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Short Communication
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Comparison of Two Challenge Models of Atrophic Rhinitis in Piglets

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Abstract

Background: Swine atrophic rhinitis (AR) is a multiple chronic respiratory diseasemainly caused by porcine *Bordetella bronchiseptica* (Bb) and Toxigenic *Pasteurella multocida* (T^+Pm). There are two challenge models using *Bordetella bronchiseptica* and *Pasteurella multocida*. The first model is to treat pigs with *Bordetella bronchiseptica* followed by Toxigenic *Pasteurella multocida* infection. Another model is to inoculate pigs with *Bordetella bronchiseptica* and *Pasteurella multocida* simultaneously. So far, there is no report about comparison of these two challenge models as for their effectiveness to establish infection.

Methods: Thirteen 42 day-old piglets were divided into three groups. Pigs in the first group were challenged with *Bordetella bronchiseptica* followed by Toxigenic *Pasteurella multocida* infection (model 1). Pigs in the second group were challenged with *Bordetella bronchiseptica* and *Pasteurella multocida* at the same time (model 2). Pigs in the third group worked as sterile controls. Clinical symptoms, turbinate lesions, lung lesions and daily bodyweight gain were used as parameters to evaluate the effectiveness of above challenge models.

Results: All challenged piglets showed different degrees of clinical symptoms, turbinate lesions, lung lesions and loss of average daily bodyweight gain. There was no significant difference in clinical symptoms and lung lesions between two challenge models. However, significant differences in turbinate lesion score and average daily gain were observed between these two challenge models.

Discussion and Conclusion: Turbinate lesions score of piglets in first group ranged from 4 to 10 and only 1/5 of piglets had a total turbinate score of 10. By contrast, turbinate lesions score of piglets in the second group ranged from 8 to 16 and 4/5 of piglets had a total turbinate score of equal or above to 10. Therefore, all above data indicated that co-infection of *Bordetella bronchiseptica* and *Pasteurella multocida* was more suitable for to establish AR infection model.

Background

Swine atrophic rhinitis (AR) is a multiple chronic respiratory diseasemainly caused by porcine *Bordetella bronchiseptica* (Bb) and Toxigenic *Pasteurella multocida* (T^+Pm). The clinical symptoms of disease include rhinitis, nasal deformation, nasal bone atrophy and growth performance decline [1].

Vaccination is an effective means of prevention and control of AR. Most of AR vaccines contain antigens of Bb and Pm [2]. According to European Pharmacopoeia, vaccines containing antigenic components of Bb and Pm should be evaluated using a challenge model combined with *Bordetella bronchiseptica* and *Pasteurella multocida* by nasal infection. There are two challenge models combined with *Bordetella bronchiseptica* and *Pasteurella multocida*. One model is to treat with *Bordetella bronchiseptica* followed by Toxigenic *Pasteurella multocida* infection [2-7]. Another model is to inoculate with *Bordetella bronchiseptica* and *Pasteurella multocida* at the same time [8-10]. There was no report about comparison of the effectiveness for above two challenge models. Therefore, in this study, we compared these two types of challenge models on 42 day-old pigs and provided a more suitable evaluation model for piglets after immunization.

Materials and Methods

Bordetella bronchiseptica strain HN8 and type D toxigenic *Pasteurella multocida* strain HB4 were isolated from a herd with clinical AR. Bb strain HN8 was cultured on Bordet-Gengou (BG) agar (Difco, Detroit, USA) at 37 for 40h and was diluted in brain heart infusion (BHI) broth (Difco, Detroit, USA) to give a suspension of about 4×10^9 colony forming units/ml (CFU/ml). Pm strain HB4 was cultured on TSA plate (Difco, Detroit, USA) containing 5% bovine serum at 37°C for 16h and was diluted in brain heart infusion (BHI) broth (Difco, Detroit, USA) to give a suspension of about 4×10^{10} CFU/ml.

Thirteen 42-day-old piglets were divided into three groups with 5 piglets in each challenged groups and 3 piglets in sterile control group. The experimental piglets were excluded from Bb and Pm infections by using RCR [11,12]. Serologically, Bb agglutination antibodies in piglet sera were less than 1:10. The method was described in Pedersen [13].

Piglet sera were also tested to be negative using OXOID PMT Antibody Assay Kit. The above pig animal trials were approved by the Animal Care and Ethics Committee of China National Research Center for Veterinary Medicine.

Piglets in group 1 (model 1) were inoculated with Bb HN8 strain at 1 ml/nostril (2×10^9CFU/ml). Three days later, piglets were inoculated with Pm strain HB4 in the same manner within next consecutive 4 days at 1 ml/nostril (2×10^{10}CFU/ml). Piglets in group 2 (model 2) were received simultaneously the mixed Bb HN8 strain and Pm HB4 strain with same bacterial titers as in model 1. The 2nd and 3rd boosts were preformed every other two days in the same amount of bacteria and inoculation way. Piglets without infection in the 3rd group worked as sterile controls throughout the study.

The inoculated piglets were observed daily for AR clinical signs such as sneezing, cough, wheezing, and eye patches. The body weight of piglets was collected at day 0 and 35 day post-inoculation to calculate daily bodyweight gain. The turbinate bone atrophy (TA), nasal septum deviation (NSD) and lung lesions were examined in a blind manner at necropsy. Turbinate bone atrophy and nasal septum deviation were assessed according to Magyar [2]. The area of lung lesions (%) was assessed according to Hannan et al. [14]. Statistical differences were determined by student-t test (Prism 5.0, GraphPad Software, SanDiego, CA). Differences were considered statistically significant when $P<0.05$

Results

Sneezing, coughing and asthma of piglets were observed after bacterial challenges. However, there was no significant difference in clinical symptoms between group 1 and group 2 as shown by Table 1. At necropsy, lung and turbinate lesion scores of each individual piglet were evaluated as previously described [5]. As shown by Figure 1, all challenged piglets had varying degrees of turbinate lesions. The mean turbinate lesion score of piglets in group 1 was 7.4 ± 2.41 and there was only one pig had score of 10 (Table 1). By contrast, the mean turbinate lesion score of piglets in group 1 was 11.4 ± 2.97 and four out five piglets had score of 10 or above. There were significant differences in turbinate lesion score between group 1 and group 2 ($P<0.05$). As expected, there were no clinical symptoms, turbinate and lung lesions in the non-challenged control. There was no significant difference in clinical symptoms and lung lesions between group 1 and group 2 as shown by Table 1.

The average daily gain of piglets in group 2 was also significantly lower than the piglets in non-challenged group and group 1 ($P<0.05$) by the end of study. The average daily gain of piglets in group 1 was slightly lower than that in non-challenged controls. But the difference was not significant (Table 2).

The different number of asterisks showed significant difference ($P<0.05$) between two groupsBI: before inoculation; PI: post inoculation.

Group	Piglet number	Symptom[a]	Lesion	
			Turbinate	Lung (%)
Group 1	16	2	9	6.8
	17	3	10	20.3
	18	1	6	4.1
	19	2	4	9.5
	66	3	8	10.8
	Mean ± SD	2.2 ± 0.84*	7.4 ± 2.41*	10.3 ± 6.16*
Group 2	5	2	8	10.8
	67	3	11	2.7
	70	3	16	13.5
	79	2	10	6.8
	81	3	12	6.8
	Mean ± SD	2.6 ± 0.55*	11.4 ± 2.97**	8.12 ± 4.15*
Non-challenged Control	6	0	0	0
	7	0	0	0
	8	0	0	0
	Mean ± SD	0	0	0
a				

Table 1: Clinical signs of AR and post-mortal findings.

Group	Piglet number	Pig body weight (kg)		Average daily gain (g)
		BI	PIw5	
Experiment 1	16	12.8	26.3	362.9*
	17	10.1	26.3	
	18	9.7	27	
	19	12.6	24.5	
	66	12	27.5	
Experiment 2	5	9.6	22.1	312.0**
	67	11.3	25.3	
	70	9.8	20.8	
	79	12.2	22.5	
	81	11.1	25.7	
Non-challenged Control	6	11.3	28.1	395.8*
	7	9.8	25.6	
	8	10.8	25.7	

Table2: Mean daily weight gain.

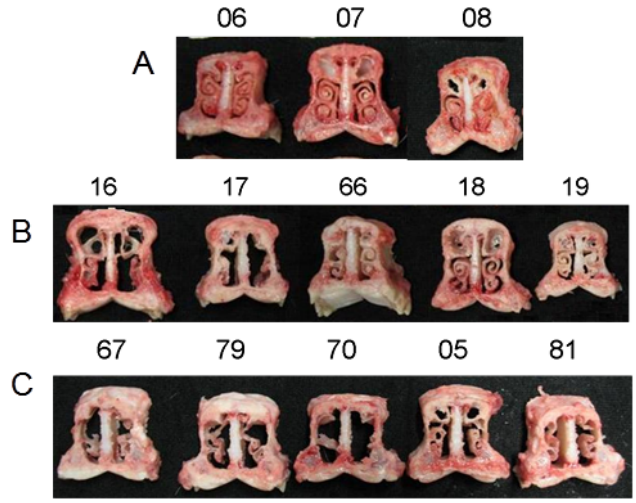

Figure 1: Porcine nasal bone picture in each group obtained at 35 days after challenge. A: Non-challenged group; B: Group1; C: Group 2.

Discussion and Conclusion

The *Bordetella bronchiseptica* treatment followed by Toxigenic *Pasteurella multocida* infection used in group 1 had been tested on 4-day-old, 34-day-old, 2-month-old and 4-month-old piglets with varying degrees of turbinate lesions [2,7,15,16]. Inoculated the *Bordetella bronchiseptica* and *Pasteurella multocida* at the same time used in group 2 had been tested in 6-day-old, 3-week-old, and 6 to 8-week-old piglets with varying degrees of turbinate lesions [8-10]. However, there is no report on the effect of above two kinds of challenge models. The purpose of this study was to compare the two different challenge models on 42 day-old piglets.

In clinical or experimental conditions, AR is often accompanied by clinical symptoms, daily weight loss, turbinate lesions, lung lesions [10,15,17,18]. Both two challenge models in group 1 and group 2 on 42-day-old pigs resulted in varying degrees of clinical symptoms, turbinate lesions, lung lesions and reductions in mean daily weight gain. The turbinate lesions were the main index of AR. In group1, the turbinate lesions score of piglets ranged from 4 to 10 and the mean turbinate score was 7.4. In group 2, the turbinate lesions score of piglets ranged from 8 to 16 and the mean turbinate score was 11.4. The mean turbinate score in group 2 were significantly higher than those in group 1 ($P<0.05$). Toxigenic *Pasteurella multocida* colonization requires Bb pre-infection [19], and younger piglets were sensitive to Bb and were less susceptible as pigs were older [20]. Since piglets in group 1 only had one time of Bb intranasal infection, it was not sufficient to colonize the ensuing toxigenic *Pasteurella multocida*. By contrast, two more boosts of Bb infection were performed on piglets in group 2 which may explain the discrepancy of scores between these two groups.

According to the European Pharmacopoeia, 80% of pigs in the unvaccinated control group have a total turbinate score of at least 10. Based on the above results, the second infection way is more suitable to establish AR disease model on 42-day-old piglets.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgments

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