Colonization of oropharynx and nasal cavity of CDCD pigs by a nontoxigenic strain of *Pasteurella multocida* type D

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Summary: *Five-week-old cesarean-derived colostrum-deprived (CDCD) pigs were inoculated intranasally with a nontoxigenic* Pasteurella multocida *type D strain. Reisolation of this organism was attempted at 1, 3, 5, 7, and 21 days post inoculation (PI). The* P. multocida *strain was isolated from both pharyngeal tonsil and nasal cavity of pigs within 7 day PI. However, isolation of* P. multocida *was more pronounced and consistent in the tonsils at 3 weeks PI. This study shows that a nontoxigenic strain of* P. multocida*, which is not considered to be a causative agent of atrophic rhinitis, can colonize the tonsil and nasal cavity of CDCD pigs for at least 3 weeks.*

Pasteurella multocida is a common commensal and pathogen of the respiratory tract of animals. In swine, toxigenic strains (both capsular types A and D) are most often associated with atrophic rhinitis, while type A strains are commonly associated with pneumonia, pleuritis, or abscessation.[1-4] However, the organism will not usually produce disease unless other microorganisms, such as *Bordetella bronchiseptica* (in the case of atrophic rhinitis) or *Mycoplasma hyopneumoniae* or pseudorabies virus (PRV) (in the case of pneumonia) predispose pigs to secondary infections by causing an initial insult to the nasal mucosa or to the lung.[5-7] Several studies have shown that *P. multocida*, especially of type D strain, can not readily colonize swine respiratory mucosal cells in vitro.[8-10] Colonization of *P. multocida* in most in vivo studies with pigs relied on *B. bronchiseptica* live organisms or cell-free sonicate, or slight chemical irritation pretreatment followed by toxigenic type D *P. multocida* infections.[5,11-12]

The objective of this study was to determine whether a nontoxigenic strain of *P. multocida* type D can colonize the oropharynx and nasal cavity of cesarean-derived colostrum-deprived (CDCD) pigs.

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We thank Ms. Judy McGillivray for her assistance in typing this manuscript.

Materials and methods

Bacterial strains

Sterile, filtered sonicate of toxigenic *B. bronchiseptica* (strain B-133) was prepared as previously described.[11] Briefly, bacteria grown for 18 hours on 5% sheep blood agar plates were harvested and suspended in PBS. The suspension was sonicated four times at 80% power for 30 seconds each time at 4°C with a W-225R sonicator (Heat Systems-Ultrasonics, Inc., Farmingdale, New York). The sonicate was centrifuged at 12,500 × g for 20 minutes, and the supernatant was filtered through a 0.22-μm-pore-size filter (Nalge Co., Rochester, New York). Protein concentration of the filtered sonicates was 1.2 mg per mL, as determined by a Protein Assay Kit (Pierce, Rockford, Illinois).

Pasteurella multocida strain P-280 which was kindly provided by Dr. Lennart Backstrom of the University of Wisconsin—Madison, was isolated from porcine nasal cavity. It is a capsular type D and nontoxigenic strain. The strain was grown on brain heart infusion (BHI) broth at 37°C for 6 hours with shaking. The culture was kept at −70°C until used and had a viability of 2.0×10^8 CFU per mL.

Experimental animals

Eleven 5-week-old CDCD pigs obtained from the Veterinary Associates (Manning, Iowa) were used for this study. The pigs were identified and assigned to three groups of three or four pigs each (Table 1). Each group of pigs was kept in separate isolation room and remained free from outside contaminants throughout the study. Pigs were fed a sterile non-antibiotic diet of Land O' Lakes feed.

Inoculation procedures

Each of three pigs in group 1 received 1.0 mL of sterile BHI broth intranasally (IN) (0.5 mL per nostril) as controls. Each of four pigs in group 2 received 1.0 mL filtered sonicates of *B. bronchiseptica* (0.5 mL per nostril) at 33 days of age, and 1.0

mL *P. multocida* type D live culture 2 days later. Each of four pigs in group 3 received 1.0 mL of *P. multocida* culture at 35 days of age. All pigs were held upright and the inoculum was instilled slowly into each nostril with a 1-mL syringe.

Reisolating and identifying *Pasteurella multocida*

All pigs were swabbed from their noses and oropharynx before inoculation, and on days 1, 3, 5, and 7 post-inoculation (PI) with *P. multocida* and at the end of the study (3 weeks after challenge) using sterile cotton swabs (Culturette; Becton Dickinson Microbiology Systems, Cockeysville, Maryland).

Reisolation of *P. multocida* from the swabs was achieved by a modified mouse inoculation technique as described previously.[2] *Pasteurella multocida* isolates were identified by standard bacteriological procedures. Capsular type D of *P. multocida* was identified by acriflavine autoagglutination test.[14]

Toxigenicity of the isolates was determined by Western blot analysis using a monoclonal antibody specific for *P. multocida* dermonecrotic toxin. The mouse hybridoma 1B2A3 was obtained from ATCC, which produces antibody reactive with *P. multocida* toxin.

Results

No *P. multocida* isolates were obtained from pigs before *Pasteurella* inoculation. The *P. multocida* strain was reisolated from both nasal and oropharyngeal swabs of all pigs at 1, 3, 5, and 7 days PI. At the end of the study, *Pasteurella* was isolated from the oropharyngeal swabs of three out of four inoculated pigs in groups 2 or 3 (Table 1). Isolation of *P. multocida* was negative from control pigs for the whole period of the study. No bacteria other than *P. multocida* were isolated from pigs. All isolates of *P. multocida* were capsular type D and were nontoxigenic.

Table 1

Reisolation of *Pasteurella multocida* from pigs

Pig #	Group	Pre-inoc.	Post-inoc.	Study end
1	1	–	–	–
2	1	–	–	–
3	1	–	–	–
4	2	–	N, T	–
5	2	–	N, T	N, T
6	2	–	N, T	T
7	2	–	N, T	T
8	3	–	N, T	T
9	3	–	N, T	T
10	3	–	N, T	T
11	3	–	N, T	–

Group 1 pigs received 1 mL of sterile BHI broth IN;
Group 2 pigs received 1 mL of filtered sonicates of *B. bronchiseptica* at 33 days of age, and 1 mL *P. multocida* live culture at 35 days of age;
Group 3 pigs received with 1 mL *P. multocida* live culture at 35 days of age.

N — *P. multocida* reisolated from nose
T — *P. multocida* reisolated from tonsil
– — reisolation negative

Discussion

Toxigenic strains of *P. multocida* have been used for studies on adhesion and colonization of the respiratory tract of pigs by the organism.[15] In contrast, this study demonstrates that a nontoxigenic strain of *P. multocida* type D can colonize the oropharyngeal tonsil of CDCD pigs for at least 3 weeks PI. Pijoan, et al.,[13] have demonstrated that toxigenic *P. multocida* strains with or without fimbriation are capable of colonizing the turbinate epithelium both in vitro and in vivo. However, colonization in the tonsils is more pronounced and consistent, indicating that tonsillar colonization may play an important role in the pathogenesis of atrophic rhinitis.[13,15]

Turbinate atrophy in swine has traditionally been reproduced by infections with *B. bronchiseptica* and toxigenic *P. multocida*, injection of *P. multocida* toxin, or infection with *P. multocida* following intranasal acetic acid treatment.[5,12] Using a *Bordetella* cell-free sonicate-live *P. multocida* model, Ackermann, et al.,[11] were able to induce atrophic rhinitis by inoculating a toxigenic *P. multocida* strain. We did not expect to reproduce disease using a nontoxigenic strain; however, this strain can colonize the tonsils of CDCD pigs with or without filtered sonicate of *B. bronchiseptica* pretreatment.

Mechanisms by which *P. multocida* colonizes the tonsil are not well understood. Most recent studies, both in vitro and in vivo, have indicated that adhesion and colonization with *P. multocida* to respiratory tract and tonsil of pigs are associated with fimbriation and/or toxin production of strains.[13,15] We do not have data to determine whether the strain used in this study is fimbriated. Toxin would not be a contributing factor to colonization since it is a nontoxigenic strain. Pigs were kept in confined isolation during the whole study. This limited the possibility that many other microorganisms competed with *P. multocida* for the colonization sites in the tonsils or respiratory tract mucus. Therefore, it is not known whether nontoxigenic *P. multocida* could colonize in the field where toxigenic *P. multocida* and other normal flora are also present. The number of the nontoxigenic *P. multocida* organisms that colonized the tonsils has not been determined.

Implications

- Nontoxigenic *P. multocida* can colonize the oropharyngeal tonsil of CDCD pigs for at least 3 weeks PI.
- Tonsillar colonization of *Pasteurella* may play an important role in the pathogenesis of atrophic rhinitis.

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