The Effect of Dexamethasone on Immune Responses of Calves to Intranasal Exposures to Live Attenuated *gdhA* Derivative of *Pasteurella multocida* B:2

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ABSTRACT

The effect of stress, created using dexamethasone on immune response by calves to intranasal exposures to *gdhA* derivative of *Pasteurella multocida* B:2 was studied. For the purpose of this study, twelve calves of 6 months old were selected and divided into 4 groups. At the start of the experiment, calves of groups 1 and 2 were intramuscularly injected with dexamethasone at the rate of 1 mg/kg body weight for 3 consecutive days. Then, the calves of groups 2 and 3 were exposed intranasal to 5 ml of the inoculums containing 10^6 cfu/ml of the *gdhA* derivative of *P. multocida* B:2. Calves of groups 1 and 4 remained unexposed control. Serum samples were collected prior to the start of the experiment and at weekly interval for a period of 7 weeks. At the end of the 7-week period, all the calves were sacrificed before the lungs were lavaged using 1L of sterile phosphate buffered saline (PBS). The sera and lung lavage fluid were subjected to ELISA to determine the levels of IgG and IgA. Significant increase in both serum and lavage IgG and IgA were observed only in group 3, which were exposed without any dexamethasone treatment. The dexamethasone-treated and exposed group 2 failed to respond to the exposures when the levels remained insignificant to those of the control untreated and unexposed group 4. The calves of group 1, which were treated with dexamethasone but remained unexposed, failed to show any response at all. In conclusion, intranasal exposures to live attenuated *gdhA* derivative of *P. multocida* B:2 must be given only to unstressed calves, since stressful condition has been found to prevent calves from responding significantly to the antigen.

Keywords: Intranasal, *Pasteurella multocida* B:2, dexamethasone, immune response

INTRODUCTION

Haemorrhagic septicaemia (HS) is an infectious disease of cattle and buffalo caused by *Pasteurella multocida* B:2 (Adler *et al.*, 1996). It is commonly fatal and is considered as one of the most economically important livestock diseases in Southeast Asia (Benkirane and De Alwis, 2002).

Subcutaneous injections of alum-precipitated or oil adjuvant vaccines have been used to control the disease. However, the vaccines were able to provide short-term immunity, approximately 4 to 6 months and up to 1 year for alum adjuvant and oil adjuvant vaccines, respectively (Verma and Jaiswal, 1997). Furthermore, the high viscosity of the oil adjuvant vaccines makes them unpopular among field users. Therefore, vaccine development was highlighted as a major area for investigation at the last International Workshop on HS (FAO, 1991). Subsequently, an attenuated *P. multocida* B:2 was successfully created following disruption

of the *gdhA* gene, a housekeeping gene, of the wild-type *P. multocida* B:2 (Sarah *et al.*, 2006). The attenuated organism was found to be able to stimulate both the mucosal and systemic immunities that protect animals from challenges (Zamri-Saad *et al.*, 2006).

Immunosuppression due to stress can increase the susceptibility to infectious disease (Concordet and Ferry, 1993; Anderson *et al.*, 1999). Similarly, outbreaks of HS have been associated with stressful conditions, particularly changes in weather (Saharee *et al.*, 2005). The administration of dexamethasone, either alone or in combination with stressful conditions, results in a systemic suppression of the immune response (Collins and Suarez-Guemes, 1985).

This study was carried out to determine the suppressive effects of dexamethasone on the systemic and mucosal antibody responses to intranasal exposures of live attenuated *gdhA* derivative of *P. multocida* B:2.

MATERIALS AND METHODS

Animals

Twelve, clinically healthy, calves (approximately 6-month-old) were randomly divided into four groups. Anthelmintic (Ivomec, MSD) was administered at the rate of 0.2 mg/kg body weight upon arrival to control internal parasitism, which might influence the development of immunity responses (Zamri-Saad *et al.*, 1994). Nasal swabs were collected at the same time after the arrival to ensure that the calves were free of *Pasteurella multocida*, prior to the start of the experiment (Zamri-Saad *et al.*, 1999). The different groups were kept in separate housing and fed cut grasses and supplement feed at the rate of 1 kg/animal/day, while drinking water was provided *ad-libitum*.

Inoculums

The *gdhA* derivative of *P. multocida* B:2, created earlier by Sarah *et al.* (2006), was used in this study. The inoculums were prepared by resuscitating the stock culture onto blood agar plates containing kanamycin (50 µg/mL) and streptomycin (60 µg/mL), and this was followed by incubation for 46 h at 37°C. At the end of the incubation period, six uniform-sized colonies were selected and inoculated into antibiotic containing 100 ml of brain-heart infusion broth, which was then incubated at 37°C for 18 h before the bacterial concentration was determined using the plate count technique method of Alcamo *et al.* (1997). The concentration was readjusted using phosphate-buffered saline (PBS) to give a final concentration of 10^6 colony-forming unit (cfu)/ml.

Experimental Procedure

At the start of the experiment, all calves of groups 1 and 2 were administered intramuscularly with dexamethasone, at the rate of 1 mg/kg body weight, for 3 consecutive days. On the day after the last dexamethasone injection, the calves of groups 2 and 3 were exposed intranasal to 5 ml of the live *gdhA* derivative of *P. multocida* B:2 inoculums which had been prepared earlier. Two weeks after the initial intranasal exposure, the calves of groups 2 and 3 were once again exposed intranasal to the same inoculums of live attenuated *gdhA* derivative of *P. multocida* B:2. Meanwhile, the calves of groups 1 and 4 remained unexposed.

All the calves were observed for signs of illnesses, while the serum samples were collected from all the calves prior to dexamethasone treatment and on weekly intervals thereafter. On week 7, i.e. after the 1st intranasal inoculation, all the calves were sacrificed and post-mortem examinations were carried out immediately on the respiratory tract. The trachea was exposed and clamped to avoid intratracheal contamination with blood, removed, and the presence, extent, and nature of superficial lesions were determined using a method developed for sheep (Gilmour *et al.*, 1983).

All lungs were lavaged by introducing 1 L cold and sterile phosphate buffered saline (PBS) into the lungs through the trachea, massaged gently before the fluid was re-collected. The

lung lavage fluid was centrifuged at 1000xg for 15 min to remove the debris before the supernatant was collected and kept at -20°C until further use.

Determination of Antibody Levels

The serum and broncho-alveolar lavage samples were used to determine the systemic and mucosal immunoglobulin IgG and IgA levels, respectively, by using the ELISA technique developed by Zamri-Saad *et al.* (2006). In brief, the microtiter plates were coated with 50 µl per well of whole cell inactivated *P. multocida* B:2 suspension prepared earlier at a concentration of 10^9 cfu/ml in carbonate-bicarbonate buffer pH9.6. The plates were dried at 37°C in an incubator for 1 hr. After 2 times washing using a washing buffer, the reactions were blocked with 200 µl PBS/BSA/Tween 20 and incubated for 1hr. Then, 50 µl of either serum or broncho-alveolar lavage samples were introduced and incubated again for 1 hr and washed 3 times.

After that, 50 µl of conjugate were put, incubated for 1 hr and washed 3 times. This was followed by the addition of substrate and incubated again. The reaction was stopped by 2 *M* H_2SO_4 and the optical density values were measured at absorbance 450 nm wavelength in an Anthos Zenyth 340 st reader.

Statistical Analysis

All the data were statistically analyzed by using the ANOVA. The probable (*p*) value of <0.05 was considered as significant.

RESULTS

Serum IgA Levels

Fig. 1 shows the serum IgA response, following intranasal exposure of calves, to *gdhA* derivative of *P. multocida* B:2. Following the dexamethasone treatment, all the groups showed insignificant differences (p>0.05) in the IgA level at week 1. However, after the first
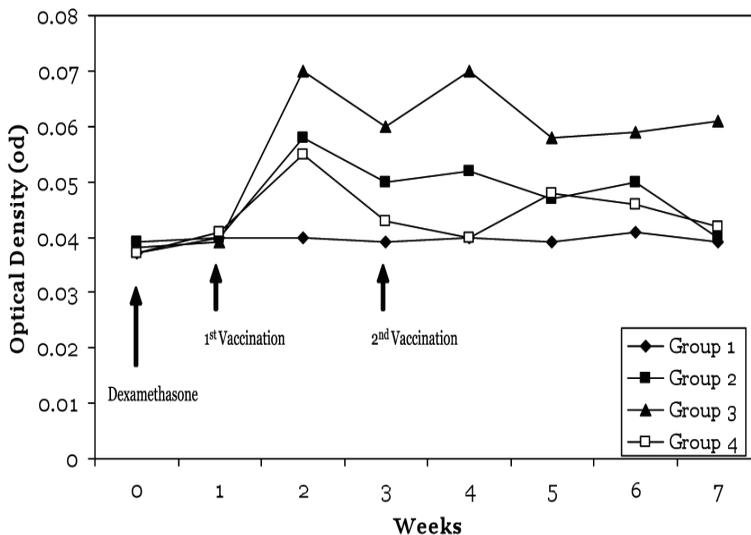

Fig. 1. The serum IgA levels in groups of calves exposed to gdhA *derivative of* P. multocida *B:2 with or without dexamethasone treatment. Group 1 was treated with dexamethasone without exposure to* P. multocida*, group 2 was treated with dexamethasone and exposed to* P. multocida*, group 3 was exposed to* P. multocida *without dexamethasone treatment and group 4 was not treated with dexamethasone and not exposed to* P. multocida

exposure, the calves of groups 2 and 3 showed a significant (p<0.05) increase in the IgA level as compared to group 1, while the level showed by group 2 was insignificantly (p>0.05) different with those of the unexposed, untreated calves of group 4.

Following the second exposure, the calves of group 3 which were without the dexamethasone treatment showed a significant (p<0.05) increase in the IgA level, which remained significantly (p<0.05) higher than all other groups (*Fig. 1*). The calves of group 2, which were treated with dexamethasone and exposed to *P. multocida*, failed to record any increment in the level of IgA after the second exposure and remained insignificantly (p>0.05) higher than the unexposed, untreated control group 4. Meanwhile, the calves of group 1, which were treated with dexamethasone but unexposed, showed no response throughout the experimental period.

Serum IgG Levels

Fig. 2 shows the serum IgG responses by the three groups. Generally, the IgG patterns were similar to those observed for serum IgA. Following the dexamethasone treatment, the serum IgG levels at the time of the first exposure were insignificant (p>0.05) among the three groups. However, after the first exposure, the calves in group 3 showed significantly (p<0.05) high IgG level, which remained until the second exposure. The levels in group 2, which was treated with dexamethasone and exposed to *P. multocida* and group 4, but not treated and exposed, remained significantly (p<0.05) low even after the second exposure until the end of this 7-week experimental period (*Fig. 2*). Group 1, which was treated with dexamethasone but not exposed to *P. multocida,* did not show any reaction throughout the study period (*Fig. 2*).
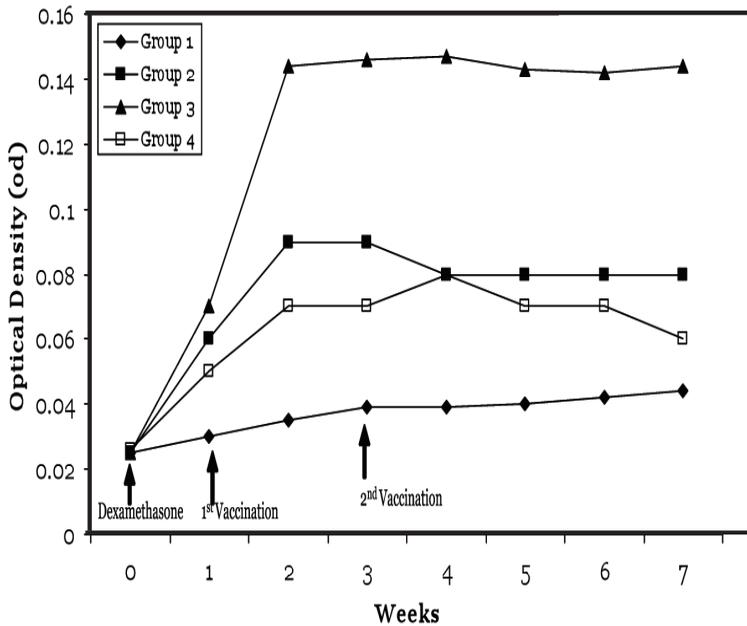

Fig. 2: The serum IgG levels in groups of calves exposed to gdhA *derivative of* P. multocida *B:2 with or without dexamethasone treatment. Group 1 was treated with dexamethasone without exposure to* P. multocida*, group 2 was treated with dexamethasone and exposed to* P. multocida*, group 3 was exposed to* P. multocida *without dexamethasone treatment and group 4 was not treated with dexamethasone and not exposed to* P. multocida

Lung Lavage IgA Levels

Fig. 3 shows the IgA response in the lung lavage fluid of calves at week 4, after the second intranasal exposure to *gdhA* derivative of *P. multocida* B:2. The IgA level in the lung lavage fluid of the calves of group 3 was significantly (p<0.05) higher than those of the calves in other groups, which appeared to be insignificantly (p>0.05) different.

Lung Lavage IgG Levels

Fig. 4 shows the IgG response in the lung lavage fluid of calves at week 4, after the second intranasal exposure to *gdhA* derivative of *P. multocida* B:2. As observed in the IgA response, the IgG level in group 3 was significantly (p<0.001) higher than the other groups, which were not significantly (p>0.05) different.
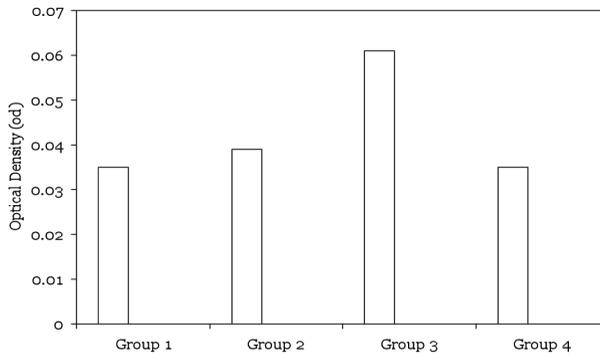

Fig. 3: The IgA level in the lung lavage fluid of calves exposed to gdhA *derivative of* P. multocida *B:2 with or without dexamethasone treatment. Group 1 was treated with dexamethasone without exposure to* P. multocida*, group 2 was treated with dexamethasone and exposed to* P. multocida*, group 3 was exposed to* P. multocida *without dexamethasone treatment and group 4 was not treated with dexamethasone and not exposed to* P. multocida

Fig. 4: The IgG level in the lung lavage fluid of calves exposed to gdhA *derivative of* P. multocida *B:2 with or without dexamethasone treatment. Group 1 was treated with dexamethasone without exposure to* P. multocida*, group 2 was treated with dexamethasone and exposed to* P. multocida*, group 3 was exposed to* P. multocida *without dexamethasone treatment and group 4 was not treated with dexamethasone and not exposed to* P. multocida
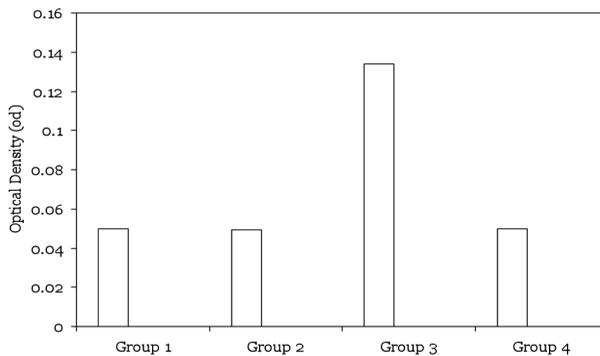
DISCUSSION

Infection by *P. multocida* B:2 has been associated with stressful situations. During rainy season and at the start of paddy planting season, cattle and buffaloes in many Asian countries are under extreme stressful condition (Interior, 1993). These stresses increase the level of hydrocortisone in the serum which leads to immuno-suppression and subsequent colonisation of bacteria, particularly in the respiratory tract (Zamri-Saad *et al.*, 1991). Dexamethasone injections have been used to replace natural hydrocortisone that mimics stressful condition on animals (Zamri-Saad *et al.*, 1991). The effect of gluco-corticoid administration has resulted in statistically significant reduction in the IgA levels and impaired the mucosal immunity (Alverdy *et al.*, 1997). Other than the local IgA level, serum IgG levels are also affected by dexamethasone administrations which lead to vaccination failure against pneumonic mannheimiosis (Zamri-Saad and Effendy, 1999). This study revealed that intranasal exposures of stressed calves to *gdhA* derivative of *P. multocida* B:2 had failed to stimulate both the mucosal and systemic immunities. This was obvious when the serum IgA and IgG levels of the exposed, stressed calves were similar to that of the unexposed calves.

On the other hand, the unstressed, dexamethasone-free calves showed significantly higher serum IgA and IgG responses following intranasal exposures to the *gdhA* derivative of *P. multocida* B:2. Similarly, the IgA and IgG levels in the lung lavage fluid of calves, which were treated with dexamethaosne, were significantly lower than those without any dexamethasone treatments. Similar poor responses of stressful animals to vaccination, including to intranasal exposures to *Mannheimia* (*Pasteurella*) *haemolytica* in goats (Zamri-Saad and Effendy, 1999) and to *Pasteurella multocida* B:2 in goats (Shafarin *et al.*, 2008), have also been reported. It was concluded that dexamethasone reduced the availability of immune cells, thus reducing immune responses.

In conclusion, exposures of calves during stressful period are of insignificant use since they remain similar as those of the unexposed calves and, therefore, are susceptible to infection. The findings gathered in this study revealed the extreme importance of vaccination against haemorrhagic septicaemia, which had been carried out prior to the stressful period. Many researchers have suggested that vaccination against haemorrhagic septicaemia in the field is done prior to the beginning of a rainy season so that a better and longer antibody response can be achieved to protect the animals during the stressful rainy season (De Alwis, 1993; Jamaludin, 1993; Phuong, 1993).

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