

Effect of *Bordetella bronchiseptica* and Serotype D *Pasteurella multocida* Bacterin-Toxoid on the Occurrence of Atrophic Rhinitis after Experimental Infection with *B. bronchiseptica* and Toxigenic Type A *P. multocida*

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ABSTRACT. In efficacy tests, 7 primary specific-pathogen-free piglets vaccinated with the *Bordetella bronchiseptica* and type D *Pasteurella multocida* bacterin-toxoid were challenged with *B. bronchiseptica* and type A *P. multocida*. Severe or moderate nasal turbinate atrophy was produced in the non-vaccinated pigs, whereas, only one of the 4 pigs in the vaccinated group had slight turbinate atrophy. Other immune sera against crude toxin of *P. multocida* type A or D were cross neutralized. The results of the present study show that the *P. multocida* serotype D bacterin-toxoid is effective against atrophic rhinitis caused by toxigenic *P. multocida* serotype A as well as toxigenic *P. multocida* serotype D. — **KEY WORDS:** atrophic rhinitis, bacterin-toxoid, *Pasteurella multocida*.

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The relationship between clinical atrophic rhinitis (AR) of swine and infections with *Bordetella bronchiseptica* and/or toxigenic capsular serotype D *Pasteurella multocida* has been reported by many investigators [2, 3, 23, 27–30, 32, 35]. In addition, it has been reported that capsular serotype A *P. multocida* is isolated predominantly from pneumonic lung in swine [26, 37]. Previous experiments have shown that the dermonecrotic toxin (DNT) of *B. bronchiseptica* and *P. multocida* are virulence factors producing AR [7, 11, 12]. Therefore, the bacterin and/or toxoid [22] containing *B. bronchiseptica* and toxigenic capsular serotype D *P. multocida* were developed, and these vaccines were prophylactic against experimental AR with *B. bronchiseptica* and toxigenic capsular serotype D *P. multocida* [1, 14, 24, 36]. Although there is little information about toxigenic capsular serotype A *P. multocida* associated with AR [4, 10, 13, 33], it has been suggested that the immunological quality of DNT of both serotype A and D *P. multocida* origin is identical by *in vitro* experiments [5, 16], the toxin gene of serotypes A and D being identical [17]. The purpose of this study was to determine the efficacy of a vaccine composed of inactivated bacterial cells of *B. bronchiseptica* and capsular serotype D *P. multocida* and their toxoid. The active immunity of the vaccines was evaluated in piglets infected experimentally with *B. bronchiseptica* and capsular serotype A *P. multocida*.

Seven primary specific-pathogen-free (P-SPF) pigs were used. They were raised in the usual P-SPF breeding manner [20] and each group was housed in a clean pig isolation room. The vaccine marketed as Ingelvac AR4 (Boehringer Ingelheim Animal Health, Inc., Missouri, U.S.A.), containing *B. bronchiseptica*, toxigenic *P. multocida* type D and their toxoid with an aluminium adjuvant, was used. Four pigs were injected intramuscularly with 1 ml of the vaccine at 6 days of age. Another 3 pigs were used as non-vaccinated controls. *B. bronchiseptica* strain S1 which is known to be pathogenic for piglets [31], grown on Bordet Gengou agar (Difco Lab., Detroit, Michigan, U.S.A.) and

containing 10% sheep blood, was suspended homogeneously in phosphate buffered saline (pH 7.2) at 8.2×10^6 colony-forming units (CFU)/ml. Toxigenic capsular serotype A *P. multocida* strain ZF-899 which has been demonstrated to be pathogenic to piglets [33], grown on dextrose starch (DS) agar (Difco Lab.), was suspended homogeneously in Mueller Hinton broth (Difco Lab.) to obtain a suspension containing approximately 1×10^{10} CFU/ml. Challenge of *B. bronchiseptica* was done in a 0.5 ml suspension instilled into each nostril on the post-vaccination week (PVW) 4, and *P. multocida* was done in the same manner as with *B. bronchiseptica* starting from PVW 5 for four consecutive days. The pigs were observed daily for clinical signs of AR. Nasal swab samples were taken from all pigs immediately before vaccination and at regular time intervals after vaccination. For the recovery of *B. bronchiseptica*, samples were plated on MacConkey agar (Eiken Chemical Co., Ltd., Tokyo) containing 25 µg/ml of furazolidone, 0.5 µg/ml gentamicin, 4 µg/ml of fradiomycin and 2 µg/ml of clindamycin, and were incubated for 3 days at 37°C. The samples were also cultured for *P. multocida* using DS agar (Difco Lab.) containing 0.1 µg/ml gentamicin and 30 µg/ml vancomycin for 18 hr at 37°C. Suspected colonies of *B. bronchiseptica* or *P. multocida* were identified using conventional biochemical tests [6, 21]. Blood samples were collected from pigs at the same times as the nasal swab samples were taken. ELISA tests for the detection of antibodies against pure DNT of *B. bronchiseptica* [8, 9] and pure DNT of *P. multocida* serotype D [18] were performed. Each pure DNT antigen showed a single band of nearly 160 kd or 140 kd by sodium dodecyl sulfate-polyacrylamide disc gel electrophoresis. The pigs were sacrificed using an intravenous injection of thiopental sodium (Tanabe Pharmaceutical Co., Osaka) on PVW 7. The snouts were cut transversely between the first molar and the canine tooth, and the turbinate atrophy was recorded as: - = normal, + = slight, ++ = moderate and +++ = severe according to the criteria described by Maeda *et al.* [15].

No clinical signs of AR were recognized in any of the pigs (Table 1). At necropsy, it was found that all of the pigs in the non-vaccinated group showed moderate or severe turbinate atrophy. In contrast, only one of 4 pigs in the vaccinated group had slight turbinate atrophy (Table 1). *B. bronchiseptica* was recovered from all pigs of both groups post-inoculation. *P. multocida* was intermittently recovered from all pigs in the non-vaccinated group, but was not isolated from any pig in the vaccinated group (Table 2). No antibody titers against pure DNT of *B. bronchiseptica* and *P. multocida* type D were detected in any pig sera collected before vaccination, but each of these titers was detected in the serum of each pig after vaccination, and increased after challenge in the vaccinated group (Table 3).

In a cross neutralization test, strain ZF-899 (serotype A) and strain ZF-848 (serotype D) of toxigenic *P. multocida* were used for the production of crude toxin (sonic-extracted sample) as described previously [34]. Both anti-crude toxin sera were produced by SPF guinea-pigs (SLC Co., Shizuoka) with each formaldehyde treated crude toxin adsorbing an alhydrogel. Both antisera and non-immune guinea-pig serum were mixed with a serial dilution of each of the above crude toxins, and left at 37°C for 2 hr with horizontal shaking. The dermonecrotic activity of each sample was detected by the method described by Nakase *et al.* [19]. In the case of mixed with non-immune serum, serotype A crude toxin: diluted 1:64, serotype D crude toxin: diluted 1:32.

On the one hand, In case of mixed with serotype A or D crude toxin and each antisera, both crude toxins :diluted < 1:2, respectively.

It has been resolved that progressive AR is caused by infection with DNT-producing *P. multocida* (serotype A and D strains) [25]. DNT of *B. bronchiseptica* and DNT of *P. multocida* have been recognized as a development of AR [7, 11, 12], and DNT of serotype A and D *P. multocida* origin were identical in an *in vitro* experiment [15, 16]. Various vaccines containing bacterin and/or toxoid of *B. bronchiseptica* and DNT-producing serotype D *P. multocida* are being used worldwide, but these vaccines have been evaluated against experimental AR with *B. bronchiseptica*

Table 1. Clinical signs of AR and post mortal findings

Treatment	No.	Clinical signs of AR	Lesion	
			Atrophy ^{a)}	Pneumonia
Vaccinated	61	No	–	No
	62	No	+	No
	63	No	–	No
	64	No	–	No
Non-Vaccinated	65	No	+++	No
	66	No	+++	No
	67	No	++	No

a) –: Normal, +: Slight, ++: Moderate, +++: Severe.

Table 2. Recovery of the organisms from the nasal cavities in pigs

Treatment	No.	<i>B. bronchiseptica</i>					type A <i>P. multocida</i>			
		BV ^{a)}	PVw4 ^{b)}	PVw5	PVw6	PVw7	BV	PVw5	PVw6	PVw7
Vaccinated	61	–	–	+	+	+	–	–	–	–
	62	–	–	+	+	+	–	–	–	–
	63	–	–	+	+	+	–	–	–	–
	64	–	–	+	+	+	–	–	–	–
Non-Vaccinated	65	–	–	+	+	+	–	–	+	–
	66	–	–	+	+	+	–	–	–	+
	67	–	–	+	+	+	–	–	+	+

–: Negative, +: Positive.

a) BV: Before vaccination.

b) PVw: Post-vaccination weeks. PVw4: Challenge of *B. bronchiseptica*. PVw5: Challenge of *P. multocida* was done for four consecutive days.

Table 3. The serum antibody titers to pure DNT of *B. bronchiseptica* and pure DNT of *P. multocida* serotype D

Antigen	Group	The serum antibody titers ^{a)}				
		BV ^{b)}	PVw4 ^{c)}	PVw5	PVw6	PVw7
Pure DNT of <i>B. bronchiseptica</i>	Vaccinated	0.045	0.207	0.290	0.548	0.594
	Non-vaccinated	0.050	0.070	0.148	0.327	0.360
Pure DNT of <i>P. multocida</i>	Vaccinated	0.009	0.211	0.212	0.397	0.573
	Non-vaccinated	0.007	0.010	0.017	0.083	0.099

a) Geometric mean of ELISA titer (405 nm).

b),c) See Table 2.

and DNT-producing serotype D *P. multocida* [1, 14, 24, 36]. In the present study, piglets were challenged with *B. bronchiseptica* and DNT-producing serotype A *P. multocida*, and consequently all of non-vaccinated group showed severe or moderate nasal turbinate atrophy at slaughter, which has been described in reports on inoculation with *B. bronchiseptica* and toxigenic *P. multocida* serotype D [23, 27–29, 32], but none of the pigs showed signs of AR. It was considered that the experimental period was too short for the development of clinical signs of AR in these pigs.

Under the experimental design of this study, severe or moderate nasal turbinate atrophy was produced in all of the non-vaccinated group pigs, however, only one of the 4 pigs in the vaccinated group had slight turbinate atrophy. Toxigenic *P. multocida* type D bacterin-toxoid is effective against AR caused by toxigenic *P. multocida* serotype A. Serum antibody titers against pure DNT of *B. bronchiseptica* and pure DNT of *P. multocida* type D were produced by piglets after vaccination. Pure DNT of *P. multocida* type D antibody titer was higher in pig sera collected after challenge with toxigenic *P. multocida* serotype A than before challenge in the vaccinated group, and in the non-vaccinated group, pure DNT of *P. multocida* type D antibody titer also rose after challenge with toxigenic *P. multocida* serotype A. *P. multocida* was not recovered from any pig in the vaccinated group post-challenge. In another study, the challenge-organisms were occasionally recovered from vaccinated pigs (unpublished data). It was suggested that the experimental bacterin-toxoid was not completely protective against colonization with *P. multocida*.

In a cross neutralization test, immune sera against crude toxin of *P. multocida* serotype A or D cross neutralized each other for both crude toxins.

The results of the present study show that the *P. multocida* serotype D bacterin-toxoid is effective against AR caused by toxigenic *P. multocida* serotype A as well as toxigenic *P. multocida* serotype D.

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