EFFECTS ON OF DEXAMETHASONE ON VACCINATION RESPONSES AGAINST PNEUMONIC PASTEURELLOSIS IN GOATS

A.R. Intan-Shameha and M. Zamri-Saad

*Faculty of Veterinary Medicine, Universiti Putra Malaysia 43400 UPM, Serdang,
Selangor Darul Ehsan, Malaysia.*

SUMMARY

A study was conducted to compare the effect of dexamethasone treatment prior to, during and post-vaccination on antibody response and protection against experimental challenge with *Mannhaemia haemolytica*. Sixteen goats of about 7 months old were divided into 4 equal groups. All goats were vaccinated twice intranasally with 1 ml inoculum containing 10^6 colony forming unit (cfu) of formalin-killed *Mannhaemia haemolytica* A2/ml. The two doses were administered at 2-week intervals. Goats in group 1 were treated with dexamethasone prior to vaccination, group 2 was treated during vaccination, group 3 was treated at 1 week-post vaccination while group 4 remained as the vaccinated control. Serum samples were collected weekly for a period of 5 weeks to detect antibody using an ELISA. Two weeks post-vaccination, all goats were challenged intratracheally with 4 ml inoculum containing live *Mannhaemia haemolytica* at a concentration of 10^9 cfu/ml before slaughtering at 2 weeks post-challenge. The extent of pneumonic lung lesions was determined. Generally, animals without dexamethasone treatment had significantly (p<0.05) high antibody response compared to other groups while the extent of pneumonic lung lesions was significantly (p<0.05) lowest. Although group 1 showed the second highest antibody response and a lower percentage of lesions, the differences were significant (p<0.05) compared to the control group. Group 2 which showed the second lowest antibody response recorded significantly (p<0.05) extensive lung lesions while group 3, which showed the lowest antibody level had insignificantly (p<0.05) more extensive lung lesions. There were strong correlations between the antibody response and the severity of lesions. The results emphasise that vaccination will not be efficient if carried out when the animal is under stressful conditions.

Keywords: Dexamethasone, pneumonic pasteurellosis, vaccination, goats

INTRODUCTION

Pneumonic pasteurellosis is one of the most common diseases of sheep and goats throughout the world. It is caused by either *Mannhaemia haemolytica* or *Mannhaemia multocida* which have been recognised as part of the normal flora of the nasopharynx (Dungworth, 1985). In Malaysia, most cases of this disease (70%) are caused by *Mannhaemia haemolytica* type A (Jamaludin, 1993) and *Mannhaemia haemolytica* serotype A2 is the most commonly isolated followed by serotypes A9 and A7 (Bahaman *et al.*, 1991; Mohamad *et al.*, 1993).

The disease has been shown to be associated with various stress factors including environmental factors such as climatic changes, transportation and nutrition deficiencies (Jubb *et al.*, 1985; Zamri-Saad *et al.*, 1989a; Jasni *et al.*, 1991). Concurrent infections such as parainfluenza type III virus and *Haemonchus contortus* have also been shown to predispose animals to this disease (Zamri-Saad *et al.*, 1994).

Under the influence of stress factors, steroids are released into the circulation leading to immunosuppression (Chiang *et al.*, 1990). Thus, pathogenic organisms such as *Mannhaemia haemolytica* are able to proliferate in the upper reapiratory tract. A great number of these

organisms are inhaled into the lungs (Gonzalez and Maheswaran. 1993), colonize in the lungs (Shewen, 1994) leading to severe fibrinous pneumonia (Jericho, 1985) and death.

Vaccination including the use of imported vaccines has commonly being used to control the occurrence of this disease with limited success (Wan Mohamad *et al.*, 1988; Zamri-Saad *et al.*, 1989c). Although an improved locally produced oil adjuvant vaccine has been shown to increase antibody response (Zamri-Saad *et al.*, 1993a) and provided satisfactory protection (Chandrasekaran *et al.*, 1991; Zamri-Saad *et al.*, 1993b), it is less popular due to its high viscosity leading to lameness in approximately 10% of the herd (Jamaluddin, 1993). Recently, a new pasteurella spray vaccine was developed, which is easily administered and provides good protection against experimental challenge (Effendy *et al.*, 1998a; b; c). However, the protective duration was short and limited to only 12 weeks (Zamri-Saad *et al.*, 1993a).

It is generally believed that the antibody response by vaccinated animals may be markedly reduced below the protective level in the presence of stressful factors. Steroids such as dexamethasone can be used to determine whether stressful conditions cause failure of animals to respond to vaccination.

Thus, this study was conducted to determine the effect of dexamethasone treatment prior, during and post-vaccination on antibody response and protection against experimental challenge of goats with *Mannhaemia haemolytica*.

MATERIALS AND METHODS

Animals

Sixteen 7-month-old goats were selected and divided into four groups. They were kept separately and were given cut grass and supplemented food at the rate of 700g/goat/day. Drinking water was provided *ad libitum* throughout the study.

Prior to the start of the experiment, nasal swabs were collected from all goats twice a week for bacteriological isolation to ensure that all of them were free of Pasteurella haemolytica for at least 2 weeks.

Preparation of formalin-killed Mannhaemia haemolytica A2 vaccine

Thirty uniform-sized colonies of *Mannhaemia haemolytica* A2 isolated from pneumonic lung were inoculated into 50ml of Brain Heart Infusion (BHI) broth and incubated at 37°C for 24 hours. The broth was serially diluted using peptone water (-1 to -10) before 100μl of each dilution was streaked onto blood agar and incubated at 37°C for 24 hours. Standard Plate Count (SPC) method was used to determine the bacterial concentration before the concentration was re-adjusted to give a final concentration of 2.3×10^6 cfu/ml. The bacteria was then killed using 10% buffered formalin to a final concentration of 0.5%.

Preparation of inoculum for experimental challenge

The procedure of growing *Mannhaemia haemolytica* A2 in the BHI and the determination of bacterial concentration (cfu) using the SPC method as described above were carried out for the preparation of the vaccine. The bacteria cells were not killed and final concentration of this inoculum was re-adjusted at a concentration of 10^9cfu/ml.

Preparation of coating antigen for ELISA

The growth and incubation procedures of *Mannhaemia haemolytica* A2 were as described earlier, except that the organisms were killed by boiling at 97°C for 20 minutes. This was followed by washing 3 times in phosphate buffered solution (PBS) by centrifugation at 12,000 rpm for 15 minutes and the cells were re-suspended in PBS coating buffer. The final product was dispersed into several Eppendorf tubes and kept at -20°C to be used in ELISA procedure.

Table 1. Summary of treatments

Group	Dexamethasone injection	Vaccination
1	Before first vaccination	Double dose of formalin-killed vaccine at 1 week interval
2	During vaccination (at the interval between first and booster dose)	Same
3	After booster dose of vaccination	Same
4	Not given at all	Same

Experimental design

Table 1 summarises the experimental design. At the start of the experiment, goats in group 1 were injected intramuscularly with dexamethasone at a rate of 1mg/kg for three consecutive days. Three days after the last dexamethasone injection, all goats were vaccinated intranasally with formalin-killed *Mannhaemia haemolytica* A2. One week after the vaccination, goats in group 2 were similarly injected with dexamethasone for three consecutive days. The booster dose of the vaccine was similarly administered to all goats 1 week after the initial vaccination. One week after the second vaccination exposure, goats in group 3 were injected intramuscularly with dexamethasone at the same dosage for three consecutive days. Goats in group 4 remained vaccinated without dexamethasone injection (control). Serum samples were collected from the jugular vein of all goats prior to the start of the experiment and at weekly intervals thereafter.

One week after the second vaccination, all goats were challenged with 4 ml inoculum containing live *Mannhaemia haemolytica* A2 at a concentration of 10^9 cfu/ml. Clinical signs of respiratory tract infection (nasal discharge, body temperature exceeding 40.5°C, coughing and respiratory difficulties) were monitored daily and scored according to Zamri-Saad *et al.* (1996).

At the end of two week post-challenge, all goats were slaughtered. The respiratory systems were examined and the extent of pneumonic lung lesions was determined according to Gilmour *et al.* (1983).

The serum samples were subjected to enzyme-linked immunosorbent assay (ELISA) to determine the antibody levels to *Mannhaemia haemolytica* A2 according to the technique described by Puspa (1997) and Effendy *et al.* (1998c).

Enzyme-linked immunosorbent assay (ELISA)

The ELISA was carried out according to the method described by Puspa (1997) and Effendy *et al.* (1998c). The plates were coated with antigen and incubated either at 37°C for 1 hour or at -4°C for 18hours prior to incubation with serum (1:40 dilution) and the rabbit anti-goat IgG conjugate (1:8000). The results were recorded at optical density (OD) at 405 nm wavelength.

Statistical analysis

The differences in the antibody response between groups were determined by means of One-Way ANOVA and Duncan Multiple Range Test, while the correlation between the extent of lung lesions and the antibody responses was determined by means of correlation coefficient. All the data were tested at 5% level of significance.

RESULTS

Antibody response

Generally, all groups except group 1, showed good antibody responses after vaccination against *Mannhaemia haemolytica* A2 (Figure 1). Prior to the vaccination, all groups showed low antibody levels against *Mannhaemia haemolytica* A2 but the antibody levels in groups 2, 3 and 4 increased significantly (P<0.05) following vaccination.
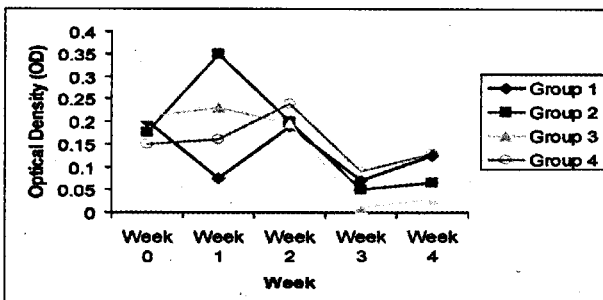
Figure 1: Antibody responses following vaccination against pneumonic pasteurellosis using spray vaccine

Following injection of dexamethasone for three consecutive days from day 0 in group 1, the antibody levels were significantly (p<0.05) decreased at week 1 before returning to pretreatment level at week 2. Group 2 showed a significantly (p<0.05) increased antibody level at week 1 following first vaccination. However, the antibody levels were decreased significantly (p<0.05) from the initial pretreatment level at week 2 following dexamethasone injection just prior to the administration of the second vaccination at the end of week 1. Similarly, group 3 responded well to the initial vaccination at

week 1 but returned to the insignificant level of pretreatment at week 2. Following dexamethasone injections and challenge by *Mannhaemia haemolytica* A2 at the end of week 2, the antibody level decreased significantly (p<0.05). The control vaccinated group showed significantly (p<0.05) increasing antibody level following first and second vaccinations at weeks 0 and 1. Thus, the vaccinated control group was the only group that had a significantly (p<0.05) higher antibody level at the time of challenge with live *Mannhaemia haemolytica* A2 at week 2.

Following challenge with live *Mannhaemia haemolytica* A2 at week 2, all groups showed significantly (p<0.05) decreased levels in their antibody levels at week 3. There were signs of antibody level recovery, particularly in groups 1 and 4 at week 4 prior to slaughter.

Gross lung lesions

All goats were slaughtered on day 14 post-challenge (week 4). Approximately 66% of the goats had gross lung lesions typical of pneumonic pasteurellosis. The affected parts of the lung appeared dark red and firm with distinct demarcation between the lesions and the normal lung areas (Plate 1). Most lesions involved the anterior and ventral parts of the lungs (Plate 2). The severity of the lung lesions produced in infected goats is summarised in Table 2.

Table 2. The average lung lesion score for groups of goats receiving dexamethasone injections at different times

| Group	% Lung lesions
1	18.5
2	45.0
3	7.75
4	5.0

Correlation coefficient

Correlation coefficient was used to determine the correlation between antibody response and the lung lesion score. Overall, the results indicated that there was strong correlation (r = 0.91). Goats with high antibody response following vaccination showed less severe lung lesions while goats under a stressful condition that had low antibody response, had more severe lung lesions.

DISCUSSION

The results indicate that double intranasal exposures to formalin-killed *Mannhaemia haemolytica* A2 stimulate good antibody response in goats. Puspa (1997) has documented that the intranasal exposures stimulate higher systemic antibody response compared to other

Plate 1: The lung of a vaccinated goat challenged with Mannhaemia haemolytica A2 following dexamethasone treatment s howing extensive areas of pneumonia.
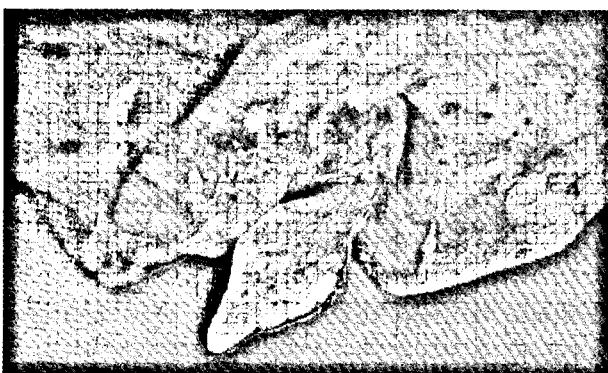
Plate 2: The lungs of a vaccinated goat challenged by Mannhaemia haemolytica A2 following dexamethasone treatment showing consolidation of the antero-ventral lobe.

type of vaccination and it also protects the goats from experimental challenge.

Generally, all groups had an average low antibody level prior to vaccination. This is probably due to the previous natural exposures to Mannhaemia haemolytica. After the first vaccination, all groups showed an immediate increase in the antibody titer except for group 1, which received dexamethasone injection prior to the vaccination. Therefore, dexamethasone injections prior to vaccination in this study significantly decreased the initial antibody level. Similar results were observed following administration of dexamethasone during (group 2) or after (group 3) vaccination. Dexamethasone has been known to produce an immunosuppressive effect on animals by suppressing neutrophil function and anti-microbial activity of alveolar macrophages (Jasni et al., 1991). It is believed that, like dexamethasone, stressful conditions compromise the defense mechanism of the respiratory tract, allowing proliferation and persistence of Mannhaemia haemolytica in the nasal cavity (Effendy, 1998), causing the loss of epithelial cilia, erosion and rhinitis. These lesions have been recognised as key factors in the pathogenesis of respiratory tract infection in animals (Baskerville, 1981). Hence,

animal exposed to several factors are more likely to develop the disease.

The results revealed a strong correlation between antibody level and the extent of lung lesions. Similar observations have been reported earlier (Zamri-Saad et al., 1996). Thus, dexamethasone-treated animals developed lower levels of antibody and eventually produced a much more severe pneumonia than those without dexamethasone treatment (Chiang et al., 1990). Gross lung lesions typical of pneumonic pasteurellosis were determined and scored according to Zamri-Saad et al. (1996). Results of the study indicated that double intranasal exposures to formalin-killed Mannhaemia haemolytica A2 significantly reducing the severity of lung lesions. It also revealed that vaccination during stressful conditions (group 2) resulted in most severe lung lesions compared to other groups. This is because administration of dexamethasone post-vaccination probably has yet to produce a maximum impact on antibody level (group 3). However, vaccinating animals prior to, during or post-stressful conditions generally leads to a failure of the host to develop protective antibody level against challenge with live Mannhaemia haemolytica.

In summary, it has been demonstrated that double intranasal exposures to formalin-killed Mannhaemia haemolytica A2 was able to stimulate good antibody response in goats. Dexamethasone had significantly reduced the ability of the respiratory tract to respond to the intranasal exposures to Mannhaemia haemolytica A2. Thus, a vaccination programme will not be efficient if carried out prior to, during or immediately post-stressful conditions.

REFERENCES

Bahaman, A.R., Nurlida, A.B., Sheikh-Omar, A.R., and Zamri-Saad, M. (1991). Biotypes and serotypes of Pasteurella haemolytica and their importance in the production of vaccine for pneumonic pasteurellosis in sheep. J. Vet. Mal. 3:33-35

Baskerville, A. (1981). Mechanisms of infection in the respiratory tract. New Zealand Vet.J. 29:235-238.

Chandrasekaran, S., Hizat, K., Zamri-Saad, M., Johara, M.Y. and Yeap, P.C. (1991). Evaluation of combined Pasteurella vaccines in control of sheep pneumonia. Br. Vet. J. 147:437-443.

Chiang, Yu-Wei, Roth, J.A. and Andrews, J.J. (1990). Influence of recombinant bovine interferon gamma and dexamethasone on pneumonia attributable to Haemophilus somnus in calves. J. Vet. Res. 51:5.

Dungworth, D.L. (1985). Pathology of Domestic Animals, Vol. 2. London: Academic Press.

Effendy, A.W.M. (1998). The role of the respiratory mucosal immunity in protection against *Pasteurella haemolytica A2* infection in goats. PhD. Thesis, FPV. Universiti Putra Malaysia.

Effendy, A.W.M., Zamri-Saad, M., Maswati, M.A., Ismail, M.S. and Jamil, S.M. (1998a). Stimulation of the bronchus-associated lymphoid tissue in the lungs of goats via oral administration of *Pasteurella haemolytica A2*. The 10th VAM Scientific Congress. pp.136-138.

Effendy, A.W.M., Zamri-Saad, M., Mohamad M., Omar, H. (1998c). Vaccination trial against pneumonic pasteurellosis in sheep using a newly developed pasteurella spray vaccine. *J. Vet. Mal.* 10: 17-20.

Gonzalez, C.T. and Maheswaran, S.K. (1993). The role of induced virulence factors produced by *Pasteurella haemolytica* in the pathogenesis of Bovine pneumonic pasteurellosis: review and hypothesis. *Br. Vet. J.* 149: 183-189.

Jamaluddin, R. (1993). Country reports: Malaysia. In: ACIAR Proceedings No 43, ed. Patten, B.E, Spencer, T.L., Johnson, R.B., Hoffman, D. and Lehane, L. pp 238-239.

Jasni, S., Zamri-Saad, M., Nurida A.B., Sheikh Omar, A.R. (1991). Experimental infection of dexamethasone-treated goats with *Pasteurella haemolytica A2*. *Br. Vet. J.* 147: 565-568.

Jericho, K.W.F. (1985). Histological changes in lungs of calves exposed to an aerosol of *Pasteurella haemolytica*. *J. Comp. Path.* 101:87-97.

Jubb, K.V.F., Kennedy, P.C. and Palmer, N. (1985). In: Pathology of Domestic Animals, Vol. 2, p.487. London: Academic Press.

Mohamad, M., Quirie, M. and Donachie, W. (1993). Prevalence of *Pasteurella haemolytica* serotypes isolated from ovine and caprine pasteurellosis in Malaysia. Proc. 16th MSAP Conference. 126-127.

Puspa, R. (1997). Serum antibody responses following either intranasal or intramuscular vaccination against pneumonic pasteurellosis in goats. Final year thesis. FKVSP.

Shewen, P.E. (1994). Host response to infection with HAP: implications for vaccine development. Abstract book of HAP94 International Conference, Edinburgh. pp.32-33.

Wan Mohamed, W.E., Mohamed, N. and Abdul Rahman, A. (1988). Proc. Sheep Symp. Mal., pp. 87-98.

Zamri-Saad, M., Sharif, H. and Basri, K. (1989a). Microbiological and pathological evaluation of vaccination against naturally occurring caprine pasteurellosis. *Vet. Rec.* 124: 171-172.

Zamri-Saad, M., Ismail, M.S., Norizah, A., Bahaman, A.R. and Sheikh-Omar, A.R. (1989b). Evaluation of an oil adjuvant vaccine for control of pneumonic pasteurellosis in sheep. *J. Vet. Malaysia* 1: 91-92.

Zamri-Saad, M., Arifah, A.K., Norizah, A., Salim, N., Ismail, M.S. and Sheikh-Omar, A.R. (1993a). detection of antibody response by enzyme-linked immunosorbent assay (ELISA) following vaccination against pneumonic pasteurellosis in sheep. *J. Vet. Mal.* 5:41-43.

Zamri-Saad, M., Ismail, M.S., Norizah, A., Bahaman, A.R., and Sheikh-Omar, A.R. (1993b). In:ACIAR Proceedings No 43, ed. Patten, B.E, Spencer, T.L., Johnson, R.B., Hoffman, D. and Lehane, L. pp.177-179.

Zamri-Saad, M., Norizah, A., Bahaman, A.R. (1993c). Serological status of pneumonic pasteurellosis in sheep in seven farms. Proceedings of the 5th VAM Congress, 29-30.

Zamri-Saad, M., Effendy, A.W.M., Maswati, M.A., Salim, N. and Sheikh-Omar, A.R. (1996). The goat as a model for studies of pneumonic pasteurellosis caused by *Pasteurella multocida*. *Br. Vet. J.* 152:453-458.