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Full Length Article

Factors Affecting Potency of Hemorrhagic Septicemia Vaccines

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Abstract

This study was aimed at investigating effects of biomass, adjuvant, storage, priming alone or with boosting of an inactivated Haemorrhagic Septicemia (HS) vaccine (prepared from dense growth of *Pasteurella multocida*) on its potency using indirect hem-agglutination (IHA) test. The vaccine containing dry mass 1.7 mg/dose or bacterial count 10^{14}/dose was sufficient to induce protective antibody titer (GMT=log $_2{}^{5.2}$). Montanide ISA 70 based HS vaccine induced better antibody response (GMT=log $_2{}^{6}$) in buffalo calves than that of HS vaccine containing aluminum hydroxide gel (GMT=log $_2{}^{3.2}$) or no adjuvant (undetectable). The oil based HS vaccine remained potent (GMT=log $_2{}^{3.8}$) even after storage at 4^0C for eight months. Boosting of calves with oil based HS vaccine may help keep the calves immunized for 12 months. It was concluded that the proposed study improved quality of the vaccine and reduced the vaccine volume per dose, cost of its production and frequency of the vaccination. © 2015 Friends Science Publishers

Keywords: *Pasteurella multocida;* Hemorrhagic septicemia; Montanide ISA 70; Buffalo calves

Introduction

Hemorrhagic septicemia (HS) is an acute and fatal bacterial disease of cattle and buffaloes. It is caused by specific serotypes of *Pasteurella multocida* (*P.multocida*) and is normally present in about 5% cattle and buffaloes in endemic areas (Wijewardana *et al*., 1986b). It is gram negative, non-motile, non-spore former and coccobacilli (De-Alwis, 1996). It grows on blood agar, brain heart infusion and casein sucrose yeast (CSY) medium while there is no growth on MacConkey's agar. It produces oxidase, catalase and indole while does not produce hydrogen sulphide and urease (Kumar *et al*., 1996; Hussain *et al.*, 2014).

Five capsular groups A, B, D, E and F of *P. multocida* have been identified using indirect hem-agglutination test (Carter, 1967; Rimler and Rhoades, 1987), while 1 to 16 somatic types have been identified by agar gel immunodiffusion (AGID) test (Brogden and Packer, 1979). The Carter's type 2:B is equivalent to Robert's type 1, Heddleston's type 2 and Namioka and Murata's type 6:B (De-Alwis, 1999; Abubakar *et al.*, 2013).

The disease is highly fatal in nature and recovery is rare after onset of the clinical signs. The disease therefore controlled by mass vaccination in the countries, where it is endemic. Failure of immunoprophylaxis of the vaccine is attributed to many factors such as amount of immunogen/ dose, adjuvant, contamination, concurrent diseases such as tick infestation, ephemeral fever, brucellosis, salmonellosis, mycotoxicosis, etc., (Ali *et al*., 2000). The present study was therefore designed to investigate the factors affecting potency of hemorrhagic septicemia vaccine.

Materials and Methods

Effect of different factors on the potency of HS vaccine was determined through Indirect Hem-agglutination (IHA) test.

Biomass Production of *Pasteurella multocida* (B:2)

The capsulated culture of *P. multocida* was obtained from Department of Microbiology for the vaccine preparation and was injected in each of the two rabbits with 0.5 mL of broth culture through intra-peritoneal (I/P) route and monitored for the response. Body temperature of the rabbits was recorded before inoculation and after every two hours post-challenge infection. After death of rabbits, the heart blood, liver, lungs and long bones were collected. Each of the samples was inoculated on CSY blood agar using streak plate method. The blood from heart was directly streaked on the agar plates. Only a small quantity of bone marrow was scoped out and streaked on the agar plates. The streaked plates were incubated at 37^oC for 24 h.

Preparation of Hemorrhagic Septicemia Vaccines

P. multocida was grown in CSY broth in a commercial biofermenter. The broth culture was inactivated with 0.5%

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formalin.To check the sterility, inactivated culture was inoculated on CSY blood agar plates and incubated at 37°C for 24 h. Safety was monitored in two sero-negative rabbits by injecting one ml of the inactivated broth culture by deep intramuscular (I/M) route. The rabbits were monitored for any kind of signs, symptoms or death for 7 days.

Thiomersal sodium was mixed in chemically inactivated bacterial culture at rate of 0.05% as preservative. The vaccines were prepared using different dry masses (1.7, 2.2, 2.7 mg/mL) and adjuvant (montanide ISA 70, aluminum hydroxide gel, no adjuvant). Moreover, oil based HS vaccine was stored at 4°C for 4, 8 and 12 months. Effect of each type of the HS vaccine on the antibody response of six months old buffalo calves at Buffalo Research Institute, Pattoki was determined using indirect hem-agglutinating (IHA) test (Das *et al*., 1998; Ali *et al*., 2000).

Experimental Design

Forty five buffalo calves (six months old) were selected and were divided into 9 groups, each having five calves. Each calf of group 1, 2, 3 and 4 was injected with oil based HS vaccine containing 0, 1.7, 2.2, 2.7 mg/dose, respectively. Each calf of group 5, 6 and 7 was vaccinated with HS vaccine (1.7 mg/dose biomass) containing Montanide ISA 70, aluminum hydroxide gel and without any adjuvant, respectively. Each calf of group 8 and 9 was primed with oil based HS vaccine (3 mL/animal) deep intramuscularly and each calf of group 8 was boosted 30 days post-priming with the same oil based vaccine using the same dose and route of vaccination. For monitoring keeping quality of the vaccine, the montanide ISA 70 based HS vaccine (1.7 mg/ dose) was stored at 4°C for 0, 4, 8 and 12 months. At each time, the vaccine was injected (0.5 mL/rabbit: deep intramuscular route: thigh muscles) to each of the five rabbits.

Blood sample (5 mL) was collected from each calf of each group on 0, 30, 60, 90, 120, 150, 180 and 210 days post priming and blood sample (1 mL) from each rabbit at each time on 0, 14, 28 and 42 days post priming in disposable syringes. Each of the blood samples was transported to the laboratory in crushed ice and was incubated at 37°C for 2 h. The serum thus oozed out from each blood sample was separated and stored at -40°C in properly labeled vials till monitoring for anti-LPS-IHA antibodies (Das *et al.*, 1998).

Indirect Hem-agglutination Test

The antigen of *P. multocida* was prepared from 16 h growth in CSY broth (Ali *et al*., 2000). Each serum sample was processed through indirect hemagglutination test (IHA) for monitoring anti-LPS-IHA antibody titer (Ali *et al.*, 2000).

Statistical Analysis

Geometric mean titer values of IHA antibody titers were calculated as by Villegas and Purchase (1989) and Burgh (1998) to compare the efficacy of vaccines.

Results

Pasteurella multocida was grown in commercial biofermenter and inactivated with 0.5% formaldehyde. Sterility of inactivated culture was monitored on CSY blood agar plates, while sero-negative rabbits were used to test the safety of inactivated culture. Thiomersal sodium was mixed in chemically inactivated bacterial culture as preservative at rate of 0.05%. The HS vaccine containing biomass 1.7 mg/dose of the vaccine showed effective antibody response (GMT=$\log_2^{5.2}$). Amount of the bacterial immunogen/dose of the vaccine was directly proportional to anti-LPS-IHA antibody response of the buffalo calves (Fig. 1). Immunogen biomass of *P. multocida* (1.68 mg/dose) showed optimum titer of anti-LPS-IHA antibody titers that persisted for more than nine months.

Bacterial vaccine without adjuvant showed poor antibody response ($\log_2^{3.2}$ units of anti-LPS-IHA antibody titer). Montanide ISA 70 based HS vaccine induced anti-LPS-IHA antibody response in buffalo calves that gradually enhanced and reached the peak level (GMT=$\log_2^{7.2}$) on 120 days post priming and declined thereafter slowly reaching to $\log_2^{6.0}$ IHA titer on 180 days post priming. The $Al(OH)_3$gel based HS vaccine induced anti-LPS-IHA antibody titer that reached at peak level ($\log_2^{6.4}$) on 60 days post priming and declined gradually thereafter, reaching to level of $\log_2^{3.2}$ IHA antibody titer on 180 days post priming. The oil based HS vaccine induced better antibody response as compared to gel based vaccines (Fig. 2). In case of oil based HS vaccine, there was gradual increase in anti-LPS-IHA GMT titer up to 60 days while decline started on day 195 post vaccination.

The primed calves when given first booster with oil based HS vaccine (1.7 mg/dose) one month post-priming, the anti-LPS-IHA antibody titer was raised to 256 after 30 days of boosting that persisted for more than 210 days (Fig. 3). The calves primed with the oil based HS vaccine showed protective antibody titer for 210 days while, alum precipitated HS vaccine for 60 days. The oil based HS vaccine when stored at 4°C remained effective (GMT=$\log_2^{3.8}$) for eight months and its efficacy declined thereafter (GMT=$\log_2^{2.4}$ on 12 months) (Fig. 4).

Discussion

Vaccination is the best way to control outbreaks of HS in cattle and buffaloes in endemic areas. It is noteworthy that *P. multocida* Robert's type-1 is cause of HS outbreaks in Pakistan (Aslam *et al*., 1988). Purified lipo-polysaccharide (LPS) of *P. multocida* or whole culture induces antibody response in mice and show resistance to challenge infection. The immunity against whole culture is more effective than that LPS alone (Adler *et al*., 1996).

P. multocida was grown in commercial biofermenter and inactivated with 0.5% formaldehyde. The formaldehyde interacts with amine group of proteins of cell wall, cell

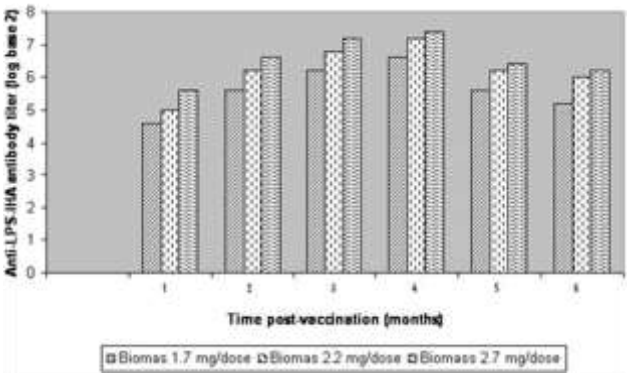

Fig. 1: Effect of bacterial mass on antibody response of buffalo calves to oil based hemorrhagic septicemia vaccine
The oil based HS vaccine was prepared using 1.7, 2.2 and 2.7 mg/dose of bacterial biomass. Each of the vaccine was injected to each of the five calves of the respective groups. Serum Anti-LPS-IHA antibody titer of each of the calves was monitored on 1, 2, 3, 4, 5 and 6 months post-priming and geometric mean titers of the antibodies at each time of each group are shown in the bar diagram

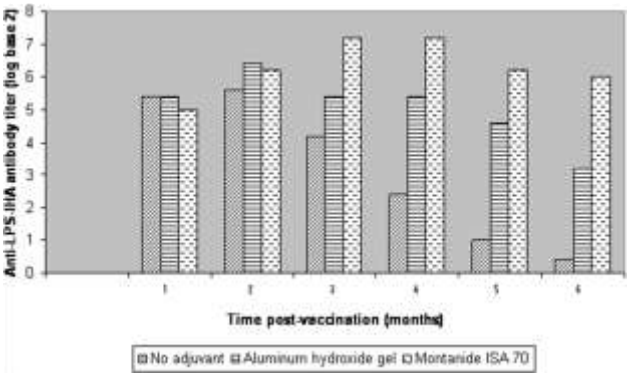

Fig. 2: Effect of adjuvant on antibody response of buffalo calves to hemorrhagic septicemia vaccine
The HS vaccine (1.7 mg/dose of bacterial biomass) was prepared using aluminum hydroxide gel, montanide ISA 70 and without adjuvant. Each of the vaccine was injected to each of the five calves of the respective groups. Serum Anti-LPS-IHA antibody titer of each of the calves was monitored on 1, 2, 3, 4, 5 and 6 months post-priming and geometric mean titers of the antibodies at each time of each group are shown in the bar diagram

membrane and cytoplasm thus inactivating the bacteria in an effective way within 24 h at 37°C. It is suggested that even higher concentrations can be utilized to inactivate the pathogen because outer most part of bacteria is LPS. Sterility of inactivated culture was monitored on CSY blood agar plates, while sero-negative rabbits were used to test the safety of inactivated culture. Thiomersal sodium is mixed in chemically inactivated bacterial culture (Kulcsar *et al*., 2008) as preservative at rate of 0.05%. However, susceptible mice can be used for safety and to evaluate the potency of the oil based vaccine against HS (Bhatti *et al*., 2005).

Amount of the bacterial immunogen/dose of the vaccine is directly proportional to anti-LPS-IHA antibody response of the buffalo calves. Immunogen biomass of *P. multocida* (1.68 mg)/dose showed optimum titer of anti-LPS-IHA antibody titers that persisted for more than nine months (Ali *et al*., 2000). However, 2 mg/dose of bacterial biomass is also suggested in HS vaccine to induce immunity for one year (Bain *et al.* 1982; Anonymous, 2012).

Bacterial vaccine without adjuvant show poor antibody response ($log_2^{3.2}$ units of anti-LPS-IHA antibody titer) so adjuvant is added in the vaccines to enhance their immunogenicity in animals. Aluminum hydroxide gel, alum, sodium alginate, saponin, lanolin, vegetable and mineral oils are commonly used adjuvant in veterinary

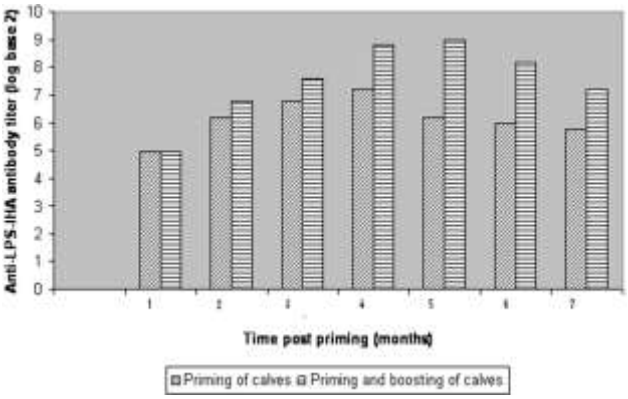

Fig. 3: Effect of boosting on antibody response of primed buffalo calves to oil based hemorrhagic septicemia vaccine
The oil based HS vaccine was prepared and primed to each of the calves of group A and B (3 mL/calf: intramuscular). Each of the calves of group B were boosted one month post priming using the same dose and route. Serum Anti-LPS-IHA antibody titer of each of the calves was monitored on 1, 2, 3, 4, 5, 6 and 7 months post-priming and geometric mean titers of the antibodies at each time are shown in the bar diagram

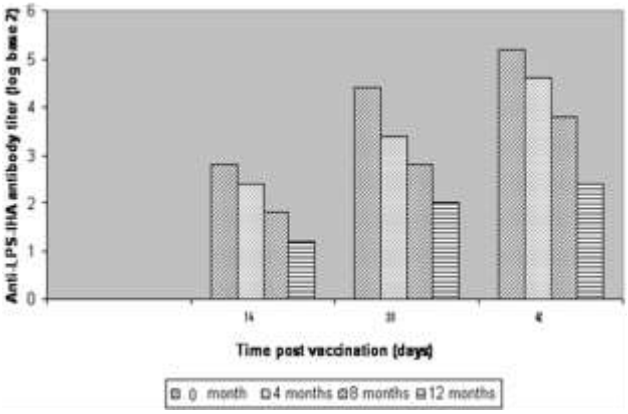

Fig. 4: Effect of storage of oil based hemorrhagic septicemia vaccine on its potency in buffalo calves
The oil based HS vaccine was prepared and stored at 4^0C for zero, 4, 8 and 12 months. At each time, the vaccine was injected to each of five rabbits. Anti-LPS-IHA antibody titer of the rabbits on 0, 14, 28 and 42 days post-vaccination was determined using Indirect hemagglutination test. Geometric mean titers of the antibodies at each time are shown in bar diagram

vaccines (Bain *et al*., 1982; Muneer *et al.*, 1994; Ali *et al.*, 2000). Gel based vaccines induce antibody response of short duration and less effective (Baig and Sheikh, 1982; Ali *et al*., 2000). In case of oil based HS vaccine, there was gradual increase in anti-LPS-IHA GMT titer up to 60 days while decline started on day 195 post vaccination. The immunity raised in calves to oil based HS vaccine persisted for one year (Muneer *et al.* 2005). Such oil based vaccine helps in slow absorbance of the immunogen from site of injection and is a source of immunogen for production of antibodies for longer period of time (Shah *et al*., 1997; Muneer *et al*., 2005). Efficacy of oil based vaccine is evaluated in calves by giving them challenge infection subcutaneously of virulent *P. multocida.* The vaccinated calves resist the challenge infection while controlled group die due to challenge infection (Patti *et al*., 1996; Shah *et al*., 1997).

The primed calves when given first booster with oil based HS vaccine (1.7 mg/dose) one month post-priming, the anti-LPS-IHA antibody titer was raised to 256 after 30 days of boosting that persisted for more than 210 days. In case of boosting of primed calves using the oil based HS vaccine with 90 days interval induced protective titer for 300 days while, alum precipitated vaccine for 120 days (Jaffri *et al.*, 2006: Anonymous, 2012). It is believed that amount of the immunogen/dose could be more than that of the routinely used gel based HS vaccines in Pakistan. The contamination in vaccine is controlled using thiomersal sodium that is antibacterial and anti-mycotic agent and is commonly used in injections/vaccines (Anonymous, 2012).

Conclusion

In crux 1.7 mg biomass of immunogen/dose and montanide ISA-70 improved the quality of HS vaccine. Boosting of the primed animals with oil based vaccine induced immunity that remained effective for more than 180 days. However, the oil based vaccine retained its potency on storage at 4^{o}C for eight months. The improvement of the vaccine reduced the volume of vaccine per dose, cost of its production and frequency of vaccination.

References

Abubakar, M.S., M. Zamri-Saad and S. Jasni, 2013. Ultrastructural changes and bacterial localization in buffalo calves following oral exposure to *Pasteurella multocida* B: 2. *Pak. Vet. J*., 33: 101–106.

Adler, B., R. Chancellor, P. Homchampa, M. Hunt, C. Ruffolo, R. Strugnell and D. Wapling, 1996. Immunity and vaccine development in *Pasteurella multocida* infections. *J. Biotechnol.,* 44: 139–144

Ali, Z., K. Muhammad, I. Hussain, E. Hameed, 2000. Antibody response of buffaloes to Haemorrhagic Septicaemia vaccine. *Int. J. Agric. Biol*., 2: 183–186

Anonymous, 2012. *Hemorrhagic Septicemia in Office of International Epizootics: Terrestrial Manual*, Chapter: 2.4.12, pp: 1–13

Aslam, M., M. Ishfaq, M. Irfan and M. Tariq, 1988. Serotyping of *Pasteurella multocida* isolated from cattle and buffaloes. *Pak. Vet. J*., 8: 146–148

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

Bain, R.V.S., M.C.L. De Alwis, G.R. Carter and B.K. Gupta, 1982. Haemorrhagic Septicaemia. *In*: *FAO Animal Production and Health Paper 33*, pp: 11–33. Food and Agriculture Organization of the United Nations, Rome, Italy

Bhatti, A.R., K. Tasneem, Z.J. Gill and A. Raza, 2005. Potency and safety testing of haemorrhagic septicemia oil adjuvanted vaccine in swiss albino mice. *Pak. Vet. J*., 25: 153–154

Brogden, K.A. and R.A. Packer, 1979. Comparison of *Pasteurella multocida* serotyping systems. *Amer. J. Vet. Res*., 40: 1332–1335

Burgh, M.A., 1998. Simple method for recording and analyzing serological data. *Avian Dis*., 2: 362–365

Carter, G.R., 1967. *Pasteurellosis: Pasteurella multocida* and *Pasteurella haemolytica*. *Adv. Vet. Sci*., 11: 321–379

Das, P., K. Muhammad and M.A. Sheikh, 1998. Standardization of indirect haemagglutination test for monitoring Pasteurella multocida antibodies. *Pak. Vet. J*., 18: 25–28

De-Alwis, M.C.L., 1999. *Haemorrhagic Septicaemia*. *ACIR Monograph*, 37: 64–65

De-Alwis, M.C.L., 1996. *Haemorrhagic Septicaemia: Clinical and Epidemiological Features of the Disease*. International Workshop on diagnosis and control of H.S. Bali. Indonesia, May 28–30

Hussain, R., M.T. Javed, F. Mahmood, T. Hussain, H.R. Chaudhry, M.S. Aslam, M.T. Ghori, A. Qayyum, W. Babar, S. Hameed and A.U. Rehman, 2014. Clinico-pathologic findings of enterotoxemia in Chinkara deer (*Gazella bennettii*) under desert conditions in Pakistan. *Pak. Vet. J*., 34: 400–402

Jaffri, K.T., Z.J. Gill, A.R. Bhatti and A. Raza, 2006. Immune response of buffalo calves to *Haemorrhagic Septicemia* oil adjuvant and alum precipitated vaccine. *Int. J. Agric. Biol.,* 8: 645–647

Kulcsar, G., K. Fabian, A. Farsang and G.Y. Virag, 2008. Evaluation of the protection provided by an inactivated trivalent *Pasteurella multocida* vaccine against experimental Pasteurella infection and Pasteurellosis in commercial environment. *In Proc: 9th World Rabbit Congress* June 10–13, 2008, Verona, Italy

Kumar, A.A., P.C. Harbela, R.B. Rimler and P.N. Kumar, 1996. Studies on *Pasteurella multocida* isolates of animal and avian origin from India. *Ind. J. Comp. Microbiol. Immunol. Infect. Dis.*,17: 120–124

Muneer, R., S. Akhtar and M. Afzal, 1994. Evaluation of three oil adjuvant vaccines against Pasteurella multocida in buffalo calves. *Rev. Sci. Tech. Off. Int. Epiz.*, 13: 837–843

Muneer, R., M. Hussain and A.B. Zahoor, 2005. Efficacy of oil based haemorrhagic septicemia vaccine: A field trial. *Int. J. Agric. Biol*., 7: 571–573

Patti, U.S., S.K. Srivastava, S.C. Roy and T. More, 1996. Immunogenicity of outer membrane protein of Pasteurella multocida in Buffalo calves. *Vet. Microbiol*., 52: 301–311

Rimler, R.B. and K.R. Rhoades, 1987. Serogroup F, a new capsule serogroup of *Pasteurella multocida*. *J. Clin. Microbiol*., 25: 615–618

Shah, N.H., N.H. Shah and F.K. De-Graaf, 1997. Protection against haemorrhagic septicemia induced by vaccination of buffalo calves with an improved oil adjuvant vaccine. *FEMS Microbiol. Lett.*, 155: 203–207

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 edition, pp: 186–191. The American Association of Avian Pathologists. Kendall/Hunt Publication Co., USA

Wijewardana, T.G., M.C.L. De-Alwis and A.A. Vipulasiri, 1986b. An investigation into the possible role of the goat as a host in hemorrhagic septicemia. *Sri Lanka Vet. J*., 34: 24–32

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