pasteurella multocida* (Robert type-1) was grown on tryptose sucrose yeast (TSY) broth in a fermenter. A sterilised air was provided that potentiated the density of the culture ( $5 \times 10^9$  organisms/ml). The culture was inactivated with formalin (0.5%). The alum precipitated and oil based haemorrhagic septicaemia vaccines (APHSV and OBHSV) were prepared and their efficacies were evaluated in rabbits. Both of these vaccines induced high level of indirect hemagglutination (IHA) antibody titer and 100 percent protection to challenge on 90 days post vaccination. OBHSV induced higher level of antibodies than that of APHSV. However, both of these vaccines increased 2°F body temperature of the vaccinated rabbits. The temperature incepted declining to normal on 6 hours post-vaccination.

### INTRODUCTION

Haemorrhagic septicaemia (HS) is one of the most formidable bacterial diseases of cattle and buffaloes. The disease is caused by a serotype of *Pasteurella multocida* which is antigenically similar to Robert type-1 that is used in preparation of HS vaccine (Aslam, 1986). The disease is seasonal in its occurrence. The environment (high ambient temperature), management (overcrowding, inadequate ventilation, transportation), and malnutrition are among the plausible factors which are incriminated to potentiate the incidence of the disease in Pakistan. Acute nature and short duration of the disease are main causes of therapeutic failure in affected animals. The disease is controlled through mass vaccination programme. The outbreaks of the disease are not uncommon in the vaccinated animals.

Present study is designed to grow the dense culture of *P. multocida* for preparation of alum precipitated and oil-based HS vaccines (APGSV and OBHSV, respectively) and to evaluate their efficacies in rabbits.

### MATERIALS AND METHODS

#### Activation of seed culture of *Pasteurella multocida*

*Pasteurella multocida* (Robert type 1) was collected from Microbiology Section, College of Veterinary Sciences, Lahore. One ml of 18 hours serum broth culture of the organism was injected (subcutaneously) in 13 months old buffalo calf. The calf showed signs of haemorrhagic septicaemia on 36 hours post inoculation. The blood smear was prepared, stained and examined microscopically. The citrated blood was collected

aseptically and stored in freezer at -20°C in small aliquots (one ml/vial). One vial was processed for checking the purity of the culture.

#### Production of mass culture

A pure colony of the organism was inoculated into the fermenter containing a modified casein sucrose yeast (CSY) broth (Anonymous, 1992). The medium contained tryptose soya broth (Difco: 30 grams), sucrose (Difco: 06 grams), yeast extract (Difco:06 grams) and phosphate buffered saline (pH 7.2; 1000 ml).

The medium was prepared in bulk (10 litre), sterilised by autoclaving at 121°C for 20 minutes and transferred aseptically to the fermenter which was incubated at 37°C for 24 hours. The culture in the fermenter aerated during incubation (Nandani-Peiris and Alwis, 1991). Purity, viable count and dry weight of the culture were determined (Anonymous, 1992).

#### Preparation of vaccine

Two types of the vaccines were prepared from this mass culture (Bokhout *et al.*, 1996).

#### Alum precipitated haemorrhagic septicaemia vaccine (APHSV)

The inactivated mass culture was diluted in saline solution to achieve 1.68 mg bacterial body weight ( $1 \times 10^9$  bacteria) per 5 ml of the vaccine. Aluminium potassium sulphate (10 percent stock solution) was admixed with the diluted culture to achieve its 0.5 percent concentration. The pH was adjusted to 7.2. The aluminised culture was incubated at 25°C for 24 hours.

### Oil-based haemorrhagic septicaemia vaccine (OBHSV)

One part of the formalin inactivated dense culture (1.68 mg bacterial body weight or  $5 \times 10^9$  bacteria per ml) was admixed with 4 part of the oil-base. The oil base was composed of Span-80 (40 ml), liquid paraffin (950 ml) and Tween 80 (10 ml). The mixture was blended for 3 minutes.

Safety of the vaccines was evaluated in rabbits. Two ml of each of the vaccines was injected to each of the five rabbits and the reaction was recorded for 7 days. No mortality indicated the safety of the vaccines.

### Efficacy trial of the vaccines

The efficacy trial of each vaccine was conducted on the adult rabbits. The rabbits free from antibodies against *P. multocida* were randomly divided into three test groups, i.e., A, B, C (each having 5 rabbits) and one control group, i.e., D. The rabbits in group D were subdivided into D-1 and D-2 (each having 5 rabbits).

These rabbits were vaccinated as follows:

- Group A & B: Two ml of APHSV was injected (subcutaneously) to each of the rabbits.
- Group C: Two ml of the OBHSV was injected (subcutaneously) to each of the rabbits.
- Group D: The rabbits of this group served as unvaccinated control.

The body temperature of the test and control rabbits were recorded at 0, 2, 4 and 6 hours post injection. The blood samples were collected on 0, 15, 30, 45, 60, 75 and 90 days postvaccination. The serum was separated from each sample and was stored in properly labelled vials at  $-20^\circ\text{C}$ . These sera were titrated for indirect hemagglutinating (IHA) antibodies (Bain *et al.*, 1982). The data regarding IHA antibody titers were processed for calculation of GMT for comparative efficacy of the vaccines (Villages and Purchase, 1989).

The rabbits of group A and D-1 (non-vaccinated control) were challenged on 45 days post vaccination while rabbits of group B, C, and D-2 were challenged on 90 days post vaccination with one ml of culture containing  $10^9$  virulent bacteria. The challenged rabbits were examined for 7 days post infection.

## RESULTS AND DISCUSSION

Haemorrhagic septicaemia is a seasonal problem and is controlled by mass vaccination programmes before rainy seasons. Outbreaks in the animals vaccinated with bacterin are not uncommon. Immune response to the bacterin is poor and is of short duration. Immune response in the vaccinated animals is required

against outermost component of the bacterial body, i.e. capsule. The capsule of *P. multocida* is composed of mainly lipopolysaccharide (LPS) and of minor fraction of proteins (Bain *et al.*, 1982). LPS induces B cell response and can't be presented along with MHC-II antigen (Immune associated antigen-Ia) by antigen presenting cells (APC) of the animal body, and hence the responsive B cells (plasma cells) can't get cooperation of T cells for enhanced antibody production. The B cell response to LPS is primary and the immunity is of low level and short duration (Abbas *et al.*, 1991). This property of the LPS in bacterin necessitated the farmers to vaccinate their animals quarterly. There is a minor fraction of bacterial proteins in the capsule, immunity against what can be potentiated by having it in required level in the dose and adding adjuvants in the bacterin. In the present study, use of enrichment media in the fermented and provision of fresh filtered air during incubation resulted  $5 \times 10^9$  bacteria or 1.68 mg bacterial dry weight per ml of the culture. These results are in agreement with Afzal and Muneer (1990).

The protein antigens in the oil-based vaccines are processed and presented by APC to specific T cells. The T cells produced interleukins which potentiate the response of the LPS specific plasma cells, induce LPS specific memory cells, and immunoglobulin (Ig) switching over. The production of interleukins is antigen specific but their action is antigen non-specific (Hamblin, 1988). The induction of protein specific interleukins presumably potentiated the activity of LPS primed B-cells and induced a high level of antibody response of animals to the adjuvant containing vaccines (Table 1). In the APHSV vaccinated rabbits, the peak level of IHA antibodies (GMT 1 : 55.7) was achieved on 45 days post-vaccination and then the titer started declining that was 24.3 on 90 days post vaccination. All the vaccinated rabbits showed 100 per cent protection to challenge infection on 45 or 90 days post vaccination. In case of OBHSV vaccinated rabbits, the antibody titer gradually increased upto 90 days after vaccination. All the OBHSV vaccinated rabbits displayed 100 per cent protection to challenge infection on three months post-vaccination. From the increasing trend of antibodies in the OBHSV vaccinated rabbits (Table 1), it can be inferred that these rabbits may remain immunised for one year. These results are in agreement to those of Afzal and Muneer (1990) who recorded higher titer in rabbits vaccinated with OBHSV or combined oil based vaccine containing *P. multocida* and foot and mouth disease virus.

There are limitations of the adjuvant containing HS vaccines. Table 2 indicates that these vaccines induced phrogenicity in the vaccinated rabbits which might be

Table 1: Antibody response of rabbits to alum precipitated and oil-based *Pasteurella multocida* vaccines

Type of vaccine	Days post-vaccination	Distribution of rabbits on the basis of indirect haemagglutinating antibody titre							GMT
		2 <sup>0</sup>	2 <sup>2</sup>	2 <sup>3</sup>	2 <sup>4</sup>	2 <sup>5</sup>	2 <sup>6</sup>	2 <sup>7</sup>	
APHSV	0	5	0	0	0	0	0	0	00.0
	15	0	0	1	2	2	0	0	18.4
	30	0	0	0	1	2	2	0	36.8
	45	0	0	0	0	1	2	2	73.3
APHSV	0	5	0	0	0	0	0	0	00.0
	15	0	0	1	3	1	0	0	16.0
	30	0	0	0	1	2	2	0	36.8
	45	0	0	0	0	2	2	1	55.7
	60	0	0	0	0	2	3	0	48.5
	75	0	0	0	0	4	1	0	36.8
	90	0	0	0	2	3	0	0	24.3
OBHSV	0	5	0	0	0	0	0	0	00.0
	15	0	0	4	1	0	0	0	09.0
	30	0	0	1	4	0	0	0	13.9
	45	0	0	0	2	3	0	0	24.3
	60	0	0	0	1	3	1	0	32.0
	75	0	0	0	0	3	2	0	42.2
	90	0	0	0	0	2	3	0	48.5

APHSV = Alum Precipitated Haemorrhagic Septicaemia Vaccine

OBHSV = Oil-based Haemorrhagic Septicaemia Vaccine

Table 2: Pyrogenesis in rabbits vaccinated with haemorrhagic septicaemia vaccines (Temperature = mean + SD, °F).

Group of rabbits	Time post-vaccination (hours)			
	0	2	4	6
n=5 in each group				
CONTROL	101.44 ± 0.39a	101.60 ± 0.51a	101.56 ± 0.36a	101.36 ± 0.26a
APHSV	101.36 ± 0.96a	102.40 ± 1.09a	102.92 ± 0.84b	102.12 ± 0.27b
OBHSV	101.16 ± 0.79a	103.28 ± 0.54b	103.32 ± 0.39b	102.24 ± 0.35b

The figures in each columns having similar superscripts are not significantly different ( $P < 0.01$ ).

due to high contents of bacterial LPS (Tortora *et al.*, 1989). However, further studies are required to investigate the dose level of the adjuvant containing vaccines with minimum pyrogenicity and maximum immunogenicity in domestic animals.

This study indicated that OBHSV can induce higher level of antibodies than APHSV. Moreover, the rabbits are a) quite susceptible to *P. multocida* infection, b) responsive to the adjuvant containing bacterial vaccines and c) can be used to evaluate the pyrogenicity of the LPS containing vaccines. This study is in agreement with Jaiswal *et al.* (1983) who found rabbits as good

experimental animals for production of disease/immunity or for evaluation of pyrogenicity of the bacterial vaccines.

### ACKNOWLEDGEMENTS

The authors are highly grateful to Chief Executive of Avicenna Laboratories, Bhikhi, Sheikhpura who funded the project and provided all laboratory facilities for executing the experimental work in the best interest of livestock industry.

## REFERENCES

- Abbas A.K., A.H. Lichtman and J.S. Pober, 1991. Cellular and molecular immunology, W.B. Saunders Company, London, pp: 417.
- Afzal, M. and R. Muneer, 1990. Development of combined vaccine of haemorrhagic septicaemia and foot and mouth disease. Pakistan Vet. J., 10: 67-69.
- Anonymous, 1992. OIE, Manual of standards of diagnostic tests and vaccines for list A and B diseases of mammals, birds, and bees. pp: 313-321.
- Aslam M., 1986. Serotyping of *Pasteurella multocida* isolated from cattle and buffaloes. M.Sc., (Hons). Thesis, Vet. Microbiology, University of Agriculture, Faisalabad.
- 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, pp: 1-54.
- Bokhout B.A., C. Van Gaalew and Ph.J. Van der Heijdet, 1996. A selected water in oil emulsion: composition and usefulness as an immunological adjuvant. Vet. Immunol. Immunopathol., 2: 491-500.
- Hamblin A.S., 1988. Lymphokines, IRL Press Ltd. Oxford, UK., pp: 71.
- Jasiwal, T.N., B.K. Gupta, and S.C. Mishra, 1983. Potency testing of haemorrhagic septicaemia alum precipitated vaccine in rabbits. Indian J. Comp. Microbiol. Immunol. Infect. Dis., 4: 152-155.
- Nandani-Peiris, L.K.P. and M.C.L. de-Alwis, 1991. Simplified techniques for haemorrhagic septicaemia oil adjuvant vaccine production. Proc. 4th Int. Workshop on Haemorrhagic septicaemia, FAO, Bangkok, Thailand, pp: 117-120.
- Tortora, G.J., B.R. Funke and C.L. Case, 1989. Mechanisms of pathogenicity, In: "Microbiology; An Introduction" 3rd Ed., The Benjamin/Cummings Publishing Company, Inc., Wokingham, UK. pp: 377-398.
- Villages, P. and H.G. Purchase, 1989. Titration of biological suspensions. In: "A Laboratory Manual for the Isolation and Identification of the Avian Pathogens" 3rd Ed., The American Association of Avian Pathologists. Kendall/Hunt Publishing Company, USA, pp: 186-191.