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VACCINATION OF SHEEP AGAINST PNEUMONIC PASTEURELLOSIS USING A NEW SPRAY VACCINE

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SUMMARY

A vaccination trial was carried out in an established sheep farm using a newly developed pasteurella spray vaccine. Fifty animals of age ranging from 1 to 3 years old in group 1 were vaccinated by spraying intranasally with 1 mL of the spray vaccine while another 50 animals in group 2 were vaccinated subcutaneously with 3 mL of pasteurella alum precipitate vaccine. Vaccination of group 1 was repeated after 2 weeks while group 2 after 4 weeks. Fifty animals of group 3 were unvaccinated control. Serum samples were collected prior to and at two-weekly intervals for a period of 4 months and subjected to enzyme-linked immunosorbent assay (ELISA) to determine the antibody levels. The lungs of dead animals were submitted for bacteriological examinations. Prior to the trial, the antibody levels in all three groups were low. Following vaccination, the antibody levels increased steadily and significantly in both vaccinated groups, and reached the peak level at week 8 post-vaccination before they declined to become significantly low at week 12 post-vaccination. The antibody levels dropped to the original prior vaccination level at week 14 post-vaccination. Re-vaccination at week 14 significantly increased the antibody levels, which lasted until the end of the study period at week 18. Stress due to haemonchosis at week 4, however, drastically reduced the antibody levels in all groups, causing 13% of the animals, all of which were from the control group except one from the alum vaccinated group, to succumb to pneumonic pasteurellosis. The results indicated that the herd must be re-vaccinated, either with spray or alum vaccine, at 12-week intervals to maintain a protective herd immunity.

Keywords: Pneumonic pasteurellosis, spray vaccine, sheep

INTRODUCTION

Pneumonic pasteurellosis is one of the most common respiratory diseases of sheep and goats throughout the world. It is usually caused by *Pasteurella haemolytica* type A although *Pasteurella multocida* types A and D have also been associated with the disease. In Malaysia, 70% of the reported pneumonic pasteurellosis of sheep and goats has been associated with *P. haemolytica* type A (Jamaludin, 1993), and *P. haemolytica* serotype A2 is the most commonly (30%) isolated serotype from pneumonic lungs. Vaccination has been used widely all over the world to control the occurrence of this disease but the protection afforded by several commercial pasteurella vaccines available in this country to control the disease is questionable (Wan Mohamed *et al.*, 1988; Zamri-Saad *et al.*, 1989). Several factors have been associated with vaccination failure such as the route of administration, the antigen and the adjuvant used in the vaccine (Bahaman *et al.*, 1991; Mosier, 1993). It is postulated that one of the major factors that resulted in the failure of vaccination in sheep farms is due to improper vaccination programme for the sheep and goat farms (Zamri-Saad, 1996). This report describes the pattern and duration of antibody response and its effect on the disease incidence following vaccination of sheep with a newly developed pasteurella spray vaccine.

MATERIALS AND METHODS

Animals

One hundred and fifty clinically healthy sheep between 1 to 3 years old were selected from an established sheep farm. The animals were kept integrated under coconut plantation and there was no history of vaccination against pneumonic pasteurellosis even though incidences of the disease have been reported to occur endemically.

The selected animals were divided into three groups of 50 animals per group. The groups were kept together and managed according to the normal practice of the farm. The animals were allowed to graze on native undergrowth of the coconut plantation during daytime and were kept in sheds at night. While in the sheds, the sheep were fed supplemented feed at the rate of 500 g per animal while drinking water was available *ad libitum*. Anthelmintic (Ivermectin, Merck) was administered according to the manufacturer's

recommendation whenever necessary to control internal parasitism.

Vaccine preparation

The intranasal pasteurella spray vaccine was prepared according to Effendy *et al.* (1998). Briefly, *P. haemolytica* A2 isolated earlier from pneumonic lungs of a goat (Zamri-Saad *et al.*, 1994) was selected from the stock culture, subcultured onto blood agar and incubated at 37°C for 24 hrs. Thirty approximately similar-sized colonies were then inoculated into 50 mL brain-heart infusion broth and incubated at 37°C for 18 hrs before the number of colony forming units (cfu) were determined by total plate count. The bacteria were killed with 0.5% formalin, centrifuged at 10,000xg for 20 min and re-suspended in phosphate buffered saline (PBS) to a final concentration of $2.3x10^5$ bacteria/mL. The vaccine was kept at 4°C until used.

The pasteurella alum precipitated vaccine was prepared at the Veterinary Research Institute, Ipoh according to the method of Chandrasekaran *et al.* (1991) and was administered as recommended. The vaccine contained *P. haemolytica* A7 and *P. multocida* types A and D.

Experimental design

Sheep in group 1 were sprayed intranasally with 1 mL of the formalin-killed pasteurella broth vaccine containing *Pasteurella haemolytica* A2 (Effendy *et al.*, 1998). A booster dose of 1 mL vaccine was repeated two weeks later. Sheep in group 2 were vaccinated subcutaneously with 3 mL of the locally prepared alum precipitate pasteurella vaccine (Chandrasekaran *et al.*, 1991). A booster dose of 3 mL was given 4 weeks later. Sheep in group 3 remained as unvaccinated control. Re-vaccinations of the respective groups were carried out when the antibody levels reached insignificant levels when compared to the unvaccinated control.

Serum samples were collected from all animals prior to and at two-weekly intervals post-vaccination and were subjected to the enzyme-linked immunosorbent assay (ELISA) (Zamri-Saad *et al.*, 1993a) to determine the antibody response. During the entire 18-week trial period, post-mortem examinations were carried out on every dead sheep from the three experimental groups. The lungs were examined for gross lesions of pneumonic pasteurellosis and its extent was determined according to Gilmour *et al.* (1983). The right apical lobe, either with or without lesion was collected for isolation of *P. haemolytica*.

Statistical analysis

The data on serum antibody levels and the incidence of pneumonic pasteurellosis were analysed using student's t test.

RESULTS

Serological response

The average antibody level in all three groups prior to the trial was similar. Following the initial exposure, the antibody level in the two vaccinated groups increased significantly ($p<0.01$) when compared with those of control (Fig. 1).

The antibody level was further enhanced following the booster dose to reach the peak level at 8 weeks post-vaccination, during which time the antibody levels in both vaccinated groups were significantly ($p<0.01$) higher than that of the unvaccinated control. The antibody levels started to decline and the difference between the three groups became insignificant ($p>0.05$) at 12 weeks post-vaccination. Further drop in the antibody response to the initial pre-vaccination level ($p>0.05$) was detected at week 14 post-vaccination. Re-vaccination at week 14, however, effectively boosted up the antibody response to highly significant ($p<0.05$) levels, which remained high until the end of the 18-week study period. In general, the average antibody level stimulated by the spray vaccine was slightly lower than the level stimulated by the alum precipitate vaccine but the difference was insignificant ($p>0.05$) (Fig. 1).

At 4 weeks post-vaccination, when the herd was suffering from clinical haemonchosis, the antibody level in the three groups dropped below the initial pre-vaccination level (Fig. 1).

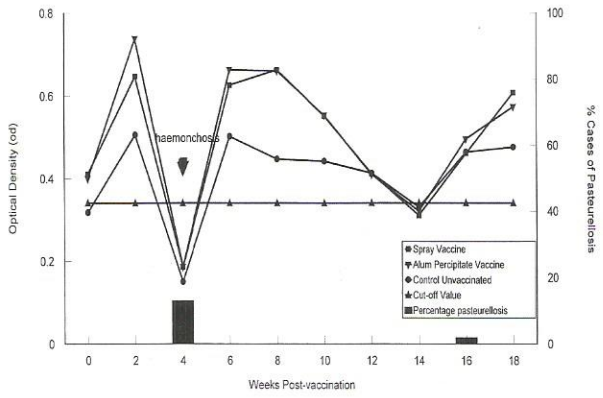

Fig. 1. Antibody responses by the different groups of sheep following vaccinations against pneumonic pasteurellosis

Incidences of pneumonic pasteurellosis

Eight animals in the control group died during week 4 post-vaccination with 5 (10%) animals showing lesions of pneumonic pasteurellosis. One more control animal died of pneumonic pasteurellosis on week 16.

An average of 30% of lung area had lesions of pneumonic pasteurellosis. Three animals in the alum precipitate vaccine group died with 2 (4%) showing lesions of pneumonic pasteurellosis affecting approximately 5% of the lung area (Fig. 1). Two animals in the group vaccinated with spray vaccine died but none showed pneumonic lesions.

Bacteriology

P. haemolytica was isolated from all lung samples with pneumonic lesions.

DISCUSSION

The antibody levels prior to and at the start of vaccination trial was low, thus the animals were susceptible to the disease. Similar low antibody level had been reported earlier in several sheep farms in Malaysia (Zamri-Saad *et al.*, 1993b). The low antibody level in this farm could be due to the fact that the animals were not exposed to any pasteurella vaccine in the last two years, thus predisposing the animals to outbreak of pneumonic pasteurellosis.

Following vaccination, the vaccinated animals in groups 1 and 2 showed high antibody response. This probably leads to the increased resistance and lowered incidence of the disease. The antibody response increased in the vaccinated groups following booster dose, thus protecting the animals from infection. The antibody levels in the control group, however, were low and declining.

At week 4, however, an outbreak of clinical haemonchosis was reported in the farm which killed eight animals and causing severe stress. As a result, the antibody response, particularly in vaccinated groups dropped markedly and significantly below the initial pre-vaccinated level. The animals were more susceptible to infection by *Pasteurella haemolytica* as observed in the alum and the unvaccinated control groups that succumbed to pneumonic pasteurellosis. Stressful conditions such as concurrent haemonchosis have been shown to predispose animals to pneumonic pasteurellosis (Zamri-Saad *et al.*, 1994) due to the increased serum steroid level that lead to immunosuppression (Collins and Suarez-Guemes, 1985).

The peak antibody levels were observed at week 8 post-vaccination. The antibody levels started to decline, became significantly low at week 12 post-vaccination and further reduced to the initial pre-vaccinated level at week 14 post-vaccination. The low antibody levels at week 14 post-vaccination, similar to the levels observed at the beginning of the trial, predisposed the animals once again to pneumonic pasteurellosis, probably causing one animal from the control group to die of pneumonic pasteurellosis at week 16. It was clear that re-vaccination, either with spray or alum vaccine, must be carried out at 12-week intervals in order to maintain the high herd immunity against pneumonic pasteurellosis. Many pasteurella vaccines have been produced, but the vaccination regiment for each vaccine is unclear (Zamri-Saad, 1996). Thus farmers vaccinate their herds at irregular intervals when they were free or when there is an outbreak of the disease. Studies on vaccination intervals or vaccination regime against pneumonic pasteurellosis have not been conducted thoroughly, which would contribute to the success of vaccination (Mosier, 1993).

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RINGKASAN

PEMVAKSINAN BEBIRI TERHADAP PASTEURELOSIS PNEUMONIA MENGGUNA VAKSIN SEMBUR BARU

Suatu percubaan pemvaksinan telah dijalankan dalam sebuah ladang bebiri mapan mengguna vaksin sembur pasteurela yang baru dikembangkan. Lima puluh ekor haiwan berumur di antara satu hingga tiga tahun dalam kumpulan 1 telah divaksinkan secara semburan intranasum dengan 1 mL vaksin sembur, sambil 50 ekor haiwan dalam kumpulan 2 divaksinkan secara subkutis dengan 3 mL vaksin mendak alum pasteurela. Pemvaksinan kumpulan 1 diulang selepas dua minggu sambil kumpulan 2 selepas empat minggu. Lima puluh ekor haiwan daripada kumpulan 3 merupakan kawalan bukan tervaksin. Sampel serum dikumpul sebelum pemvaksinan dan pada setiap selang dua minggu seterusnya selama empat bulan, dan ditentukan aras antibodinya melalui assai imunoserap terangkai enzim (ELISA). Peparu haiwan yang mati dikenakan pemeriksaan bakteriologi. Sebelum percubaan, aras antibodi dalam kesemua tiga kumpulan adalah rendah. Berikutan pemvaksinan, aras antibodi meningkat secara berterusan dan tererti dalam kedua kumpulan tervaksin, dan mencapai aras puncak pada minggu 8 pascapemvaksinan sebelum ianya menjadi tererti rendah semula pada minggu 12 pascapemvaksinan. Aras antibodi jatuh pada aras asal sebelum pemvaksinan pada minggu 14 pascapemvaksinan. Pemvaksinan semula pada minggu 14 meninggikan secara tererti aras antibodi, dan ianya kekal tinggi sehingga akhir tempoh kajian pada minggu 18. Bagaimanapun, tekanan disebabkan hemonkosis pada minggu 4 mengurangkan aras antibodi secara mendadak dalam semua kumpulan, menyebabkan 13% daripada haiwan ini, kesemuanya daripada kumpulan kawalan kecuali satu daripada kumpulan tervaksin alum, mati kerana pasteurelosis pneumonia. Hasil kajian menunjukkan gerompok haiwan perlu divaksin semula, sama ada dengan vaksin sembur atau vaksin laum, pada selang 12 minggu untuk mnegekalkan keimunan grompok pelindung.