ROLE OF *Bordetella bronchiseptica* IN THE ATROPHIC RHINITIS CHALLENGE MODEL USED FOR RHINISENG® PRE-CLINICAL EFFICACY TESTING

J. Montané, M. Monells, D. Torrents, R. March, M. Sitjà, A Camprodon

HIPRA, Avda La Selva, 135 - 17170 Amer (Girona) – Spain, daniel.torrents@hipra.com

INTRODUCTION

The current European Pharmacopoeia (EP) monograph for inactivated atrophic rhinitis vaccines containing *Pasteurella multocida* toxin (PMT), with or without cells of *P. multocida* (Pm), must be tested against a toxigenic Pm challenge. Vaccines containing PMT, with or without cells of Pm, and cells or antigenic components of *Bordetella bronchiseptica* (Bb) must be tested against a combined Bb + Pm challenge. No Bb specific challenge test is needed, therefore considering Bb as a predisposing factor for infection with toxigenic Pm strains. The Bb strain used in the combined infection Bb + Pm is not required to be a dermonecrotoxin (DNT) producing strain.

In this work, the *B. bronchiseptica* strain to be used for RHINISENG® pre-clinical efficacy testing was characterized in an *in vivo* model to evaluate its ability to cause atrophic rhinitis lesions by its own.

MATERIALS AND METHODS

Five-day-old piglets were weaned from dams free of antibodies against Bb and PMT and transported to the experimental facilities for the challenge procedure. At 5 and 6 days of age, all piglets were instilled with 0.5 ml of acetic acid in PBS (10 g/L) per nostril. The experimental design of the infection is summarized in Table 1.

Table 1. Experimental design of the infection.

		Inoculation (at days of age)*	
Group	**Pigs/group**	**7 days**	**10 days**
Bb	10	Bb	HI
Pm	10	HI	Pm
Bb + Pm	10	Bb	Pm
Control	10	HI	HI

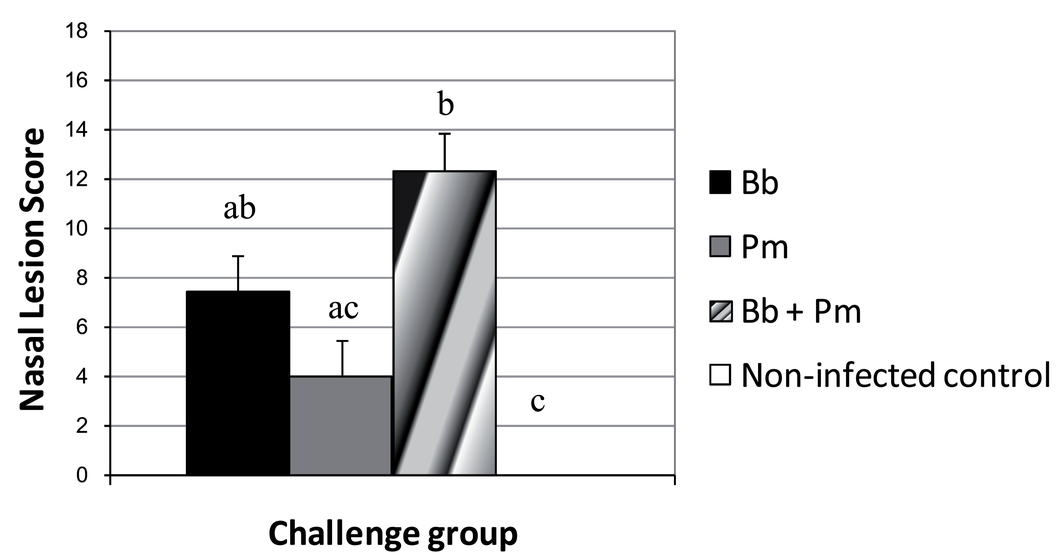
Bb = *B. bronchiseptica*; Pm = *P. multocida*; HI = Heart Infusion broth.
* Each animal received 0.5 ml of the product in each nostril.

The toxigenic Bb strain had been provided by Dr. K.B. Register (Ackermann *et al.*, 1991). The Bb inoculum had a titre of $1{,}2 \cdot 10^8$ CFU/ml in *Bvg*$^+$ phase. The Pm strain was a toxigenic capsular type D strain isolated from the nasal turbinates of a pig. The Pm inoculum had a titre of $1{,}6 \cdot 10^9$ CFU/ml.

At 6 weeks of age, pigs were euthanized and atrophic rhinitis lesions were scored for turbinate bone atrophy and nasal septum deviation from 0 to 18, as described in the EP monograph and by Magyar *et al*. (2002), resulting in a total Nasal Lesion Score (NLS). A One-Way ANOVA was used for statistical comparison ($p<0.05$).

RESULTS

Figure 1. Nasal Lesion Score (Mean ± SEM) in the different challenge groups at 6 weeks of age.



CONCLUSIONS AND DISCUSSION

The toxigenic Bb strain used in the challenge model for RHINISENG® pre-clinical efficacy testing demonstrated its ability to cause atrophic rhinitis lesions by its own, even more than the toxigenic Pm strain used in this study (Figure 1). It has been described that expression of the dermonecrotic toxin by Bb is not necessary for predisposing to infection with toxigenic Pm (Brockmeier *et al.*, 2007), therefore the Bb strain selected for vaccine efficacy testing was more pathogenic than required.

Moreover, the infection dose of Bb used in RHINISENG® studies was higher than the commonly used for reproducing non-progressive atrophic rhinitis (NPAR), where one milliliter of a suspension of $\leq 1 \cdot 10^6$ CFU/ml is administered (Magyar *et al.* 1988, 2002; Brockmeier *et al.* 2002).

REFERENCES

1. Ackermann M. R. 1991. Infect Immun 59: 3626-3629.
2. Brockmeier, S.L., *et al.* 2002. Infect Immun 70: 481-490.
3. Brockmeier, S.L., *et al.* 2007. Vet Microbiol 125: 284-289.
4. Magyar, T. *et al.* 1988. Vet Microbiol 18: 135-146.
5. Magyar, T. *et al.* 2002. Vaccine 20: 1797-1802.

Laboratorios Hipra, S.A.
Avda. la Selva, 135
17170 Amer (Girona)
Spain

Tel (34) 972 43 06 60
Fax (34) 972 43 06 61
hipra@hipra.com
www.hipra.com