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Comparison Prior and Post Vaccination of Inactivated Recombinant Vaccine Against Mannheimiosis in Boer Goats Farm in Sabah

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Abstract

The study was conducted to evaluate the efficacy of inactivated recombinant vaccine of *Mannheimia haemolytica* (MH) against naturally occurring mannheimiosis in a breeding Boer goat farm in Sabah, Malaysia. From the inception of Rural Development Corporation (RDC) farm in year 2005, the farm suffered a high incidence of mannheimiosis, one of the major caprine respiratory diseases in Malaysia. In order to reduce the incidence of the disease, the efficacy of the laboratory tested inactivated recombinant vaccine for mannheimiosis (RVM) was carried out on the farm goats. Goats more than 6-months-old were given the vaccine intranasally, followed by a booster dose on day 14, and subsequently a second booster dose was administered at 6 month interval (for two years). The data of goats that died of mannheimiosis were collected based on gross pathology and subsequent bacterial isolation to confirm the diagnosis. The effect of vaccination was evaluated by review of mortality pattern attributable to mannheimiosis during the three year study that spanned between 2006 and 2008. There was significant reduction of incidence and mortality attributable to mannheimiosis in RDC farm with a resultant drop in mortality rate from 10-22 death per month to 3-2 per month after vaccination also 3.71% in 2006 prior to the introduction of the vaccination regime to 0.08% after the introduction in 2008. This is clearly showed that the RVM is suitable to be use on the farm goats.

Keywords: *Mannheimia haemolytica*; Inactivated recombinant vaccine; Boer goats

Introduction

Goats were one of domesticated animal used for food and milk in the world, including Malaysia. In 1960s, many small holders owned 5 to 10 goats in Malaysia, however the number of head has reduced significantly in 1980s due to lack of local goats production and supply. At the same time, the lack of knowledge of rearing and diseases of goats had greatly led to the reduction in number of goats in Malaysia. Since year 2000, the Malaysian government has promoting the agriculture sector, particularly in the animal industry by increasing the number of farm animals through the importation of Boer goats from Australia and South Africa to augment the local supply. The selection of this goat was based on the fast growth rate and excellent carcass qualities; this selection was to enhance the production of meat rather than milk production. With this rare quality, the breed is gradually the most popular breed of meat goat in Malaysia. Although there are had been claims that the Boer goats have high resistance to disease and that they adapt well to hot and dry semi-deserts but this claim has not been elucidated previously in Malaysia.

Pneumonic mannheimiosis is one of the major problems in goats in Malaysia and this has affected RDC which has established a Boer goat breeding farm since year 2005. Various control strategies has been employed but vaccination is still the best alternative to combat or to reduce the incidence of pneumonic mannheimiosis in goats and sheep. Despite the fact that several available *Mannheimia* vaccines which contained *M. haemolytica* A2 are available in the market, the disease still abound possibly due to the poor immunogenicity, antigenicity of *M. haemolytica* A2 [1-4] and the fact that the serotype possesses no cross-reactivity with *M. haemolytica* A7 and A9 [4] or with *M. haemolytica* A1 and A6 [5] which makes vaccination of goats using vaccine containing whole-cell *M. haemolytica* A2 to fail to cross-protect against challenge with live *M. haemolytica* A7 and A9 [5,6].

In order to combat and control this obvious problem of this disease in Malaysia, a vaccine which has potential to provide protection against *M. haemolytica* serotypes A2, A7 and A9 is essential [7]. In search for this, the use of outer membrane proteins (Omps) of *M. haemolytica* that have been shown to be immunogenic could be useful tool in vaccine preparations [8-10]. With the development of a recombinant vaccine for mannheimiosis (RVM) since year 2003 and subsequent satisfactory laboratory evaluation [11], the need for a field evaluation against naturally occurring mannheimiosis is paramount. A trial using this RVM vaccine was conducted in a goat farm in Sabah and the result obtained is hereby presented.

Materials and Methods

Farm history and animal management

The RDC goat farm was established by the Sabah State government to augment the local goat production by importation of Boer goats from Australia. The farm was located at Papar district of Sabah. At the inception in 2005, the total number of animal was 3630 and by 2008, the farm has population of 12908 goats of more than 6 months of age. All animals were kept in the same paddock. The goats were left to graze during daytime and kept in confined areas at night. While in confinement, they were fed supplemented feed at the rate of 300 g/animal/day while drinking water was available *ad libitum*.

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Goats aged 6 month and above were enrolled into this study which spanned between year 2006 and 2008. The emphasis of this investigation was mortality due to pneumonic mannheimiosis, hence mortality pattern and survival rates prior to and after the introduction of vaccination against mannheimiosis were recorded over the period of 2006 and 2008 to assess the impact of vaccination with RVM vaccine against mannheimiosis in this flock.

Preparation of inactivated recombinant vaccine

Briefly, the PCR was used to generate OMP_{31} gene and the sequencing was done to ensure the right gene to be obtained. Merck pETBlue-2 blunt vector was used and propagated in the NovaBlue cells before expressing in Tuner (DE3) pLacI. The overexpression of the proteins was checked by SDS-PAGE and Western immunoblotting. The OMP31 was undertaken by Malaysian patent (PI 20070305) in the name of Universiti Putra Malaysia. The cultures of the recombinant cell prepared were harvested and killed in 0.5% formalin-PBS overnight. This was followed by three times washing in sterile PBS to ensure that the formalin was completely removed. Finally, the recombinant cells were re-suspended in sterile PBS as stock vaccine seed. Adequate amount of sterile phosphate buffer saline (PBS) was added to the stock vaccine seed to give a final concentration of 1.0×10^5 CFU/mL. The sterility of the vaccine was tested by inoculating 0.1 mL of the vaccine onto blood agar followed by incubation at 37°C for 24 h. The vaccine was considered sterile when no bacterial growth appeared on blood agar.

Vaccination, bacterial isolation and post-mortem procedure

The vaccine was given intranasally approximately 1 mL into the nostril of goats. After 2-weeks interval, the booster dose was given again. Then the RVM was repeated after 6-months interval for two years. All goats were observed daily for signs of respiratory infection and death.

Throughout the study, all dead animals were subjected to post-mortem examination, where the entire respiratory tract was the priority. The lung portions with pneumonic lesions were cut, transferred into small sterile plastic bags (9 cm×13 cm) and processed on the same day for isolation of *M. haemolytica* at the Kepayan Bacteriology Laboratory, Department of Veterinary Services and Animal Industry, Sabah.

Mortality pattern

The mortality was noted monthly between year 2006 and 2008. The mortality rate was calculated based on the percentage of mortality due to mannheimiosis and the total number of survivors.

Statistical analysis

The MedCalc ver 11.6.0.0 (Mariakerke, Belgium) was employed by plotting the Kaplan-Meier survival analysis for mortality and survival between the year prior-vaccination and year post-vaccination groups. The mean mortality data was analysed using a one-way ANOVA and further signified in Student-Newman-Keuls test pairwise comparisons. The significant differences were determined at $p<0.05$.

Results

Clinical observations

Throughout the two years observations period, 40% of the animals showed nasal discharge while 30% showed mild to moderate cough prior-vaccination period. Some animals were emaciated and culled. No other respiratory signs were observed during the post-vaccination period.

Bacterial isolations

M. haemolytica were successfully re-isolated from all lungs with the percentage pneumonic lung lesions varying between 5-10%. Standard laboratory practice was used such as the Gram-stain and biochemical tests for identification of the bacterial species. Serotyping was not attempted.

Mortality pattern

The mortality pattern over the period of 2006 and 2008 was presented in figure 1 while the mortality rate in relation to vaccination was presented in figure 2. The percentage mortality per year was presented in table 1. The mortality prior to the introduction of the vaccination had various peaks varying between 10-22 death per month while after the vaccination the mortality dropped marked with occasional peaks varying between 3-2 per month. The highest peak occurred during the peak of the raining season April. The percentage

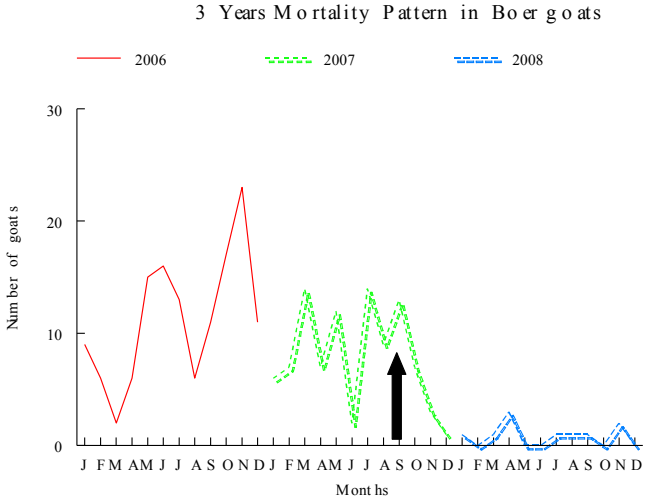

Figure 1: Mortality pattern associated with mannheimiosis prior vaccination (2006/2007) and after vaccination (2007/2008). (⬇Signifies inception of vaccination).

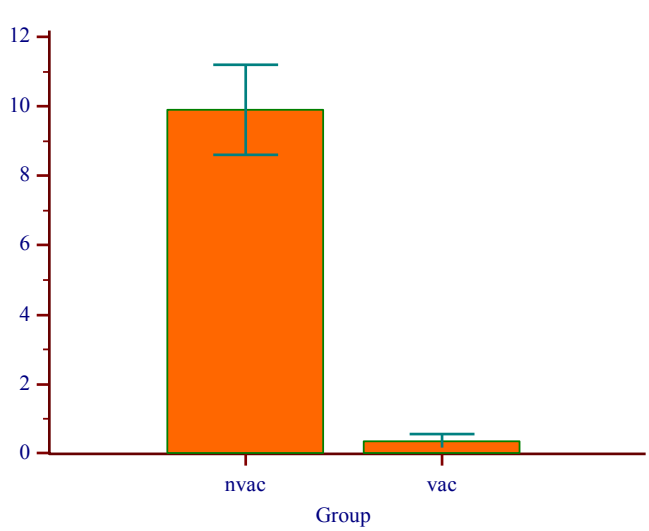

Figure 2: SEM mortality rate of goats due to pneumonic mannheimiosis prior vaccination (2006/2007) and after vaccination (2007/2008).

	2006	2007	2008
No. of mortality	135	95	10
No. of animals	3630	6506	12908
Percentage %	3.71	1.46	0.08

Table 1: Percentage Mortality due to pneumonic mannheimiosis over three years 2006-8.

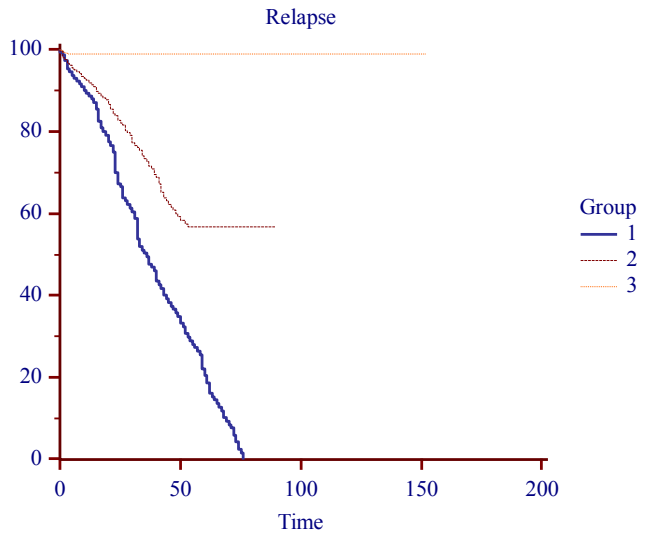

Figure 3: Kaplan-Meier survival curves shows the cumulative mortalities and survivors of goat population of Group 1 (Year 2006), Group 2 (Year 2007) and Group 3 (Year 2008). The vaccination was commenced in Sep 2007 (⬆), followed by a 6-month interval booster dose in January 2008 (▼).

mortality also dropped from 3.71% in 2006 prior to the introduction of the vaccination regime to 0.08% after the introduction in 2008. The mortality rate of unvaccinated animals (Prior to vaccination, 2006–August 2007) was 10.1% while after vaccination (After to vaccination, September 2007–2008), it dropped to 0.42% (Figure 2). The cumulative mortalities and survivors in the goat population for three years of assessment were shown in figure 3.

Discussion

This investigation described the field evaluation of inactivated recombinant vaccine against mannheimiosis using RDC farm in Sabah. This farm that has Boer goats had earlier recorded high mortalities due to pneumonic mannheimiosis prior this intervention despite the use of vaccination programme which utilizes other vaccines [12]. This encouraging result with lower mortality and respiratory signs with this newly developed recombinant vaccine (RVM) against mannheimiosis may possibly be attributed to the intranasal administration which has been reported to elicit a better mucosal or humoral immune response [12,13]. At the same time, the less handling of the goats may possibly reduce stress leading to better uptake of the vaccine and enhanced protection against mannheimiosis.

The vaccine performance may also be due to its ability to induce memory which is particularly evident in the early post-challenge period at week-7 than after the booster dose [10]. The vaccine could be preferred to injectable *Mannheimia* vaccine which abounds in small holder farms in spite of its varying protection [14]. Since the use of injectable vaccines in farms of this magnitude is less practicable, time consuming, increases the labor cost and could be stressful to both animals and operators, the use of intranasal could be found easy and cheap to employ.

The mortalities recorded before and after the commencement of the RVM further elucidate the efficacy of this vaccine and its ability to protect goats from *M. haemolytica* infection. This observation was also reported for a recombinant haemorrhagic septicaemia (HS) in goats in a separate study where the vaccine was reported to protect the goats against *Pasteurella multocida* B: 2 infection [13].

Conclusion

This study re-emphasized that RVM vaccine for mannheimiosis can provide significant protection and reduction in mortalities attributable to mannhemiosis on goat farm. With the ease of application, this third generation of vaccine has been proven to be less time consuming, reducing the labor cost and stress to animals as well as the operators.

Acknowledgments

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