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Original Research Article

Efficacy and cross-protectivity of live intranasal aerosol hemorrhagic septicemia vaccine in buffalo calves

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A B S T R A C T

Keywords

Calves, Challenge, Efficacy, Immunity, Vaccine.

Present study was carried out to determine the efficacy and cross-protectivity of live intranasal aerosol hemorrhagic septicemia (HS) vaccine in buffalo calves, and to investigate need of booster vaccination. Efforts were also made to establish correlation between the humoral immune response and active protection. Twenty four calves (4–6 months) were divided into three groups (A, B and C), each having eight calves. Group A served as unvaccinated control. Animals in group B were given single dose of live intranasal aerosol HS vaccine, while group C was treated with booster dose after one month of first vaccination. The absorbance values of group B sharply rose gradually till 80th day following vaccination then it started declining and reached to minimum level on 260th day. The mean absorbance values of group C showed that the rise in antibody titers declined after second shot of vaccination at 80th day of first dose, and the highest peak was observed at 140th day post vaccination. Antibody levels gradually decreased up to 260th day. Overall population mean with inclusion of all sampling period depicted that three groups are highly significantly different ($P< 0.05$). In twelve months post vaccinated challenge protection study, only in the group C all calves survived with a slight rise in temperature. The results of this study revealed that boosting of immunity via intranasal route in calves provided better protection against HS. Thus said study may have implications for developing better vaccinations strategies to reduce the intensity of infection within the host body.

Introduction

Haemorrhagic septicemia (HS) is the most common contagious bacterial infection of cattle and buffaloes in Pakistan and other south East Asian countries (Khan *et al.,* 2006; Tarek *et al.,* 2014). The disease is resulting in high mortality and morbidity because of its catastrophic epizootics occurrence in many Asian countries

(Waheed ullah *et al.,* 2009). Two serotypes i.e. *P. multocida* B: 2 and E: 2 are involved in disease production. The disease is acute in nature, having short incubation period and is characterized by high fever with in appetence and salivation, edematous swelling in the throat region which extends to ventral cervical region and brisket, at the end, respiratory distress leads to collapse and death of the animal (Dutta *et al.,* 2005; Subhash and Shiv, 2011). Hemorrhagic septicemia is ranked as the most common contagious disease in Pakistan as 34.4% of all deaths in susceptible stock are due to HS (Benkirane and De Alwis, 2002). Therefore, the disease cannot be overlooked as it causes heavy annual economic loss worth of Rs. 2.17 billion only in Punjab Province (Anonymous, 1996). Antibiotic treatment is effective only during early stages but due to sudden onset of the disease and short incubation period; the disease often escapes early detection (Kedra and Borkowska-Opacka, 2001). Vaccination is an effective means of control of the disease. Various types of killed vaccines i.e. broth bacterin, oil adjuvant vaccine (OAV), alum precipitated vaccine (APV), are being used in Pakistan for the prevention of HS (Ali *et al.,* 2000). Virulent strain of *P. multocida* (B: 2) have been used for the preparation of killed vaccines. APV gives protection for shorter duration i.e. only for four to six months while OAV confers immunity for 9 to 12 months but is slowly absorbed, difficult to inject and occasionally produces marked local reactions (Bain *et al.,* 1982). Currently, emphasis is being placed on the production potential of a vaccine which should be economical, stable in tropical environment and easy to administer. *P. multocida* serotype B: 3, 4 are considered to be safe to use as live vaccine against HS in cattle (Myint *et al.,* 2005). A novel vaccine prepared from live heterotypic strain prevented losses in cattle and buffaloes in natural outbreaks in endemic areas of Myanmar and animals were protected against direct challenge by serotype B:2 even after 12 months of vaccination (Jones *et al.,* 2002). The objectives of this work were to determine the efficacy and cross-protectivity of live intranasal aerosol HS vaccine in buffalo calves and also investigate the need of booster vaccination in calves and establish the correlation between the humoral immune response and active protection.

Materials and Methods

Bacterial strains

The *P. multocida* serotype B: 3, 4 was used as a live vaccinal strain while serotype B:2 was used as the challenge strain.

Source of bacteria

Isolates of bacterium *P. multocida* serotype B: 3, 4 and B: 2, were recovered from the stocks kept at -20°C in the lyophilized form at Bacteriology Lab of Animal Health Institute, NARC, Islamabad.

Experimental design

Twenty four calves of four to six months of age were included in the study. These animals were divided into three groups (A, B and C), each having eight calves. Group A served as unvaccinated control. Animals of group B were given single dose of live intranasal aerosol HS vaccine while in group C booster dose was given after one month of first vaccination. Dagleish *et al.* (2007) represented a clear dose-dependent response. According to him, animals receiving a higher vaccine dose being less affected clinically, bacteriologically and pathologically. Therefore, to give the best combination of high immune response,

protection and safety, the dose used for vaccination was 10^9cfu (Hodgson *et al.,* 2005).

Preparation of vaccine and challenge strains

The vaccinal and challenge strains, *P. multocida* B: 3, 4 and B: 2 respectively, were revived in mice and grow in bulk in Tryptose Soya Broth (TSB). After adjustment of number of organisms the suspension for respective strains were either used for vaccination (10^9cfu) or challenge infection (10^9cfu) of animals.

Vaccination procedure

A simple aerosol spray was used as an intranasal applicator. Live vaccine was introduced into the bottle. Vaccination was carried out by bringing the tip of the spray nozzle close to the nostril of the animal and sprayed quickly so that vaccine was forced to the upper respiratory tract of the animals.

Safety test

Five buffalo calves of six to eight months of age, each were given the 100 times the recommended dose of live haemorrhagic septicemia vaccine. The animals were observed for a week for any adverse reaction (Myint *et al.,* 2005).

Blood sampling and serum separation

The blood was withdrawn from experimental animals before and after vaccination on monthly basis. Serum was separated and stored at -20°C until used.

Preparation of antigen

Whole bacterial antigen was prepared as described by Afzal *et al.* (1992).

Protein estimation

The amount of protein, present in the antigen, was tested by using Lowry's method (Lowry *et al.,* 1951).

Enzyme-linked immunosorbent assay (ELISA)

An indirect ELISA for detecting antibodies against *P. multocida* was carried out according to Afzal *et al.* (1992). The optical density of the plates was read at 492 in ELISA reader.

Challenge infection

Four calves from each group were challenged by injecting subcutaneously 2×10^7 cfu viable organisms of *P. multocida* B: 2 after seven month of vaccination and four from each group after twelve months. The animals were observed for 5 days for clinical signs. The rectal temperature was also recorded after every two hours. The jugular blood was withdrawn from all calves before and after challenge infection, after every four hours in EDTA coated tubes and the estimation of white blood cells was carried out.

Statistical analysis

The rectal temperature and WBCs count were measured at each intervention (7 and 12 months challenge). The absorbance values at each point were represented as mean with standard error means. ANOVA was used to investigate the significance of the treatments. Differences were considered significant when $P<0.05$.

Results and Discussion

The mean absorbance values and standard deviations recorded for each group are

depicted in Fig. 1. The patterns of response were different among the three groups. Data revealed that IgG titers increased steadily in both the vaccinated groups. However, in group C (two shot of vaccine) absorbance values remained substantially high with greater antibody titer than that of group A and B throughout the study. The antibody titers of group B sharply rose till 80th day post vaccination and then started declining and reached at minimum level at 260th day. A decline in absorbance values in groups C and B was observed after 80 and 120 days post vaccination, respectively. Thereafter, the absorbance values became static in group C with slight rise and fall in levels till 360 days. In group B, the titers were below the cutoff point at 260 days post vaccination. A comparatively higher antibody titer up to 360 days was observed in experimental animals vaccinated with two shots of vaccine, throughout the trial period. While no effective rise in antibody titers were observed in unvaccinated calves. Overall population mean with inclusion of all sampling period depicted that three groups were significantly different ($P< 0.05$). The difference can also be seen through 95% confidence limit Fig. 2.

Challenge protection study

The patterns of clinical responses differed among three groups after challenge. In seven month post vaccinated challenge, the rise in mean rectal temperature was comparatively high in group A (104.87±0.48) and B (104.74±0.47) while increase in temperature in group C (101.5±0.261) was less than A and B. The mean increase observed during the period of 0 to 10 h post challenge was 7.4˚F (for group A), for B it was 7˚F and 2.2˚F for group C. The temperature remained 106.2˚F in group A even after 32 hours of challenge, in group B it reduced to 103˚F but in group C it came to normal within 32 hours post challenge. In groups A the highest peak was observed at ten hours post challenge while in group B and C it was at 6 hours as shown in Fig. 3.

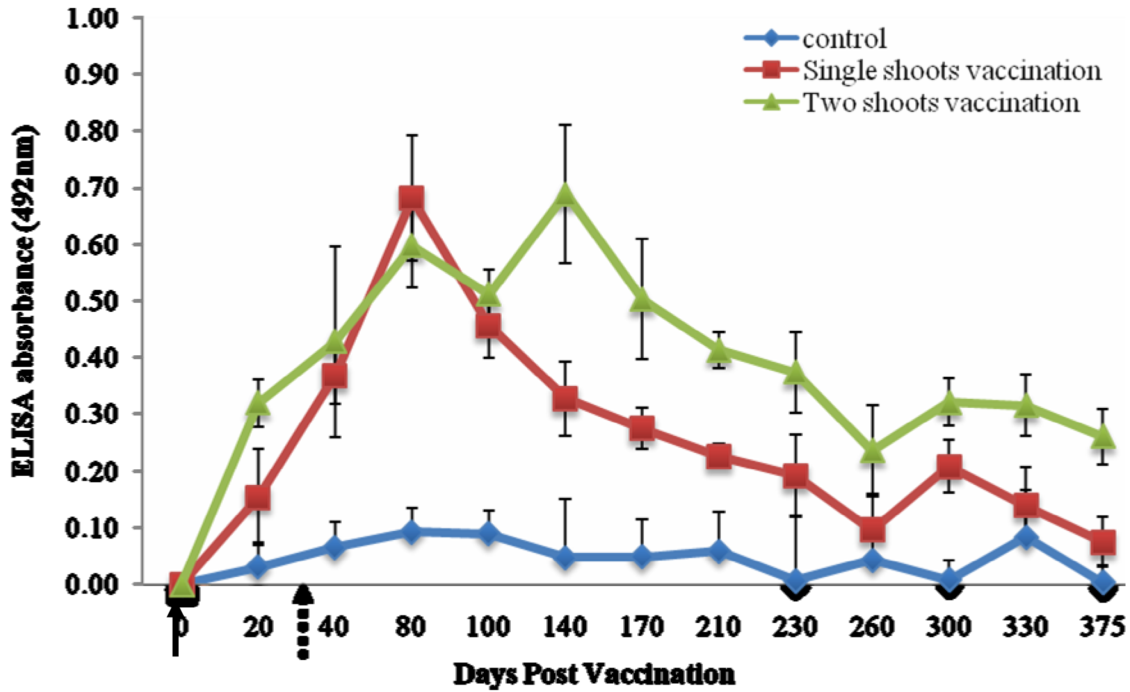
Haematological analysis of seven months post vaccinated challenge revealed that the calves of group A showed a sudden rise in the WBCs counts and then the marked leucopenia was observed immediately before death. While, the WBCs counts remained stable in both group B and C even 32h of post challenge. In seven months post vaccinal challenge protection study two of four from group B and four of four calves from group C i.e. from single and double shot vaccinated group, respectively, survived from the challenge infection whereas, all the unvaccinated controls were died within 37h post challenge as shown in Fig. 4. In the case of twelve months post vaccination challenge, the rise in mean rectal temperature from the normal was lower in group C (102.19±0.350) than the other two groups. The temperature elevation during 0 to 10 h from the normal was 5.3˚F, 6˚F and 3.6˚F for Groups A, B and C, respectively. Elevated temperature came to normal after about 0 to 28 hours of challenge. The highest temperature recorded at 8 hours post challenge in group A (106.2˚F) and group B (105.3˚F) but at 8 hours in group C (105.2˚ F). On the other hand, the mean WBC count was lowest in group A after 4 hours of challenge. Minor variations in WBCs counts were observed in group B and C with the progression of the disease. In twelve months post vaccinated challenge protection study, three of four calves from the unvaccinated control and three of four calves from single shot vaccinated group were died. But all the calves from the group C survived from the challenge with a slight rise in temperature.

General demeanor

Calves showed the sign of illness, in 6h of post challenge. The general demeanor varies among the groups. All the C grouped animals showed temporary pyrexia but in group B in addition to pyrexia the animals looked dull and depressed. General demeanors of group A and one of group B calves included elevation of temperature with salivation and reduction in appetite. In addition to this swelling in the neck region nasal and eye discharge with dyspnea was also observed. These symptoms led to the recumbence and finally to the death of the animal.

This study revealed that primary and booster vaccination with 2×10^7 viable organisms of *Pasteurella multocida* B3, 4 strains could be given safely through aerosol route to buffalo calves of 4 to 6 months old. This is in accordance with Mynt *et al.* (2005) who reported the intranasal route with fine aerosol spray was safer than subcutaneous injection in younger cattle and buffaloes. The study further indicate that buffalo calves given single aerosol intranasal spray of live vaccines remain protected against experimental challenge only for seven months post vaccination while the calves given booster dose after one month of priming remain protected against direct subcutaneous challenge even after 12 months of vaccination. This is the first report on the need of booster vaccine in younger calves between four to six months of age. Our results are also in contrast with the findings of the Priadi and Natalia (2001) who showed that cattle and buffalo calves between six months to two years old, vaccinated intranasally with *P. multocida* B: 3, 4 were protected against the direct subcutaneous challenge with *P. multocida* B: 2. The live vaccines when administered intranasally as fine aerosol vaccine mimic natural route of infection by targeting the natural stimulatory factors and generates the circulatory (IgG) and mucosal (IgA) antibodies. Like IgG, IgM and IgA are also involved in immunity (Hodgson *et al.,* 2005). The previous studies conducted on OAV (Chandrasekaran *et al.,* 1994) revealed that there was poor correlation between the humoral immunity and active protection. We also could not find the positive correlation between IgG levels with protection as the well protected animals at 12 month of challenge did not exhibited considerably higher titers than non protected animals thus depicting the positive role of cell mediated as well as mucosal immune responses in protection (Benkirane and De Alwis, 2002). Following the challenge infection studies, the general demeanor observed in infected animals including dull, depressed, swelling in the neck region with pyrexia and dyspnea. These findings are in concordance with the findings of Dutta *et al.* (2005). The results have indicated that a booster dose had significant effect on the duration of immunity. It points out the importance and need of booster vaccination for prolonged immunity. The intranasal live HS vaccine is effective, safe and primes systemic responses with dose 2×10^7 viable organisms. This dose did not produce any undesirable reaction in the vaccinated animals and proved to be safe for the buffalo calve of 4–6 months of age. Moreover, the live aerosol HS vaccine induced long term immunity for more than a year, by targeting the stimulatory factors of the disease. Vaccine trials with greater number of animals, however, are needed in order to establish that the safety and protective properties in the target species.

Fig.1 Mean and standard deviation of antibody titer of buffalo calves vaccinated with live haemorrhagic septicaemia vaccine



The Primary vaccination was given to buffalo calves at day zero (↑) and Secondary vaccination was given after one month (⇡).

Fig.2 Mean antibody level of different groups with 95% confidence limit

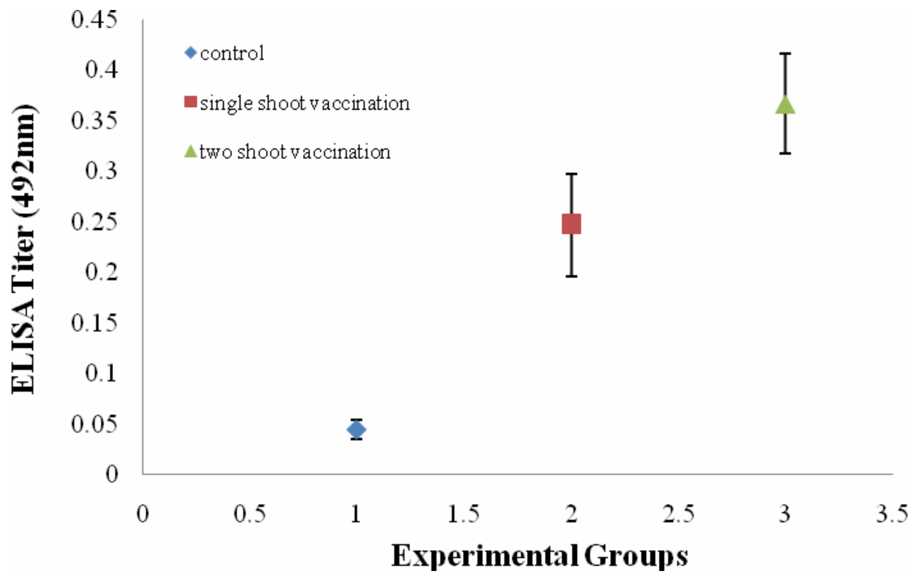


Fig.3 The pattern of retal temperature (^{o}F) for 12 months among three experimental groups after challenge

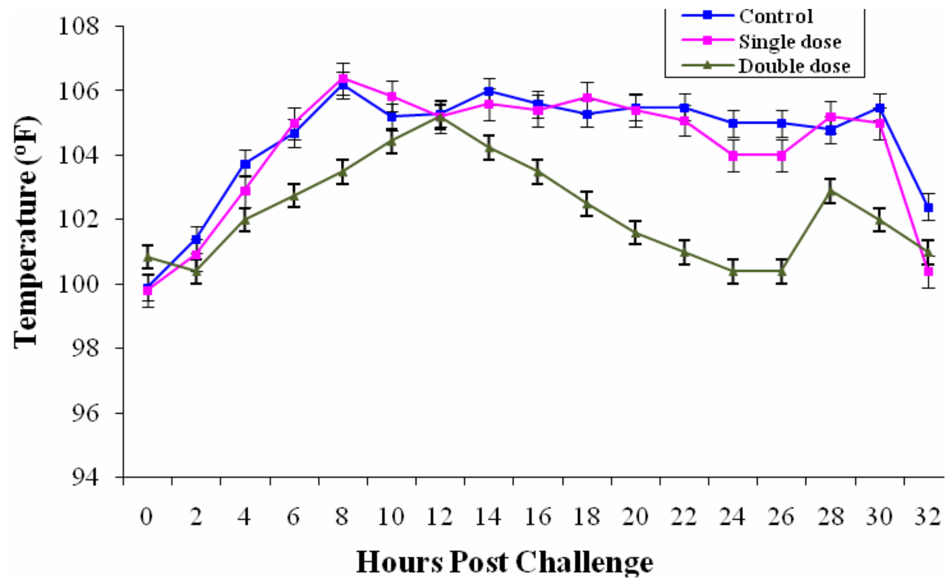
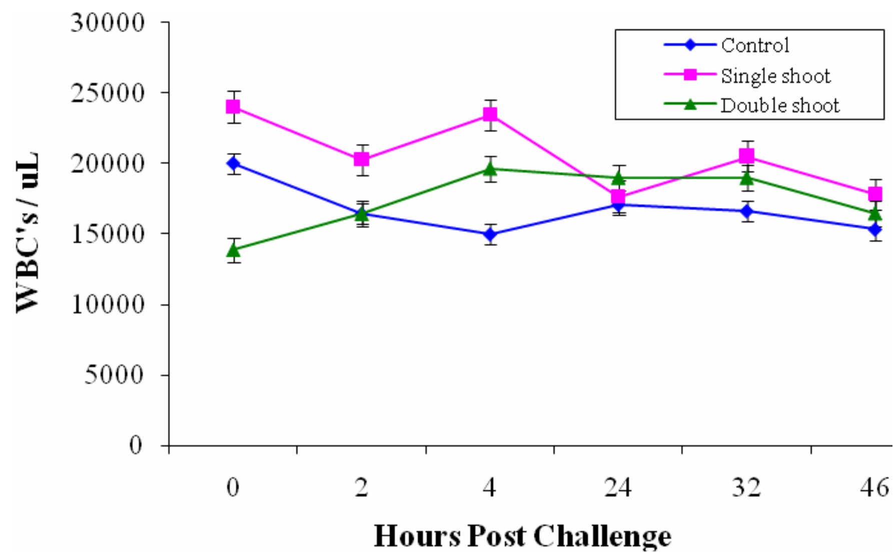


Fig.4 The profile of WBC for seven months post vaccinated challenge among three experimental groups



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