# Toxigenic Type A *Pasteurella multocida* as a Causative Agent of Nasal Turbinate Atrophy in Swine

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ABSTRACT. Although no clinical signs of atrophic rhinitis (AR) were recognized in 2- and 5-week-old pigs, approximately 60% of 2- to 6-month-old pigs showed clinical signs of AR in an affected pig farm. None of the pigs had normal turbinate at slaughter. *Bordetella bronchiseptica* was not isolated from any of the pigs before onset and incipient stage of the outbreak (2-week to 2-month-old). *Pasteurella multocida* of capsular type D was not isolated from any of those pigs. However, toxigenic *P. multocida* of capsular type A was isolated from a number of the pigs immediately before onset and incipient stage of the outbreak. Thirty-six-day-old primary specific-pathogen-free pigs were inoculated intranasally with a toxigenic type A *P. multocida* isolated from a 5-week-old pig. Severe nasal turbinate atrophy was observed in those pigs which were necropsied at 3 weeks post-inoculation. This is the first report on outbreak of severe nasal turbinate atrophy induced by toxigenic type A *P. multocida* in Japan.—KEY WORDS: atrophic rhinitis, toxigenic type A *Pasteurella multocida*.

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The relationship between atrophic rhinitis (AR) in swine and infections with *Bordetella bronchiseptica* and/or toxigenic *Pasteurella multocida* of capsular type D has been reported by several investigators [4, 5, 16, 17]. Piglets inoculated with *B. bronchiseptica* together with toxigenic type D *P. multocida* developed severer turbinate atrophy than those inoculated with *B. bronchiseptica* alone [3, 4, 16].

It has been reported that a predominance of type A *P. multocida* isolates from pneumonic lungs in swine [15, 20], whereas type D isolates have more often been associated with AR [5, 16, 17]. Although there is a little information about toxigenic *P. multocida* of capsular type A found in association with AR in Europe [6–8], the significance of this toxigenic strains has not been reported in Japan.

The main purpose of the present study was to define development of described lesions of progressive AR [14] in pigs from herds infected with toxigenic type A *P. multocida* in Japan. In addition, the authors attempted to reproduce severe AR in primary specific-pathogen-free (caesarean section-produced, colostrum-deprived: P-SPF) pigs by experimental inoculation with an isolate of type A *P. multocida* alone.

## MATERIALS AND METHODS

### *An affected pig farm*

*Status of farm*: About three-hundred fifty sows were reared on this farm located in Chubu district. Their piglets were weaned 3 weeks after birth, litters were moved to the growing houses 6 weeks after birth, and growers were moved to the finishing houses at 4-month-old.

The sows were injected sequentially with commercially available inactivated *B. bronchiseptica* vaccines; all sows were vaccinated intramuscularly with 10 m*l* of vaccine 3 months and 4 weeks before parturition. The geometric mean (GM) of agglutinin titer in serum of sows against *B. bronchiseptica* reached at 1:3,104. The piglets were nebulized into nasal cavity with kanamycin or oxytetracycline at 0-, 1-, 2- and 3-week-old, and their feed was medicated with oxytetracycline and sulfamonomethoxine up to 35th day after weaning.

Clinical signs of AR such as distortion and/or shortening of the snout have been observed in about 60% of growing and finishing pigs at least for the last 3 years.

*Post mortem findings*: Ten pigs weighing about 105 kg (about 7-month-old) were slaughtered in 1990. Their snouts were cut transversely between the first molar and a canine tooth, and the turbinate atrophy was classified as; − = normal, + = slight, ++ = moderate, +++ = severe, and ++++ = very severe according to the criteria described by Maeda *et al.* [10].

*Isolation of organisms and clinical observation*: Nasal swab samples were taken from ten pigs each five times; two and five weeks, two, four and six months after birth, and all pigs were examined for

presence of clinical signs of AR.

For the recovery of *B. bronchiseptica*, samples of these swabs were placed on MacConkey agar plate (Eiken Chemical Co., Ltd., Tokyo, Japan) containing 25 μg/m*l* of furazolidon, 0.5 μg/m*l* of gentamycin, 4 μg/m*l* of fradiomycin and 2 μg/m*l* of clindamycin, and were cultured for 3 days at 37°C. The nasal swab samples were also cultured for *P. multocida* using dextrose starch (DS) agar (Difco Lab., Detroit, Michigan, U.S.A.) containing 0.1 μg/m*l* of gentamycin and 30 μg/m*l* of vancomycin for 18 hr at 37°C.

Suspected colonies of *B. bronchiseptica* or *P. multocida* grown on agar media were transferred to Bordet-Gengou agar (Difco Lab.) containing 10% sheep blood (BG medium) or DS agar and were identified using conventional biochemical tests [12, 13]. The capsular serotypes of *P. multocida* isolates were identified by the hyaluronidase test [2] and the indirect-hemagglutinion (IHA) test [1]. The dermonecrotic toxin (DNT) activity of *P. multocida* isolates was detected by the method of intracutaneous injection in guinea pig [19].

*Antibody tests*: Blood samples and nasal swab samples were taken simultaneously. Agglutinating antibody titers against *B. bronchiseptica* were determined using the tube-agglutination test with AR antigen (Kitasato, Tokyo, Japan). Serum antibody titer against type A *P. multocida* was examined by the IHA test [18].

*Experimental infection*

*Bacterial strain*: Strain ZF-899 of *P. multocida* isolated from a 5-week-old pig with clinical signs of AR was identified as capsular serotype A and demonstrated to produce the DNT. The organism was grown on DS agar for 18 hr at 37°C. It was suspended in Mueller Hinton broth (Difco Lab.) to obtain a suspension containing approximately $10^9$ colony-forming-units (CFU)/m*l*.

*Inoculation for pigs*: Four P-SPF Landrace pigs (produced in our laboratory) were used. Three pigs, 36 days of age, were instilled with 0.5 m*l* of the bacterial suspension described above through each nostril for 5 consecutive days. Another pig was used as the uninoculated control. The two groups of pigs were housed separately in clean pig chambers.

*Clinical observation and recovery of organisms*: Pigs were observed for their signs and symptoms of AR such as sneezing, distortion of the snout and other abnormalities. Recovery of organisms from nasal swab samples were conducted immediately before inoculation and at intervals of certain time after the inoculation. The materials of recovery of *P. multocida* and *B. bronchiseptica* were examined as mentioned above.

*Necropsy findings*: Pigs were killed at 61 days old (21 days post-inoculation). Of the pigs showing any macroscopic signs of disease, the turbinate atrophy was recorded.

## RESULTS

*An affected pig farm examined*: No clinical signs of AR were recognized in 2- and 5-week-old pigs. In contrast, a number of 2- to 6-month-old pigs had clinical signs of severe AR such as distortion and/or shortening of the snout; 6 out of 10 pigs (2- and 4-month-old), and 7 out of 10 pigs (6-month-old) (Table 1).

None of the pigs had normal turbinates with the filled nasal cavity. Five out of 10 pigs at slaughter showed severe or very severe nasal lesions (Table 2). The pulmonary lesion were present in 9 of these pigs.

*B. bronchiseptica* was not isolated from all of the 2-week, 5-week, and 2-month-old pigs, but was positive in 6 of ten 4-month-old pigs, and 1 of ten 6-month-old pigs. Capsular serotype D strain of *P. multocida* was not isolated from all the pigs.

Although capsular serotype A strain of *P. multocida* was not isolated from all the 2-week-old pigs, it was isolated from many pigs on and after the age of 5-week-old. In addition, DNT-producing *P. multocida* of capsular serotype A was isolated from the most pigs before onset and incipient stage of outbreak (Table 1).

GM of agglutinating antibody titers against *B. bronchiseptica* in the sera was quite variable among pig herds, depending on their ages. The GM of IHA titers against type A *P. multocida* in the sera were 1:2 in 2-, 5-week and 2-month-old pigs, 1:3.2 in 4-month-old pigs, and 1:6.9 in 6-month-old pigs (Table 1).

*Experimental infection*: None of the pigs showed clear clinical signs of AR (Table 3). *B. bronchiseptica* was not isolated from any of the pigs. *P. multocida* was not isolated from any of the pigs before inoculation, but was recovered from all of the pigs inoculated (Table 3).

In regard to necrosis of the pigs, it was found that turbinate of a pig in uninoculated control appeared normal with the filled nasal cavities. All of the

Table 1. Clinical signs of AR, recovery of the organisms from the nasal cavities and serum antibody titers against antigen from *B. bronchiseptica* and type A *P. multocida* in pigs from an affected farm

| Stage (old) | Sampled | Antibody titer[a] B.b[b] | Antibody titer[a] P.m A[c] | Clinical signs of AR[d] | Isolation of organisms[e] B.b | P.m D | P.m A(DNT+)[f] |
|---|---|---|---|---|---|---|---|
| 2-week | 10[g] | 955 | 2 | − 10 | 0 | 0 | 0 |
| | | (≧1,024~512) | (<4) | | | | |
| 5-week | 10 | 256 | 2 | − 10 | 0 | 0 | 5( 5) |
| | | (512~128) | (<4) | | | | |
| 2-month | 10 | 128 | 2 | − 4 | 0 | 0 | 2( 1) |
| | | (256~64) | (<4) | + 6 | 0 | 0 | 5( 5) |
| 4-month | 10 | 20 | 3.2 | − 4 | 4 | 0 | 3( 0) |
| | | (64~8) | (8~<4) | + 6 | 2 | 0 | 1( 0) |
| 6-month | 10 | 20 | 6.9 | − 3 | 1 | 0 | 3( 0) |
| | | (32~8) | (16~4) | + 7 | 0 | 0 | 2( 1) |
| Total | 50 | | | | 7 | 0 | 21(12) |

a) Geometric mean of titer, it was calculated that antibody titer ≧1,024 was converted to 1,024 and <4 to 2.
( ): a range of titer.
b) Agglutinin titer of *B. bronchiseptica* in the serum.
c) Indirect-hemagglutination titer of type A *P. multocida* in the serum.
d) −: Normal, +: Distortion and/or shortening of the snout.
e) B.b: *B. bronchiseptica*, P.m D: type D *P. multocida*, P.m A: type A *P. multocida*.
f) Number of pigs from which dermonecrotic toxin producing strains were isolated.
g) Number of pigs.

Table 2. Clinical signs of AR, and the occurrence of nasal and lung lesion in slaughter pigs from an affected farm

| Number of pigs | | Turbinate atrophy[a] | | | | | Pneumonic lesion | |
|---|---|---|---|---|---|---|---|---|
| Tested | Positive AR signs[b] | − | + | ++ | +++ | ++++ | Negative | Positive |
| 10 | 8 | 0 | 2 | 3 | 3 | 2 | 1 | 9[c] |

a) −: normal, +: slight, ++: moderate, +++: severe, and ++++: very severe.
b) Distortion and/or shortening of the snout.
c) DNT-non-producing strain of type A *P. multocida* was isolated from 7 of 9 pigs.

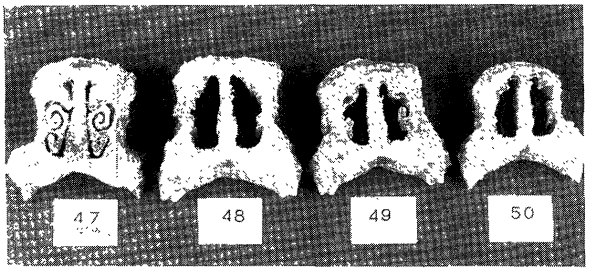


Fig. 1. Resulting turbinate atrophy in pigs given type A *P. multocida* ($10^9$ CFU/day) for 5 consecutive days from 36-day-old; pigs were killed at 57-day-old (see Table 3).

Table 3. Clinical signs of AR, post mortem findings and recovery of the organisms from the nasal cavities in pigs inoculated with type A *P. multocida*

| Group | No. | Clinical signs of AR | Lesion | | Recovery of type A *P. multocida* from the nasal cavities[c] | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Turbinate atrophy[a] | Pneumonia[b] | BI | PIw1 | PIw2 | PIw3 |
| Uninfected | 47 | No | − | − | − | − | − | − |
| Infected | 48 | No | +++ | − | − | + | + | + |
| | 49 | No | +++ | +[d] | − | + | + | + |
| | 50 | No | +++ | − | − | + | + | + |

a) −: normal, +++: severe.
b) −: normal, +: slight.
c) BI: Before inoculation, PIw: Post-inoculation week(s).
−: negative, +: positive.
d) : Type A *P. multocida* (inoculated strain) was recovered from the right lobus medius.

inoculated pigs indicated severe turbinate atrophy (Table 3, Fig. 1).

## DISCUSSION

In an affected pig farm, a number of pigs older than 2 months had clinical signs of progressive AR [14], and more than moderate turbinate atrophy was observed in 8 out of 10 pigs at slaughter. With respect to the recovery of *B. bronchiseptica*, the organism was not isolated from any of the pigs before onset (2- and 5-week-old) and incipient stage of the outbreak (2-month-old), but some of the pigs showed positive at the later stage of outbreak (4- and 6-month-old). However, no macroscopic turbinate atrophy was observed in 13- to 18-week-old pigs inoculated with *B. bronchiseptica* [9]. Type D *P. multocida* was not isolated from any of the pigs. It has been suggested that type D *P. multocida* and *B. bronchiseptica* are not causative agents of progressive AR in this farm.

In contrast, DNT-producing type A *P. multocida* was isolated from a number of the pigs before onset (5-week-old) and the incipient stage of the outbreak (2-month-old). Therefore, it was revealed that progressive AR occurred due to the infection of DNT-producing type A *P. multocida* in this pig farm. However, it was not known why DNT-producing type A *P. multocida* was isolated more frequently from pigs before onset and incipient stage of the outbreak than from those at the later stage in this pig farm.

Nakagawa *et al.* [11] reported that the germ-free piglets were intranasally inoculated with tyep A *P. multocida* (strain Kobe 5) at 8, 11, an 15 days of age, and they had no AR lesions. However, strain Kobe 5 has not shown DNT activity in our experiment (unpublished data). Eilling *et al.* [7] have shown that pre-treatment of the nasal mucosa with 1% acetic acid, followed by inoculation of a DNT-producing type A *P. multocida*, causes turbinate atrophy similar to that produced by natural infection of DNT-producing type D strain. In our experiment, 36-day-old pigs were inoculated with a DNT-producing type A *P. multocida* which was isolated from 5-week-old pig with severe clinical AR. All of the inoculated pigs seemed clinically normal. However, they had severe turbinate lesions at slaughter. It was considered that experimental period was too short to develop clinical signs of AR in those experimental pigs.

The results of this study revealed first in Japan that DNT-producing type A *P. multocida* alone could cause turbinate atrophy similar to that produced by DNT-producing type D *P. multocida* or *B. bronchiseptica*.

## REFERENCES

1. Carter G. R. 1955. Studies on *Pasteurella multocida* 1. A hemagglutination test for the identification of serological types. *Am. J. Vet. Res.* 16: 481–484.
2. Carter, G. R. and Rundell, S. W. 1975. Identification of type A strains of *P. multocida* using staphylococcal hyaluronidase. *Vet. Rec.* 96: 343.
3. Chanter, N., Magyar, T., and Rutter, J. M. 1989. Interaction between *Bordetella bronchiseptica* and toxigenic *Pasteurella multocida* in atrophic rhinitis of pigs. *Res. Vet. Sci.* 47: 48–53.
4. Chen, C., Lu, C., Lai, J., Chang, T., and Chan, I. 1989. Experimentally induced of swine atrophic rhinitis by *Bordetella bronchiseptica*. *Pasteurella multocida* and their combined infection. *J. Chin. Soc. Vet. Sci.* 15: 129–137.

5. Cowart, R. P., Backstrom L., and Brim, T. A. 1989. *Pasteurella multocida* and *Bordetella bronchiseptica* in atrophic rhinitis and pneumonia in swine. *Can. J. Vet. Res.* 53: 295–300.
6. de Jong, M. F. and Akkermans, J. P. W. M. 1986. Investigation into the pathogenesis of atrophic rhinitis in pigs. 1. Atrophic rhinitis caused by *Bordetella bronchiseptica* and *Pasteurella multocida* and the meaning of a thermolabile toxin of *P. multocida*. *Vet. Q.* 8: 204–214.
7. Eilling, F. and Pedersen, K. B. 1983. Atrophic rhinitis in pigs induced by a dermonecrotic type A strain of *Pasteurella multocida*. pp. 123–135. *In*: Atrophic Rhinitis in Pigs (Pedersen, K. B. and Nielsen, N. C. eds.), Commission of the European Communities, Brussels.
8. Kielstein, P. and Elias, B. 1985. The significance of *Bordetella bronchiseptica* and *Pasteurella multocida* in swine atrophic rhinits. *Zentrabl. Veterinaemed., B* 32: 694–705.
9. Koshimizu, K., Kodama, Y., and Ogata, M. 1973. Studies on the etiology of infectious atrophic rhinitis of swine. V. Experimental *Bordetella bronchiseptica* infection in conventional piglets. *Jpn J. Vet. Sci.* 35: 223–229.
10. Maeda, M., Inui, S., and Konno, S. 1969. Lesions of nasal turbinates in swine atrophic rhinitis. *Natl. Inst. Anim. Health Q. (Jpn.)* 9: 193–202.
11. Nakagawa, M., Shimizu, T., and Motoi, Y. 1974. Pathology of experimental atrophic rhinitis in swine infected with *Alcaligenes bronchisepticus* or *Pasteurella multocida*. *Natl. Inst. Anim. Health Q. (Jpn.)* 14: 61–71.
12. Nakase, Y. 1957. Studies on *Hemophilus bronchisepticus*. III Differences of biological properties between phase I and phase III of *H. bronchisepticus*. *Kitasato Arch. Exp. Med.* 30: 79–84.
13. Namioka, S. 1978. *Pasteurella multocida*—Biochemical characteristics and serotypes. pp. 271–292. *In*: Methods in Microbiology, 10 (Bergan, T. and Morris, J. R. eds.), Academic Press, New York.
14. Pedersen, K. B., Nielsen, J. P., Foged, N. T., Eilling, F., Nielsen, N. C., and Willeberg, P. 1988. Atrophic rhinitis in pig: proposal for a revised definition. *Vet. Rec.* 122: 190–191.
15. Pijoan, C., Morrison, R. B., and Hilley, H. D. 1983. Serotyping of *Pasteurella multocida* isolated from swine lungs collected at slaughter. *J. Clin. Microbiol.* 17: 1074–1076.
16. Rhodes, M. B., New, C. W., Baker, P. K., Hogg, A., and Underdahl, N. R. 1987. *Bordetella bronchiseptica* and toxigenic type D *Pasteurella multocida* as agents of severe atrophic rhinitis of swine. *Vet. Microbiol.* 13: 179–187.
17. Rutter, J. M., Taylor, R. J., Crighton, W. G., Robertson, I. B., and Benson, J. A. 1984. Epidemiological study of *Pasteurella multocida* and *Bordetella bronchiseptica* in atrophic rhinits. *Vet. Rec.* 115: 615–619.
18. Sawada, T., Rimler, R. B., and Rhoades, K. R. 1982. Indirect hemagglutination test that uses glutaraldehyde-fixed sheep erythrocytes sensitized with extract antigen for detection of *Pasteurella* antibody. *J. Clin. Microbiol.* 15: 752–756.
19. Sawata, A., Nakai, T., Tsuji, M., and Kume, K. 1984. Dermonecrotic activity of *Pasteurella multocida* strains isolated from pigs in Japanese field. *Jpn. J. Vet. Sci.* 46: 141–148.
20. Yamamoto, J., Sakano, T., and Shimizu, M. 1990. Drug resistance and R plasmids in *Pasteurella multocida* isolates from swine. *Microbiol. Immunol.* 34: 715–721.