INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2000/02–3–183–186

Antibody Response of Buffaloes to Haemorrhagic Septicaemia Vaccine

ZULFIQAR ALI, KHUSHI MUHAMMAD, IRSHAD HUSSAIN AND EJAZ HAMEED
Microbiology Section, College of Veterinary Sciences, Lahore, Pakistan

ABSTRACT

Pasteurella multocida was isolated and characterized on the basis of cultural biochemical, serological and pathogenicity tests. The dense culture of the organism was achieved in a fermenter that was provided sterilized air during incubation. Two types of the formalin inactivated *Pasteurella multocida* vaccines (oil-based and alum precipitated) were prepared and their efficacy was evaluated in bovines. It was observed that oil-based haemorrhagic septicaemia (HS) vaccine induced high level of indirect haemagglutinating (IHA) antibodies in the vaccinated buffaloes which persisted for more than six months. In contrast, alum precipitated HS vaccine induced immunity breakdown in the buffaloes with high titres of IHA antibodies while induced low level of IHA antibodies in the buffaloes with undetectable level of antibodies, which persisted for four months.

Key Words: Antibody response; Buffaloes; Haemorrhagic septicaemia; Vaccine

INTRODUCTION

Haemorrhagic Septicaemia (HS) in bovine is caused by specific serotypes of *Pasteurella (P) multocida* Robert type I (Islam & Cheema, 1975). The serotype of this pathogen prevailing in Pakistan is 6-B (Aslam, 1986). The disease causes substantial economic losses to the livestock industry which are estimated to be more than 1.887 billion annually (Chaudhry & Khan, 1978).

P. multocida is a normal inhabitant of the respiratory tract of the bovine. High ambient temperature, overcrowding, inadequate ventilation, transportation, and malnutrition are factors, incriminated in potentiating outbreak of the disease (Buxton & Fraser, 1977). The disease is an acute infection mostly of buffaloes and cattle, with high mortality in clinical cases. The infected animals are treated with a variety of therapeutic agents. Peracute nature of disease, febrile condition of animals and development of resistance against antibiotics usually result in therapeutic failure. Therefore, an effective control of disease could only be achieved by vaccination.

Routinely used vaccines are plain bacterin, alum precipitated vaccines and oil adjuvant vaccines. In Pakistan, alum precipitated HS vaccine is in practice. The outbreaks of the disease in vaccinated animals are not uncommon. Poor antigenicity, improper storage of the vaccine, and immunosuppressing biological agents are incriminated to be the cause of immunoprophylaxis failure in the vaccinated animals. The present project was designed to prepare alum precipitated and oil based HS vaccines and to evaluate their antigenicity in buffalo calves.

MATERIALS AND METHODS

Source of samples. Long bones of animals died at Bhader Nagar Farm, Okara, as a result of clinical cases of haemorrhagic septicaemia, were collected and transported to Microbiology Laboratory, College of Veterinary Sciences, Lahore. The bones in the laboratory were stored at 4°C for one month.

Isolation of *P. multocida*. Marrow (2 g) from the bones was collected aseptically and admixed in 10 mL sterilized physiological saline (0.85% aqueous solution of sodium chloride: pH 7.2). The marrow suspension was processed for isolation of the causative agent which was identified as *P. multocida* on the basis of cultural characteristics, biochemical reactions, pathogenicity test, and serological test according to techniques described by Buxton and Fraser (1977), and Sarma and Boro (1980).

Production of vaccines. The purified organism was injected into the rabbit for its activation, which was then reisolated and purified. A pure colony of the organism was inoculated into the fermenter containing sterilized tryptose soya broth sucrose (Anonymous, 1992) prepared in phosphate buffered saline (pH 7.2). The fermenter was incubated at 37°C for 24 hours. The fermenter was provided with fresh filtered air. Purity and titration of the culture (viable count and dry weight of the culture) was also determined (Anonymous, 1992).

The culture of the bacteria was inactivated by addition of final concentration formalin 0.12% and by keeping it for 48 hrs. The sterility and safety of the inactivated culture was determined (Anonymous, 1992).

Two types of the vaccines were prepared from the

inactivated mass culture (Bokhout *et al*., 1996). The inactivated culture was diluted to achieve 1.68 mg bacterial body weight/5 mL of vaccine. Then potassium aluminium sulphate (10% stock solution) was added in the culture to achieve final 0.5% alum concentration. The pH of the culture was adjusted to 7.2. The aluminized culture was incubated for 24 hours at 25°C.

One part of formalin inactivated dense culture (1.68 mg/mL bacterial dry weight) was mixed with four parts of oil base. The oil base was composed of Span-80 (4 mL), liquid paraffin (95 mL) and Tween-80 (1 mL). The mixture was blended for five minutes.

Evaluation of vaccine in buffaloes. The efficacy trial of Alum Precipitated HS Vaccine (APHSV) and Oil based HS Vaccine (OBHSV) was conducted in buffaloes. The buffaloes were divided into two test groups i.e. A, B and third (C) as control group (each having 14 animals). These animals were vaccinated as follows:

- Group A: Five ml of APHSV was injected subcutaneously to each of the animals.
- Group B: Five ml of OBHSV was injected subcutaneously to each of the animals.
- Group C: The animals of this group served as unvaccinated control.

The body temperature of the test and control animals was recorded at 0, 2, 4 and 6 hours post injection. The blood samples were being collected on 0, 15, 30, 45, 60, 70, 90 days post vaccination. The serum was separated from each sample and was titrated for indirect haemagglutinating (IHA) antibodies against HS (Fraser *et al*., 1993).

The data regarding IHA antibody titres was processed for calculation of GMT for comparative efficacy of the vaccine (Villegas & Purchase, 1989).

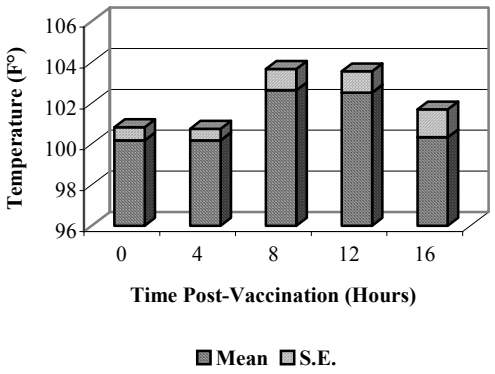
RESULTS AND DISCUSSION

Buffaloes showed antigenic response to APHSV and OBHSV vaccines. The vaccines were prepared from local isolate of the *P. multocida*. The local isolate was antigenically related with the vaccinal strain of *P. multocida* Robert type-1. The vaccinal strain being used in Pakistan is 6-B (Aslam, 1986). These results are in accordance to Ahmad and Ajmal (1972).

The bacterin induces poor immune response (Carter, 1950) that might be due to rapid absorbance of the antigen from the inoculated site and unable to induce the effective response. To potentiate the immune response, adjuvants are added in the inactivated cultures. Adjuvants commonly used are aluminium salts, linolin, sodium alginate, saponin, mineral and vegetable oils (Bain *et al*., 1982; Muneer, 1993). Adjuvant containing vaccines are alum precipitated HS vaccine, oil based HS vaccine and Linolin or saponin containing vaccine etc. (Mittal *et al*., 1977).

It was found that addition of alum (10% stock solution) in the culture resulted in a white precipitation and acidic pH. The pH was readjusted upto 7.2 with stock solution of 10% sodium hydroxide. The storage of alum containing culture at ambient temperature (25°C) resulted white precipitation at the base which was resuspended on vigorous stirring.

Fig. 1. Pyogenic response of buffaloes to oil based Haemorrhagic septicaemia Vaccine



Similarly, mixing of oil based in the culture in a shaker resulted a whitish liquid. It was observed that mineral oil emulsifier that was Span-80 played key role in preparation of homogenous vaccine. Addition of span-80 at rate of 10% in oil base resulted separation of the fluid in the supernatant while adding the emulsifier at rate of 1% resulted separation of the culture fluid at the base. Addition of the emulsifier at rate of 4% resulted in stable and homogenous vaccine. The results are not in accordance with Muneer (1993), Bakhout *et al*. (1996) who used 10% emulsifier in the base to achieve stable vaccine. This may be due to varying level of tween-80, or quality of emulsifier or mineral oil of the oil base. However, physical mixing also plays key role in the homogeneous stable vaccine.

APHSV is commonly used in Pakistan for immunization of cattle and buffaloes before the rainy seasons. APHSV does not induce long lasting and effective immunity (Baig & Sheikh, 1988). In fact, immunogenic property of the vaccine is directly proportional to the quantity of the immunogens per mL of the vaccine. Each vaccinal dose contained a minimum of 2 mg dry weight of the bacteria for effective immunoprophylaxis (Bain *et al*., 1982). In the present study, use of enrichment media in the fermenter and

provision of fresh filtered air during incubation resulted in the dense culture of organism ($5x10^9$ organism/mL) and high level of bacterial body weight/mL of the culture (1.68 mg/mL) which is in accordance with Nandani-peiris and de-Alwis (1993).

The bovines showed immune response to both of these vaccines (Table I). All the experimental animals unexpectedly showed quite high antibody titres at day of vaccination. It might be due to unofficial revaccination by the farm authorities or natural exposure of animals to the pathogen in form of some outbreak (Glass & Beasley, 1989). It was noted that the existing GMT titre was dropped down soon after vaccination with APHSV. Alum might be toxic to the antigen presenting cells (APC) such as macrophages or B cells. It might be a plausible reason of immunity breakdown in animals with high level of antibody titre.

Immune response, in the vaccinated animals is required against outermost components of the bacterial body i.e. capsule which is composed of mainly lipopolysaccharide (LPS) and minor fraction of proteins - exotoxins (Bain *et al*., 1982; Shah & Shah, 1998). Purified form of LPS induces B cell response and can not be presented alongwith MHC II antigen (immune associated antigen-la) by APC of the animal body, and hence the responsive B cells (plasma cell) can not get co-operation of the T cell so response to LPS is primary and the immunity is of low level and short duration (Abbas *et al*., 1991). This information is further supported by Anonymous (1992) who reported that the antibody response of animals without or low antibody titre to bacterin containing aluminium is relatively short lived and antibody level decreases rapidly at 3-4 weeks after injection.

In case of OBHSV vaccinated buffaloes, the antibodies titre (GMT 157.6) gradually increased upto 60 days after vaccination and then titre started declining (GMT 1:24.3) on 195 days post vaccinations. These results are in agreement to Muneer (1993) who recorded higher antibody titre in buffalo calves vaccinated with OBHSV that persisted for about one year.

There are limitations of the adjuvant containing HS vaccines. Fig. 1 indicates that these vaccines induced pyrogenic response in the vaccinated animals which might be due to high content of the bacterial LPS as it is pyrogenic in nature (Tortora *et al*., 1989). Another limitation is that granuloma occur at the injection site which disappear after few months. OBHSV is an oil based vaccine. The oily preparations are toxic and necrotizing (Stephen *et al*., 1984). This might be possible reason of granuloma formation in some animals and skin eruption in others. However, because of oils in vaccine, the antigen is slowly absorbed from the injection site and provides a continuous source of antigen for antibody production over a long period of time. However, further studies are required to investigate the dose level of the adjuvant containing vaccine with minimum pyrogenicity and maximum immunogenicity in domestic animals.

Table I. Antibody response of buffaloes to aluminized and oil-based Haemorrhagic Septicaemia vaccines

Type of Vaccine	Days post Vaccination	Distribution of buffaloes on basis of indirect haemagglutinating antibody titres									GMT
		4	8	16	32	64	128	256	512	1024	
Alum precipitated haemorrhagic septicaemia vaccine*	0	0	0	0	3	5	3	1	2	0	97.00
	60	0	1	3	1	4	0	0	0	0	36.80
	105	2	5	3	1	0	0	0	0	0	09.30
Oil based haemorrhagic septicaemia vaccine	0	0	0	0	3	5	3	1	2	0	97.00
	60	0	0	1	1	2	4	3	1	2	157.60
	105	0	0	0	2	5	2	1	0	0	104.00
	195	0	1	3	1	2	0	0	0	0	024.30

Note: The samples * collected on 195 days post vaccination of buffaloes were spoiled and so were not processed

CONCLUSION

It is concluded that the growth of the *P. multocida* can be improved by providing sterilized air to the culture in fermenter. An economical and quality oil based vaccine can be prepared and evaluated in laboratory animals. Oil based vaccine induces high level of immunity that remain over a long period of time as compared to that of alum precipitated vaccine.

REFERENCES

Abbas, A.K., A.H. Lichtman and J.S. Pober, 1991. Cellular and molecular immunology, W.B. Saunders Company, London, p. 417.

Ahmed, K.D. and M. Ajmal, 1972. Serotyping of *Pasteurella multocida* strains isolated from natural outbreak in Lahore Region. *Pakistan J. Sci. Res.,* 24: 98–100.

Anonymous, 1992. Office of International Epizootics (OIE), Manual of standards diagnostic tests and vaccines for list A and B diseases of mammals, birds and bees, pp: 313-21.

Aslam, M., 1986. Serotyping of *Pasteurella multocida* isolated from cattle and buffaloes. M.Sc. (Hons) Thesis, Vet. Microbiology, Univ. of Agric., Faisalabad.

Baig, M.S. and M.A. Sheikh, 1982. Post HS-vaccination serum antibody titre profile in buffalo calves. M.Sc. Thesis, College of Vet. Sci., Lahore.

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.

Buxton, A. and G. Fraser, 1977. Animal Microbiology, Vol.1, Blackwell Scientific Publications Ltd., London, pp: 121–8.

Carter, G.R., 1950. Studies on *Pasteurella multocida* chick embryo vaccine. 1. The comparative immunizing value broth bacterins and chick embryo vaccine in mice. *Am. J. Vet. Res.,* 11: 252–5.

Chaudhry, N.A. and B.B. Khan, 1978. Estimation of economic losses due to animal diseases in Pakistan. Final technical report. Univ. of Agric., Faisalabad.

Fraser, J., W. Donachie, M. Quire and N.J.L. Gilmour. 1983. Rapid indirect haemagglutination test for serotyping *Pasteurella haemolytica*. *J. Clin. Microbiol.,* 118: 206–7.

Glass, L.G. and J.N. Beasley, 1989. Infection with and antibody response to *Pasteurella multocida* and Bordetella bronchiseptica in immature rabbits. *Lab. Anim. Sci.,* 39: 406–10.

Hudson, J.R., 1954. Typing of strain of *Pasteurella septica* and a note on a living attenuated vaccine. *Bull. Int. Expix.* 42: May, pp: 267–77 (*Vet. Bull.,* 25: 3132).

Islam, M. and R.A. Cheema, 1975. Studies on the isolation and characterization of *Pasteurella multocida* strains from livestock in Pakistan. M.Sc. Thesis, Deptt. of Microbiol., Univ. of Agric., Faisalabad.

Mittal, K.R., T.N. Jaiswal, B.R. Gupta and B.K. Gupta, 1977. Studies in HS water in oil and multiple emulsion adjuvants. 1. Immunity trial in Rabbits. *Indian Vet.,* 54: 773–9.

Muneer, R., 1993. Final Technical Report (January 1990-January, 1993). Animal Sciences Institute, National Agricultural Research Centre (NARC), Islamabad, Studies on improvement of haemorrhagic septicemia vaccine.

Nandani-peiris, L.K.P. and M.C.L. de-Alwis, 1991. Simplified techniques for haemorrhagic septicaemia oil adjuvant vaccine production. Proceedings of 4th International Workshop on Haemorrhagic septicaemia, FAO, Bangkok, Thailand, pp: 117–20.

Sarma, G. and B.R. Boro, 1980. Isolation of Pasteurella multocida from sporadic outbreaks of bovine pasteurellosis in Assam. *Vet. Res.,* 106: 57–8.

Shah, N.H. and N.H. Shah, 1998. Identifiation and immunigenicity of polysacchride antigens of Pasteurella multocida strains involved in haemorrhagic septicaemia. *Pak. Vet. J.,* 18: 192–6.

Stephen, L.R., P.B. Little, B.N. Wilkie and D.A. Barnum, 1984. Isolation of Haemophilus somnus antigens and their use as vaccines for prevention of bovine throboemboli meningecephalitis. *Am. J. Vet. Res.,* 45: 234–9.

Tortora, G.J., B.R. Funke and C.L. Case, 1989. Mechanisms of pathogenicity, in "Microbiology: An introduction" 3rd Edition, The Benjamin/Cummings Publishing Company Inc., Workingham, UK, pp: 377–98.

Villegas, 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 Publ. Co., USA, pp: 186–91.

(Received 07 August 1999; Accepted 15 May 2000)