Tonsil and Turbinate Colonization by Toxigenic and Nontoxigenic Strains of *Pasteurella Multocida* in Conventionally Raised Swine

Mark R. Ackermann, Mary C. DeBey, Karen B. Register, David J. Larson and Joann M. Kinyon

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13. Ling GV, Biberstein EL, Hirsch DC: 1979, Bacterial pathogens associated with urinary tract infections. Vet Clin North Am [Small Anim Pract] 9:617-630.
14. Mancianti F, Poli A, Bionda A: 1989, Analysis of renal immune-deposits in canine leishmaniasis. Preliminary results. Parasitologia 31:213-230.
15. Marcussen N, Vetner M, Kristensen HM: 1989, Interstitial nephritis and glomerulonephritis in visceral leishmaniasis in a dog. Acta Pathol Microbiol Immunol Scand 97:1137-1140.
16. Mauel J: 1990, Macrophage-parasite interactions in *Leishmania* infections. J Leukocyte Biol 47:187-193.
17. Poli A, Abramo F, Mancianti F, et al.: 1991, Renal involvement in canine leishmaniasis. A light-microscopic, immunohistochemical and electron-microscopic study. Nephron 57:444-452.
18. Pinelli E, Killick-Kendrick R, Wagenaar J, et al.: 1994, Cellular and humoral responses in dogs experimentally and naturally infected with *Leishmania infantum.* Infect Immun 62:229-235.
19. Salata RA, Lemer PI, Shalaes DM, et al.: 1989, Infections due to Lancefield group C streptococci. Medicine 68:225-239.
20. Slappendel RJ, Green CE: 1990, Leishmaniasis. *In:* Infectious diseases of the dog and cat, ed. Greene CE, pp. 769-777. WB Saunders Co., Philadelphia, PA.

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Tonsil and turbinate colonization by toxigenic and nontoxigenic strains of *Pasteurella multocida* in conventionally raised swine

Mark R. Ackermann, Mary C. DeBey, Karen B. Register,
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Pneumonia and upper respiratory tract infections, such as atrophic rhinitis, are common and insidious diseases of swine. They are often considered causes of decreased rate of weight gain, inefficient feed conversion, and increased time to market, although these parameters do not absolutely correlate with the severity of lesions. *Pasteurella multocida* is associated with lower and upper respiratory infections, based on results of lung cultures at necropsy and cultures from swabs of the nasal cavity.[4,8,9,15] In the lung, one study showed that nontoxigenic strains were most commonly isolated from acute to subacute pneumonic areas, and toxigenic strains were most commonly isolated from granulomas.[9] In atrophic rhinitis, toxigenic strains are associated with severe, progressive turbinate atrophy.[2-4,8,12] Experimentally, purified toxin induces turbinate atrophy when aerosolized into the nasal cavity or injected into the subcutis, muscle, or peritoneum. 1,6-8,11

Although isolation of *P. multocida* from pneumonic lungs and nasal cavities reflects its etiologic importance, other anatomical locations, such as tonsil, may be a reservoir for *P. multocida* in swine.[2,3,14,15] *Pasteurella multocida* can be isolated from turbinate[6,8] and tonsi1,[2,3,14] however, one study in gnotobiotic pigs and two studies in specific-pathogen-free pigs demonstrated that tonsil is colonized to a greater degree than turbinate.[2,3,13,14] The purpose of this study was to use conventionally reared swine to investigate the relative affinity of *P. multocida* for nasal turbinate mucosa and tonsil and the prevalence of toxigenic strains in a random population of Iowa swine.

Tonsil and turbinate were collected from 53 young and growing swine (20-80 kg) submitted live for necropsy at Iowa State University's Veterinary Diagnostic Laboratory and the National Animal Disease Center (NADC), Ames, Iowa, and from 21 sows killed following routine caesarean sections at the NADC. Tissues were collected (washed and rinsed instruments) and stored frozen at -80 C for up to 2 months. Clinical histories and necropsy findings of pigs from the Diagnostic Laboratory varied greatly, and some pigs had evidence of turbinate atrophy according to visual inspection at necropsy (Table 1). Sows from the NADC were clinically healthy and had no evidence of turbinate atrophy as determined by gross examination.

Thawed tissues were ground in 0.02 M phosphate-buffered saline (pH 7.0, 10% w/w), and 10-fold dilutions were inoculated onto duplicate blood agar (BA) plates (tryptose blood agar base + 5% citrated bovine blood)[a] without or with antimicrobials (Kinyon *P. muhocida* type D protein [KPMD]; plate concentration = bacitracin, 3.75 U/ml; clindamycin, 5 µg/ml; gentamicin, 0.75 µg/ml; amphotericin B, 2.25 µg/ml) to select for *P. multocida.* Suspect colonies on KPMD and additional suspect colonies from BA were subcultured to obtain single colonies on dextrose starch agar[a] and identified by standard methods.[5,15] Tests of over 200 tonsil and turbinate cultures by the Clinical Microbiology Laboratory at Iowa State University demonstrated superior recovery of *P. multocida* from KPMD when compared with BA. In addition, only a small decrease (roughly 10%) in numbers of colony-forming units (CPU) of *P. multocida* were seen in KPMD plates when equal bacterial suspensions were plated onto KPMD and BA. Colonies identified as *P. multocida* were transferred to duplicate nylon membranes and tested for expression of toxin as described with a colony-blot assay.[10] No differences in toxin expression, as determined by the membrane-lift procedure, were seen in bacterial colonies grown on KPMD or BA. *Pasteurella multocida* strains P1683 (toxigenic) and P4214 (nontoxigenic) served as positive and negative controls, respectively, for the colony-blot assay.

Pasteurella multocida was isolated from 35 (66%) of 53

From the Atrophic Rhinitis Project, USDA, ARS, National Animal Disease Center, Ames, IA 50010 (Ackermann, DeBey, Register), and the Veterinary Diagnostic Laboratory (Larsen) and the Clinical Microbiology Laboratory (Kinyon), Iowa State University, Ames, IA 50011.

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Table 1. Health status, number of colony forming units (CFU) of *Pasteurella multocida* in tonsil and turbinate, and toxin production from 35 growing pigs.

Pig no.	Clinical diagnosis*	CFU *P. multocida*†		Toxin production
		Tonsil	Turbinate	
1	meningitis	1.0	0.6	+ (both)
2	IBR	0.6	0.2	–
3	proliferative ileitis	1.2	0.4	–
4	edema disease	0.1	0.1	–
5	AR herd	2.2	1.9	–
6	AR herd	1.9	2.2	–
7	AR herd	1.8	1.0	–
8	AR herd	0.3	0.1	–
9	none	4.3	0.1	–
10	none	0.1	0.1	–
11	none	0.4	0.7	–
12	none	3.2	0.2	–
13	none	1.6	0.1	–
14	none	0.7	0.9	–
15	none	0.1	0.1	–
16	none	1.2	1.2	–
17	none	0.5	0.1	–
18	pneumonia	4.1	0	–
19	mycoplasmosis	1.2	0	–
20	mycoplasmosis	0.5	0	–
21	undetermined	0.6	0	–
22	proliferative ileitis	0.1	0	–
23	*Streptococcus cholerasuis*	0.2	0	–
24	AR herd‡	0.2	0	–
25	AR herd‡	0.1	0	–
26	AR herd	0.2	0	–
27	none	0.7	0	–
28	none	0.5	0	–
29	none	0.3	0	–
30	none	1.7	0	–
31	none	3.2	0	–
32	none	0.7	0	–
33	AR herd	0	0.1	–
34	AR herd	0	0.1	–
35	none	0	0.1	+
Average		1.0	0.5	

* IBR = inclusion body rhinitis; AR = atrophic rhinitis.
† CFU/gram tissue × 10^3.
‡ Pigs with turbinate atrophy at necropsy.

growing pigs and 4 (19%) of 21 sows in this study (Tables 1, 2). Of the 35 growing pigs that carried *P. multocida*, 17 (48%) carried both tonsil and turbinate isolates, 15 (42%) carried isolates only in tonsil, and 3 (8%) carried isolates only in turbinate. Pigs carried *P. multocida* in tonsil tissue more commonly than in turbinate. Of the 35 growing pigs carrying *P. multocida*, 32 had isolates from tonsil and 20 carried turbinate isolates. Higher numbers of CFU/gram were generally isolated from tonsil, averaging 1.0×10^3 CFU/g as compared with the turbinate average of 0.5×10^3 CFU/g.

In the sows, 4 of 21 carried *Pasteurella multocida*, and all 4 had tonsil isolates averaging 1.0×10^3 CFU/g (Table 2). Sow No. 1 also carried toxigenic *P. multocida* in the turbinate.

Only two of the growing pigs (Nos. 1, 35) and 1 sow (No. 1) carried toxigenic strains (Tables 1, 2). Toxigenic strains were isolated from both the tonsil and turbinates from pig No. 1 and from only turbinate from pig No. 35. Sow No. 1 was the only animal from which both nontoxigenic (tonsil) and toxigenic (turbinate) strains were isolated. No obvious trends were present in pigs that were ill, pigs from atrophic rhinitis herds, and clinically normal pigs. Toxigenic strains were not isolated from the 3 pigs with turbinate atrophy. Few contaminant bacteria were present on KPMD plates; whereas BA plates frequently contained *Proteus*, *Bacillus* sp., *Escherichia coli*, and *Streptococcus* sp.

Colonization of the tonsil by *P. multocida* in conventionally reared swine in this study is similar to that in experimental studies with gnotobiotic and specific-pathogen-free pigs.[2,3,13,14] In all of these studies, *P. multocida* were isolated in greater frequency and in higher numbers per gram in tonsil than in turbinate. In this study, 15 of 53 pigs carried *P. multocida* in tonsil only and thus infection would be missed from examination of routine nasal swabs. The tonsil may be an important reservoir for *P. multocida* strains that eventually have a role in bronchitis, pneumonia, pleuritis, and atrophic rhinitis. Turbinate, in contrast, is colonized to a

Table 2. Health status, number of colony-forming units (CFU) of *Pasteurella multocida* in tonsil and turbinate, and toxin production from 4 sows.

Sow no.	Clinical diagnosis	CFU *P. multocida** Tonsil	CFU *P. multocida** Turbinate	Toxin production
1	none	1.1	0.1	+†
2	none	1.2	0	–
3	none	0.7	0	–
4	none	1.1	0	–
Average		1.0	0.1	

* CFU/gram tissue × 10^3.
† Turbinates only.

lesser degree. *Pasteurella multocida* adheres weakly to ciliated respiratory epithelial cells[8,14] and does not efficiently colonize turbinate mucosa experimentally unless there is damage caused by *Bordetella bronchiseptica* cytotoxin or acetic acid (experimentally).[8]

Toxigenic strains also colonize tonsil, although few pigs in this study carried these strains. Even pigs from herds with atrophic rhinitis and pigs with turbinate atrophy failed to carry toxigenic strains. This finding suggests that either the animals eliminated toxigenic *P. multocida* responsible for the turbinate lesions or the atrophy was caused by another agent such as *B. bronchiseptica.* Toxigenic strains of *B. bronchiseptica* produce reversible forms of turbinate atrophy and are associated with irreversible, severe forms when combined with toxigenic strains of *P. multocida.*[6,8] In our experience, toxin production by *P. multocida* strains is stable and consistent. That is, toxigenic strains produce toxin and nontoxigenic strains do not spontaneously acquire toxin production, even after freezing or after in vivo inoculation and reisolation.

Colonization of the tonsil is likely due to the microenvironment of the tonsil crypt, which despite the presence of large numbers of neutrophils is conducive to growth of *P. multocida.* [2,3] In ultrastructural studies of pigs, few bacteria were present on the surface of squamous epithelium between crypts.[2] Therefore, at necropsy, whole ground tonsil should be cultured to identify *P. multocida* carriers rather than relying on tonsil swabs. Swabs streak the surface of the tonsil and do not sample the deep portions of tonsil crypts where largest numbers of bacteria localize.

Both tonsil and turbinate have a large surface area for bacteria to colonize. The deep crypts present in tonsil increase the surface area for bacterial colonization and growth. Although the surface area of tonsil is increased by crypts, turbinate tissue also has a large surface area because two sides of the turbinate are exposed to the nasal cavity, scrolling of the turbinate increases the surface area in a given volume, and the turbinate epithelium has microscopic crypts that are not as deep as tonsil crypts but are more numerous.

A limited number of sows were studied; however, a similar trend of colonization (tonsil) by nontoxigenic strains was present. From this random sampling, it does not appear that sows have an increased rate of colonization or that a higher percentage of sows carry toxigenic strains. Further testing of sows may indicate whether there is a higher carrier rate of toxigenic strains in animals with life spans longer than that of slaughter pigs.

Sources and manufacturers

a. Difco Laboratories, Detroit, MI.

Disclaimer: Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

References

1. Ackermann MR, Adams DA, Gerkin LL, Rimler RB: 1993, Purified *Pasteurella multocida* protein toxin reduces acid phosphatase positive osteoclasts in the ventral nasal concha of gnotobiotic pigs. Calcif Tissue Int 52:455-459.
2. Ackermann MR, Cheville NF, Rimler RB: 1991, Colonization of the pharyngeal tonsil and respiratory tract of the gnotobiotic pig by a toxigenic strain of *Pasteurella multocida* type D. Vet Pathol 28:267-274.
3. Ackermann MR, Rimler RB, Thurston JR: 1991, Experimental model of atrophic rhinitis in gnotobiotic pigs. Infect Immun 59: 3626-3629.
4. Bowersock TL, Hooper T, Pottenger R: 1992, Use of ELISA to detect toxigenic *Pasteurella multocida* in atrophic rhinitis in swine. J Vet Diagn Invest 4:419-422.
5. Carter GR: 1986, *Pasteurella multocida. In:* Essentials of veterinary bacteriology and mycology, pp. 160-164. Lea and Febiger, Philadelphia, PA.
6. Chanter N, Rutter JM: 1989, Pasteurellosis in pigs and the determinants of virulence of toxigenic *Pasteurella multocida. In: Pasteurella* and pasteurellosis, ed. Adlam C, Rutter JM, pp. 161-195. Academic Press, Orlando, FL.
7. Dominick MA, Rimler RB: 1988, Turbinate osteoporosis in pigs following intranasal inoculation of purified *Pasteurella* toxin: histomorphometric and ultrastructural studies. Vet Pathol 25:17-27.
8. Foged NT: 1992, *Pasteurella multocida* toxin: the characterization of the toxin and its significance in the diagnosis and prevention of progressive atrophic rhinitis in pigs. Acta Pathol Microbiol Immunol Scand 100 (suppl. 23).
9. Iwamatsu S, Sawada T: 1988, Relationship between serotypes, toxin production of *Pasteurella multocida* isolates and pneumonic lesions of porcine lung. Jpn J Vet Sci 50:1200-1206.
10. Magyar T, Rimler RB: 1991, Detection and enumeration *Pasteurella multocida* with a colony-blot assay. J Clin Microbiol 29:1328-1332.
11. Martineau-Doize B, Frantz JC, Martineau GP: 1990, Effects of purified *Pasteurella multocida* dermonecrotoxin on cartilage and bone of the nasal ventral conchae of the piglet. Anat Rec 228:237-246.
12. Nielsen JP, Foged NT, Sorensen V, et al.: 1991, Vaccination against progressive atrophic rhinitis with a recombinant *Pasteurella multocida* toxin derivative. Can J Vet Res 55: 128-138.
13. Piffer IA, Castro AFP, Brito JRF: 1992, Association of toxigenic type D *Pasteurella multocida* (Pm) to the upper respiratory tract of swine. Int Perspect Vet Sci 1:172.
14. Pijoan C, Trigo F: 1990, Bacterial adhesion to mucosal surfaces with special reference to *Pasteurella multocida* isolates from atrophic rhinitis. Can J Vet Res 54:S16-S24.
15. Rimler RB, Rhoades KR: 1989, *Pasteurella multocida. In: Pasteurella* and pasteurellosis, ed. Adlam L, Rutter JM, pp. 37-73. Academic Press, Orlando, FL.