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Comparison of local reaction at injection site following intramuscular administration with three commercial atrophic rhinitis vaccines in pigs

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Abstract

Bordetella bronchiseptica and *Pasteurella multocida* are two main pathogens responsible for atrophic rhinitis (AR), which causes considerable economic losses in swine industry worldwide. Commercial vaccine has been widely used to prevent the damage from AR in Korea. Adverse effects of vaccination at the injection site have been reported, which results in the numerous complaint from farms. However, data on about local reaction at the injection site remains limited. In this study, we compared the local adverse effects of three commercial vaccines following intramuscular injection. The results showed that no gross lesion was founded at the injection sites of all three vaccines. In histopathologic examination, a various level of lesions was identified. Especially, the local reaction of vaccine including saponin as an adjuvant showed the lowest level of histopathological lesions, when compared to those of oil-based and vitamin E-based vaccines. Therefore, this study would provide the information about the extent of local reaction at the injection site and help the farmer to select AR vaccine in order to avoid adverse reaction due to vaccination.

Key words : Atrophic rhinitis, Vaccine, Local reaction

INTRODUCTION

Atrophic rhinitis (AR) is one of complex bacterial respiratory diseases which causes considerable economic losses in swine industry worldwide. The clinical manifestations include nasal discharges and sneezing in early phase, and the atrophy of nasal turbinate, facial deformity and nasal bleeding in pigs. AR is caused by two respiratory bacteria, *Bordetella bronchiseptica* (*B. bronchiseptica*) and *Pasteurella multocida* (*P. multocida*) (Ackermann et al, 1991). Primarily, toxigenic *B. bronchiseptica* attaches nasal mucous membrane, where it colonizes and generates dermonecrotic toxin (Brockmeier et al, 2002). This toxin has effect on nasal turbinate, which results in osteopathy and hypoplastic rhinitis. Secondary infection of *P. multocida*, mainly type D, aggravates the pathological lesions of AR. *P. multocida* produces the potent toxin which induces progressive osteopathy of facial and turbinate bone (Pedersen and Elling, 1984). To reduce damages from AR, many pig farms use both antimicrobial agents and vaccines for a prevention and treatment. Recently, government's policy on antibiotics ban in feed has highlighted the importance of vaccination against two etiological agents.

Vaccine companies have developed new vaccines with various combination of antigens and adjuvants. Most commercial AR vaccines consist of *B. bronchiseptica* bacterins in combination with *P. multocida* bacterins or toxoids as antigen combination (Haesebrouck et al, 2004). For the adjuvant, various substances are used to

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increase the immune response, but adjuvants may act as an irritant to cause local inflammatory reaction at the injection site (Petrovsky, 2015). Especially, local reaction at the injection site such as granuloma and aseptic necrosis has emerged as a major interest since vaccination against FMDV was applied in Korea (Lyons et al, 2016). Severe inflammatory reaction at the injection sits induces the formation of granuloma, which results in the decreased efficacy of vaccine as well as economical loss for farmers due to condemnation of the affected parts (Park et al, 2017). For these reasons, vaccine companies have developed a variety of new types of adjuvants to reduce adverse effects. Therefore, the aim of this study was to compare the local reaction at the injection site following the administration with three different vaccines.

MATERIALS AND METHODS

Vaccines and animal study

The experimental protocol was approved by the Konkuk University Institutional Animal Care and Use Committee (IACUC) with IACUC number KU16181. Three of commercially available vaccines were selected in this study. RHINISENG contains immunogens with saponin as an adjuvant. The vaccine A contains with oil-based adjuvants. The vaccine B uses vitamin E as an adjuvant. Eight of pigs were used in this study. The pigs were randomly selected as similar ages and normal health conditions. The neck muscles behind the ear of the pigs were equally divided into four areas, at which 2 mL of three different vaccines and PBS were administrated intramuscularly. After vaccination, two animals from each group were euthanized at 3 days post-vaccination (DPV), 7 DPV, 14 DPV, and 28 DPV. All pigs were reared in the same pen and marked with plastic ear tag. Tattoo was used to visualize the injection sites.

Gross evaluation of injection site

To evaluate the local reaction, it was determined whether gross lesions were developed at the injection sites after vaccination. The gross lesions included necrosis, bleeding, abscess, cyst, and other abnormal pathological findings.

Histopathological evaluation of injection site

To evaluate the local reaction, histopathological changes of the injection sites were determined. Injection sites were fixed in 10% neutral buffered formalin, and normal H&E staining method was used. Briefly, fixed tissues were washed for 12 to 24 hours and dehydrated using 70 to 100% alcohol. After the clearing tissue using xylene, tissues were embedded in paraffin. 5 μm-sectioned tissues were mounted on slide. The slide was stained with hematoxylin and eosin. After dehydration, the slide was examined using microscopy. Histopathological evaluation was classified into four categories; granulomatous inflammation, infiltration of neutrophils, calcification, and muscular degeneration/necrosis. The score of each category ranged from 0 (no remarkable lesions) to 4 (severe) (Table 1).

Statistical analysis

Statistical analysis of average histopathological scores was performed using Mann-Whitney test in SPSS 21 program.

Table 1. Histopathological scores in the injection site

Scores	Grade	Histopathologic features and distribution
0	None	No remarkable lesions associated with injection
1	Minimal	Focal lesions Small numbers of inflammatory cells in inflammatory lesions
2	Mild	Focal lesions (larger lesions than minimal grade) Moderate numbers of inflammatory cells in inflammatory lesions
3	Moderate	Multifocal to confluent lesions Moderate to large numbers of inflammatory cells in inflammatory lesions
4	Severe	Diffuse lesions Severe diffuse inflammatory reaction

RESULTS

Gross lesion

Pathological lesion including necrosis, bleeding, abscess, and cyst was not observed grossly in all injection site.

Histopathology lesion

Based on histopathological results, an inflammation was observed in muscular tissues or connective tissues between muscles with infiltration of inflammatory cells and cellular necrosis (Fig. 1). Inflammatory cells were mainly round-shape mononuclear cell with large nucleus, and most of them were considered as lymphocytes or histiocytes. In some cases, inflammatory cell and fibroblast started to proliferate, leading to hyperplasia as time went by. These findings were considered as granulomatous inflammation. The infiltration of polymorphic nuclear cells was also observed. In addition, dystrophic calcification was seen in the injection site, where histiocytes and multinucleated giant cells were infiltrated around calcification.

At 3 DPV, the grades of granulomatous inflammation and infiltration of neutrophils at RHINISENG-injected site were higher than those at PBS-injected site (Fig. 2). However, difference of adverse effect scores between RHINISENG and PBS-injected sites was not statistically significant. The scores in vaccine A and vaccine B-injected sites were similar to those at PBS-injected site. At 7 DPV, the scores of granulomatous inflammation and infiltration of neutrophils at RHINISENG-injected site were decreased. However, the higher level of inflammation was observed at vaccine B-injected site than those at RHINISENG and vaccine A-injected sites ($P<0.05$). At 14 DPV, the score of vaccine B slightly reduced, but that at Vaccine A-injected site increased. The level of inflammation at both sites were statistically higher than that at PBS-injected site ($P<0.05$). At 28 DPV, the histopathological lesions were minimal at RHINISENG-injected site and the similar results were obtained at vaccine B-injected site. The mild lesions were observed at vaccine A-injected site, rather than PBS-injected site ($P<0.05$).

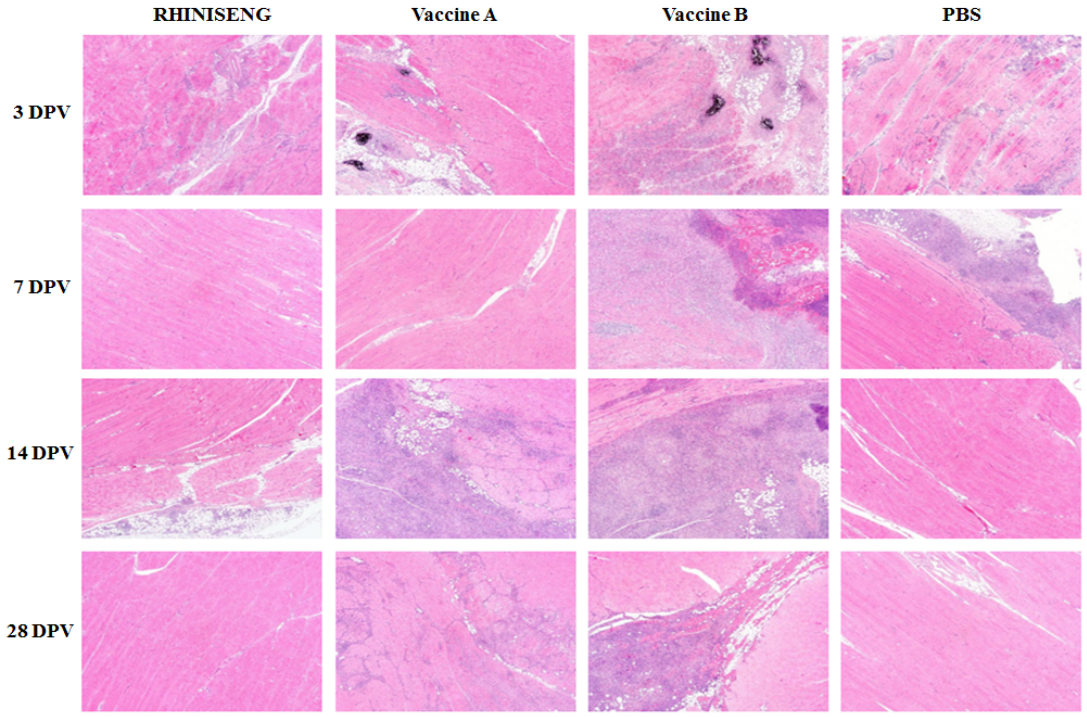

Fig. 1. Histopathology of local reaction at injection sites following three different atrophic rhinitis vaccines and PBS. DPV: days of post-vaccination.

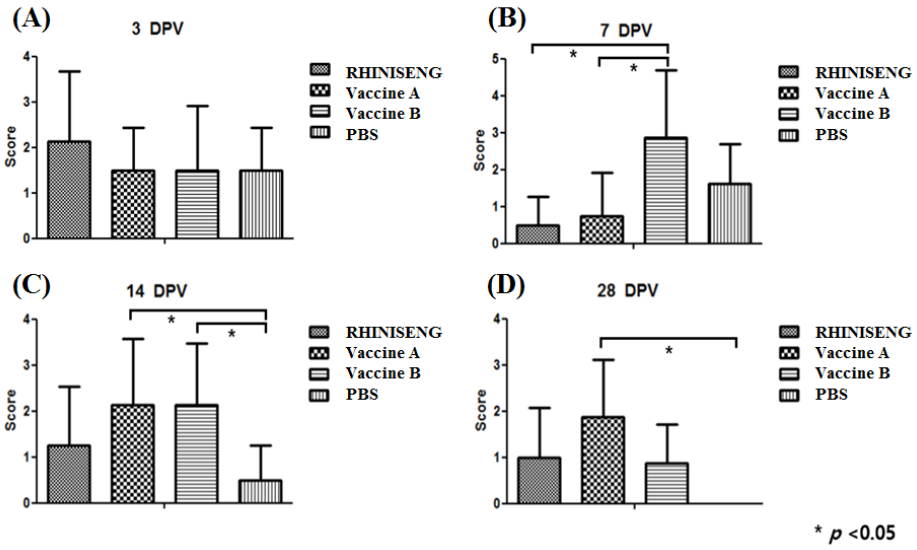

Fig. 2. Comparison of histopathological scores of three commercial atrophic rhinitis vaccines in the injection sites. (A) 3 days of post-vaccination (DPV), (B) 7 DPV, (C) 14 DPV, (D) 28 DPV.

DISCUSSION

Atrophic rhinitis is one of the most important respiratory diseases and results in huge economical losses in swine industry. More importantly, co-infection with other respiratory pathogen induces severe respiratory distress, called porcine respiratory complex syndrome because atrophy of turbinate gives an opportunity for other pathogens to bypass upper respiratory tracts (Opriessnig et al, 2011). For these reasons, many farms take measurements to control and prevent AR with antibiotics and/or vaccines. Vaccination of sows and gilts for passive immunization to new born piglets through colostrum is commonly used strategy to control AR in Korea (Lee and Yoo, 2015). However, intramuscular injection is a common method for the administration of AR vaccine despite the advance of the modern technology. In this study, three vaccines were compared in order to evaluate the local reaction at the injection site. The formulation of RHINISENG is based on aqueous adjuvant, but two other vaccine contains vitamin E or oil adjuvants. The main findings in this study are 1) three AR vaccines did not induce gross lesions at the injection sites and 2) hydrophilic adjuvant-based vaccine induced the lowest level of histopathological lesions, when compared to those of other vaccines.

Up to date, numerous compounds are widely used as adjuvants to stimulate immune response; mineral salts, emulsions, toll-like receptor agonists, cytokines, saponins, and polymers (Burakova et al, 2018). The roles of adjuvant include immunostimulation through antigen presenting cell and/or cytokine, preservation of conformation of antigens, and long-lasting stimulation by slow release. For inactivated vaccine, the selection of adjuvant plays a critical role in determining the efficacy of vaccine because it primarily stimulates humoral immune response (Lee and Nguyen, 2015). Oil-based emulsion is frequently used in animal vaccines because of simple production, cost-effectiveness, and good efficacy (Cox and Coulter, 1997). However, nationwide vaccination with oil-based FMDV vaccine caused massive adverse effect, especially granuloma, at the injection site, which makes farmers be reluctant to use other oil-based vaccines. In this study, all vaccines, including oil-based vaccine did not induce gross lesions. This result postulated that the intramuscular administration with these vaccines does not lead to economic burdens by the formation of abnormal meat. No local adverse effect may impact positively on the farmer's choice on use of AR vaccination, which may contribute successful eradication of AR in Korea. However, because the local reaction was evaluated in 6-week-old piglets, further study in sows and gilts is needed to determine more sophisticated local reaction in the injection site.

There are many causes of local reaction at the in-

jection sits, including organisms introduced with a contaminated needle, live contaminating organisms in the vaccine, adjuvant induced reactions, cytokine release, hypersensitivity reactions (type I, II, III, or IV), trauma, and hemorrhage (Roth, 1999). In histopathology, all tissues exhibited moderate local reaction, including PBS-injected site. This might be attributed to the physical damage by injection needle. When compared between three different AR vaccines, the local reaction at RHINISENG-injected site showed the lowest level of histopathological lesions. In contrast, vaccination of oil-based vaccine resulted in moderate local reaction until 30 DPV. This is because that oil vaccine tends to remain for a long time in the injection site, rather than other aqueous vaccines.

In conclusion, we compared the local reaction of three commercial atrophic rhinitis vaccines at the injection site. No gross lesion was founded, but a various level of histopathological changes was identified.

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