# Enhanced Adherence of *Pasteurella multocida* to Porcine Tracheal Rings Preinfected with *Bordetella bronchiseptica*

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## ABSTRACT

**Adherence of 25 isolates of *Pasteurella multocida* to porcine tracheal rings was evaluated. Results indicated that adherence was not related to the isolate's origin, capsular or somatic types, dermonecrotoxin production or hemagglutination activity. The effect of a preinfection with *Bordetella bronchiseptica* on the colonization by *P. multocida* was then studied. On rings infected with *P. multocida* alone, bacteria initially adhered to the epithelium, but within a few hours, the level of colonization decreased progressively. On rings preinfected with *B. bronchiseptica*, or pretreated with a cell-free *B. bronchiseptica* culture supernate (or filtrate), a high level of *P. multocida* colonization was maintained for at least 24 hours. Results indicate that *B. bronchiseptica* appears to facilitate upper respiratory tract colonization by *P. multocida* by a process which involves a low molecular weight (≤ 1000) heat-stable substance, possibly the tracheal cytotoxin.**

## RÉSUMÉ

**Nous avons évalué l'adhérence de 25 isolats de *Pasteurella multocida* à des anneaux de trachée de porcelets. Les résultats indiquent que le degré d'adhérence ne semble pas relié à l'origine de l'isolat, ni à son type capsulaire ou somatique, ou encore à sa capacité d'hémagglutiner ou de produire une toxine dermonécrotique. Nous avons par la suite étudié l'effet d'une préinfection par *Bordetella bronchiseptica* sur la colonisation de *P. multocida*. Les anneaux de trachée infectés uniquement avec *P. multocida* ont montré une colonisation qui décroissait progressivement. Par contre, les anneaux de trachée pré-infectés avec *B. bronchiseptica*, ou exposés à un surnageant stérile d'une culture de *B. bronchiseptica*, ont permis une bonne colonisation de *P. multocida* durant une période d'au moins 24 heures. Les résultats indiquent que *B. bronchiseptica* semble faciliter la colonisation des voies respiratoires supérieures par *P. multocida* en élaborant une molécule thermostable de faible poids moléculaire (≤ 1000) qui pourrait être la cytotoxine trachéale.**


Porcine atrophic rhinitis is a multifactorial disease characterized by atrophy of the nasal turbinates and snout, and necrosis in the epithelia of the upper respiratory tract (1). The etiological agents involved are *Bordetella bronchiseptica* and *Pasteurella multocida* (1–3). All *Bordetella* species are known to adhere preferentially to ciliated epithelial cells and to produce several toxic factors (4–6). *Bordetella bronchiseptica* produces a thermolabile dermonecrotic toxin which seems to be implicated in the nasal lesions found in neonatal piglets (7–9). *Bordetella bronchiseptica* also produces a tracheal cytotoxin (10); this low molecular weight glycopeptide is ciliostatic and causes extrusion of ciliated cells from tracheal organ cultures. This cytotoxin, produced by all species of *Bordetella* (11), has been mainly studied in *Bordetella pertussis* (10,12,13).

A thermolabile dermonecrotic toxin is also produced by *P. multocida*, although this toxin is serologically different from the one produced by *B. bronchiseptica*, they share similar biological activities (14). The lesions caused by *P. multocida* are irreversible and the turbinate bones do not regenerate (15). *Pasteurella multocida* is encapsulated (16) and may produce a hemagglutinin (17), but little is known about the chemical determinants responsible for its colonization in pigs (18). *Bordetella bronchiseptica* adheres well to epithelial cells, whereas *P. multocida* adheres poorly and requires predisposing conditions to colonize the mucosa (19–22). Some studies have demonstrated that an infection with *B. bronchiseptica* permits colonization by toxigenic *P. multocida* and leads to more severe lesions than does infection with *B. bronchiseptica* alone (1,22).

Although the mechanism is not clear, adherence seems to be an important first step for successful colonization and infection of the porcine nasal cavity by these two bacteria (1,23). Tracheal organ culture permits the study of the pathogenesis of organisms which damage mucosal cells by attachment and toxin production (24). The purpose of the present study was to quantitate the adherence of *P. multocida* to porcine tracheal rings and to evaluate the effect of a *B. bronchiseptica* preinfection.

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A total of 26 isolates of *P. multocida* were used in this study. Reference strain *P. multocida* 4-4056 (capsular type D) was provided by M. de Jong (Veterinary Health Service, Zwolle, The Netherlands). All other isolates were retrieved from the nasal cavities of pigs affected (18 strains) or not (seven strains) with atrophic rhinitis. Their phenotypes have been previously described by Fortin and Jacques (17). *Bordetella bronchiseptica* 276, originally isolated from a pig with severe atrophic rhinitis (25), was provided by J.M. Rutter, Institute for Research on Animal Diseases, Compton, U.K. Bacteria, grown overnight on blood agar plates at 37°C, were resuspended in Eagle's minimal essential medium (MEM) at a concentration of $10^8$ CFU/mL. The suspensions were diluted and 0.1 mL aliquots were plated on blood agar to obtain exact inoculum counts.

Tracheal ring cultures prepared according to Collier (26) were adapted for newborn piglets (27). Briefly, tracheae were excised and divided into rings which were placed in culture dishes. Rings were incubated for 5 h at 37°C in a 5% $CO_2$-air atmosphere, with MEM containing amphotericin B (2.5 μg/mL), ampicillin (0.1 mg/mL), and gentamicin sulfate (0.5 mg/mL). Rings were then rinsed three times with fresh MEM without antibiotics, incubated overnight, and rinsed a fourth time just before infection. At each step of the experiment, the rings were examined for ciliary activity using an inverted microscope. Before infection, only rings with normal vigor, i.e. those which exhibited a ciliary activity over more than 80% of the epithelial border were used.

The first part of the study was to quantitate the adherence to tracheal rings of 25 isolates of *P. multocida* of various phenotypes. Experiments were initially performed to determine the effects of various parameters, including the inoculum size and the incubation time, on adherence of *P. multocida*. Tracheal rings were infected with 1.5 mL of a bacterial suspension of one of the 25 field isolates. One hour after infection, rings were washed in PBS and agitated for 1 min in PBS containing 1% Triton X-100 (a treatment which did not affect *P. multocida* viability) to dislodge adherent bacteria (28,29); dilutions were then made and inoculated onto agar plates. To permit comparisons of the adherence among the isolates of *P. multocida*, an adherence index (28,29) was calculated to compensate for slight variations in concentration of bacterial inocula: Antilog [(log CFU/ring) − (log CFU/inoculum) +5]. Results were compared for statistical significance using *t*-test.

After an incubation period of one hour, microorganisms adhered to the epithelium and approximately $10^5$ CFU were recovered per tracheal ring, which corresponds to an adherence index of approximately 70. Comparisons were made among the isolates according to phenotype. Eighteen of the 25 isolates were retrieved from nasal cavities of pigs from herds with clinical signs of atrophic rhinitis. The mean adherence index of these 18 isolates (65 ± 52) was not statistically different ($p > 0.6$) from the mean adherence index of the seven other isolates originating from herds without clinical signs of atrophic rhinitis (79 ± 94). The 25 isolates were then grouped according to their capsular or somatic types. The mean adherence indexes of the ten capsular type A isolates (77 ± 76) and of the 15 capsular type D isolates (64 ± 58) were not significantly different ($p > 0.6$). Furthermore, the mean adherence indexes of the isolates possessing somatic antigens (30) 3 and 16, 4 and 7, or other somatic antigens were also not significantly different ($p > 0.1$).

Adherence of the isolates to porcine tracheal rings was then compared to their ability to agglutinate human type O erythrocytes. The isolates of *P. multocida* were tested for their ability to agglutinate human type O erythrocytes, according to Fortin and Jacques (17). No significant difference ($p > 0.6$) was found between the mean adherence indexes of the hemagglutination-positive (59 ± 31) and hemagglutination-negative isolates (74 ± 76). Finally, the isolates were grouped according to their production of dermonecrotic toxin. The dermonecrotic activity of the isolates was determined by the guinea pig skin test as described by Lugtenberg *et al* (31). Briefly, bacteria-free broth culture supernates of *P. multocida* were injected intracutaneously in volumes of 0.2 mL. The hemorrhagic necrotizing reactions were judged daily for three days. *Pasteurella multocida* 4-4056 was used as a positive control. The mean adherence index of the 15 isolates not producing the toxin (69 ± 65) was not significantly different ($p > 0.9$) from the mean adherence index of the ten toxin-producing isolates (69 ± 67). Results indicated that adherence was not related to the isolate's origin, capsular or somatic types, dermonecrotoxin production or hemagglutination activity. Our results are in agreement with those obtained by Vena *et al* (32) using tracheal epithelial cells.

In the second part of the study, the effect of *B. bronchiseptica* preinfection on the adherence of *P. multocida* was evaluated since *in vivo*, *B. bronchiseptica* is known to facilitate *P. multocida* colonization (15,22). The adherence of *B. bronchiseptica* to the porcine trachea maintained in organ culture has been described (27). Reference strain *P. multocida* 4-4056 was used for this part of the experiment. Tracheal rings were first infected with a suspension of *B. bronchiseptica* 276 containing $10^8$ CFU/mL. Either at the same time, 2 h, or 4 h after infection with *B. bronchiseptica*, rings were infected with a suspension of $10^8$ CFU/mL of *P. multocida* 4-4056. Control rings were infected with *P. multocida* 4-4056 alone. One, 2, 4, 6 and 8 h postinfection with *P. multocida*, rings were prepared for bacterial counts as mentioned above. Distinct colonial morphologies allowed the two bacteria to be easily counted.

*Pasteurella multocida* 4-4056 adhered to the trachea maintained in organ culture, and approximately $10^5$ CFU were recovered per tracheal ring after an incubation period of 1 h, as also observed with the field isolates. Within a few hours, the level of colonization decreased progressively to $10^1$–$10^3$ CFU per ring (Fig. 1). On rings simultaneously infected with both *B. bronchiseptica* and *P. multocida*, the adherence of *P. multocida* was not influenced by the presence of *B. bronchiseptica* and bacterial counts decreased after a few hours (Fig. 1A). On rings preinfected with *B. bronchiseptica* 2 h prior to infection with *P. multocida*, adherence of *P. multocida* was different; approximately

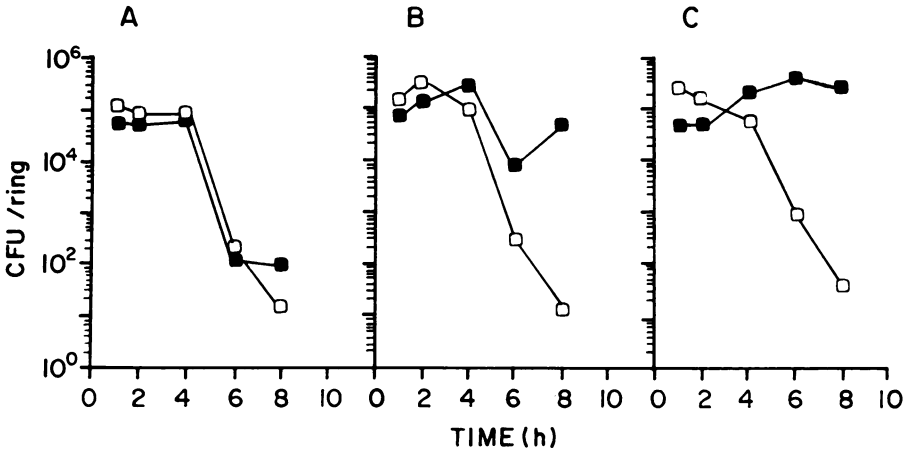


Fig. 1. Adherence of *P. multocida* 4-4056 to control tracheal rings (□) or to porcine tracheal rings infected with *B. bronchiseptica* 276 (■). Rings were infected with *B. bronchiseptica*: (A) simultaneously; (B) 2 h; or (C) 4 h prior to infection with *P. multocida*.

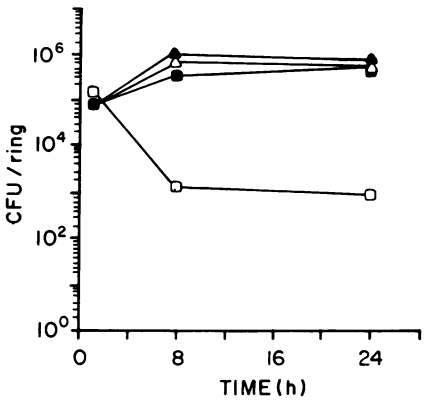


Fig. 2. Adherence of *P. multocida* 4-4056 to control porcine tracheal rings (□) or to porcine tracheal rings pretreated with *B. bronchiseptica* 276 (■), a cell-free culture supernate (▲), or a heated cell-free culture supernate (△) of *B. bronchiseptica* 4 h prior to infection with *P. multocida*.

$10^5$ CFU were recovered per tracheal ring and the bacterial colonization was maintained through the 8 h duration of the experiment (Fig. 1B). The level of colonization was even higher on rings preinfected with *B. bronchiseptica* 4 h prior to infection with *P. multocida* (Fig. 1C). Adherence of *B. bronchiseptica* was not influenced by the presence of *P. multocida* in organ culture (data not shown).

The effect of preinfection on the adherence of field isolates of capsular types A and D isolates, either producing or not producing the dermonecrotic toxin, was then evaluated. Adherence of the isolates was enhanced when the tracheal rings were preinfected with *B. bronchiseptica* 4 h prior to their inoculation, as previously observed with the reference strain (data not shown).

It is unlikely that the enhancement of adherence observed might be due to growth factors provided by *B. bronchiseptica*, since simultaneous infection with both microorganisms did not enhance *P. multocida* adherence. Furthermore, mixed culture of these two bacteria did not result in an increased growth of *P. multocida* (data not shown). *Bordetella bronchiseptica* seemed instead to predispose the mucosa and allow successful colonization by *P. multocida* capsular types A and D, either producing or not producing dermonecrotoxin. In order to study the relative specificity of the enhancement of bacterial colonization by *B. bronchiseptica*, the effect of a preinfection by this microorganism on the adherence of another porcine respiratory pathogen member of the *Pasteurellaceae, Actinobacillus pleuropneumoniae*, was evaluated. In this experiment reference strains K17 and WF83 of *A. pleuropneumoniae* were used. They were grown overnight on PPLO agar plates at 37°C. Adherence of *A. pleuropneumoniae* to porcine tracheal rings was not influenced by a preinfection by *B. bronchiseptica* (data not shown), suggesting that the predisposing effect of *B. bronchiseptica* was relatively specific for *P. multocida*.

To see if the enhancement of the colonization by *P. multocida* after a preinfection with *B. bronchiseptica* was due to extracellular products, tracheal rings were pretreated with a cell-free *B. bronchiseptica* culture supernate 4 h prior to infection with *P. multocida* instead of being infected with a live culture. *Bordetella bronchiseptica* 276 was grown overnight in MEM. Bacterial cells were centrifuged, and the supernate was filtered through a 0.22 μm-pore-size membrane filter unit (Sartorius, Göttingen, Germany). A similar effect was obtained and a high level of colonization by *P. multocida* 4-4056 was observed for at least 24 h (Fig. 2). A fraction of the filtrate was then heated for 30 min at 56°C to destroy the dermonecrotic activity; the guinea pig skin test confirmed the absence of dermonecrotic activity. This filtrate fraction was then used to treat the tracheal rings before the infection with *P. multocida*. Heating of the filtrate did not alter its effect on *P. multocida* adherence. Another fraction of the filtrate was applied to a 1.0 mL volume of prepacked Detoxi-Gel affinityPak column (Pierce Chemical Company, Rockford, Illinois) to remove endotoxin, and was used to treat the tracheal rings before infection with *P. multocida*. A high level of colonization of *P. multocida* was observed when the filtrate was used for the rings' pretreatment after removal of endotoxin. Finally, a fraction of the filtrate was ultrafiltered through a membrane with a 1000 MW cut-off (Amicon, Danvers, Massachusetts) to approximate the molecular weight of the active extracellular product of *B. bronchiseptica*. The enhanced adherence of *P. multocida* 4-4056 was also observed when tracheal rings were pretreated with the ultrafiltrate (i.e. components of MW $\leq 1000$).

Previous studies tended to demonstrate that the dermonecrotoxin produced by *B. bronchiseptica* could play a major role (9,23). This toxin has a molecular weight of approximately 145 000 daltons, and is thermolabile, i.e. its activity is lost upon heating at 56°C (33). Results we obtained with heated cell-free culture supernate devoid of dermonecrotic activity, with an ultrafiltrate (composed of components with MW $\leq 1000$), or with an endotoxin-free filtrate indicated that

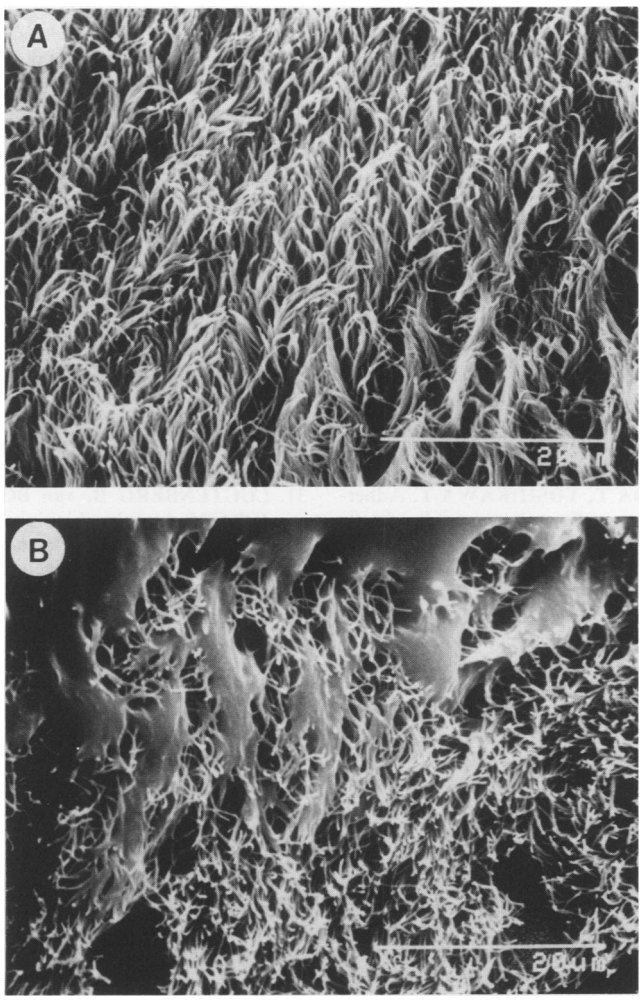


**Fig. 3. Scanning electron micrographs of critical point-dried preparations of porcine tracheal rings. (A) Control ring; (B) Ring exposed during 4 h to a bacteria-free *B. bronchiseptica* culture supernate; note the accumulation of mucus.**

the dermonecrotoxin as well as the endotoxin (or lipopolysaccharides) were probably not involved.

Just before infection with *P. multocida*, control rings exhibited a normal ciliary activity. With the rings pretreated with *B. bronchiseptica*, or a cell-free culture supernate, an important accumulation of mucus was observed and ciliary activity was greatly reduced. Control rings, as well as rings pretreated with the cell-free culture supernate of *B. bronchiseptica* were retrieved from tracheal cultures just before infection with *P. multocida*, and prepared for scanning electron microscopy, as previously described (27). The controls showed a normal mucosa entirely covered by ciliated cells (Fig. 3A), while pretreated rings were partially covered by mucus, sticking cilia together (Fig. 3B). Since *P. multocida* has been shown to have an affinity for porcine respiratory mucus (34), the ciliostasis and the mucus accumulation induced by *B. bronchiseptica* might explain the enhanced adherence of *P. multocida*.

A tracheal cytotoxin (TCT) produced by all *Bordetella* spp. was purified from *Bordetella pertussis*; and its primary structure and biological activities were recently described (10,12,13). Tracheal cytotoxin is a 921 dalton monomeric disaccharide-tetrapeptide derived from peptidoglycan. It appears to cause ciliostasis and to destroy specifically ciliated epithelial cells. Tracheal cytotoxin was present at 3–6 pmol/mL of *B. bronchiseptica* culture supernate. Analysis for TCT was accomplished by solid-phase extraction from filtered culture supernates, followed by reversed-phase high-pressure liquid chromatography. Quantitation was based upon comparison to purified TCT standards (13). Furthermore, porcine tracheal rings were first exposed to 3 $\mu$M of purified TCT from *B. pertussis* (a gift from William E. Goldman) for 4 h and then inoculated with *P. multocida*. A high level of colonization of *P. multocida*, similar to the one obtained with *B. bronchiseptica*, was observed.

Our results suggest that low molecular weight, heat-stable, substances produced by *B. bronchiseptica*, possibly TCT alone or in combination with a cofactor, could act as predisposing factors by inducing a ciliostasis of the tracheal epithelium with a concomitant accumulation of mucus which allows long-term colonization by *P. multocida*.

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## REFERENCES

1. **RUTTER JM.** Atrophic rhinitis in swine. Adv Vet Sci Comp Med 1985; 29: 239–279.
2. **MAGYAR T, CHANTER N, LAX N, RUTTER JM, HALL GA.** The pathogenesis of turbinate atrophy in pigs caused by *Bordetella bronchiseptica*. Vet Microbiol 1988; 18: 135–146.
3. **MOES J, DEWAELE A.** Rhinite atrophique: conceptions actuelles. Ann Med Vet 1987; 131: 229–262.
4. **BEMIS D.** *Bordetella*. In: Gyles CL, Thoen CO, eds. Pathogenesis of Bacterial Infections in Animals. Ames: Iowa State University Press, 1986: 137–146.
5. **SEKIYA K, FUTAESAKO Y, NAKASE Y.** Electron microscopic observations on tracheal epithelia of mice infected with *Bordetella bronchiseptica*. Microbiol Immunol 1988; 32: 461–472.
6. **YOKOMIZO Y, SHIMIZU T.** Adherence of *Bordetella bronchiseptica* to swine nasal epithelial cells and its possible role in virulence. Res Vet Sci 1979; 27: 15–21.
7. **GOODNOW PA.** Biology of *Bordetella bronchiseptica*. Microbiol Rev 1980; 44: 722–738.

8. **KUME K, NAKAI T, SAMEJIMA Y, SUGIMOTO C.** Properties of dermonecrotic toxin prepared from sonic extracts of *Bordetella bronchiseptica*. Infect Immun 1986; 52: 370-377.
9. **ROOP RM II, VEIT HP, SINSKY RJ, VEIT SP, HEWLETT EL, KORNEGAY ET.** Virulence factors of *Bordetella bronchiseptica* associated with the production of infectious atrophic rhinitis and pneumonia in experimentally infected neonatal swine. Infect Immun 1987; 55: 217-222.
10. **COOKSON BT, GOLDMAN WE.** Tracheal cytotoxin: a conserved determinant of all *Bordetella* species. J Cell Biochem 1987; 11B (Suppl): 124.
11. **WARDLAW AC.** Virulence factors and species specificity in *Bordetella*. In: Donachie W, Griffiths E, Stephen J, eds. Bacterial Infection of Respiratory and Gastrointestinal Mucosae. Oxford: IRL Press, 1988: 41-56.
12. **COOKSON BT, TYLER N, GOLDMAN WE.** Primary structure of the peptidoglycan-derived tracheal cytotoxin of *Bordetella pertussis*. Biochemistry 1989; 28: 1744-1749.
13. **COOKSON BT, CHO H-L, HERWALDT LA, GOLDMAN WE.** Biological activities and chemical composition of purified tracheal cytotoxin of *Bordetella pertussis*. Infect Immun 1989; 57: 2223-2229.
14. **NAKAI T, SAWATA A, KUME K.** Intracellular locations of dermonecrotic toxin in *Pasteurella multocida* and in *Bordetella bronchiseptica*. Am J Vet Res 1985; 46: 870-874.
15. **CHANTER N, RUTTER JM.** Pasteurellosis in pigs and the determinants of virulence of toxigenic *Pasteurella multocida*. In: Adlam C, Rutter JM, eds. *Pasteurella* and pasteurellosis. London: Academic Press, 1989: 161-196.
16. **JACQUES M, FOIRY B.** Electron microscopic visualization of capsular material of *Pasteurella multocida* types A and D labelled with polycationic ferritin. J Bacteriol 1987; 169: 3470-3472.
17. **FORTIN M, JACQUES M.** Hemagglutination by *Pasteurella multocida* of porcine origin. J Clin Microbiol 1987; 25: 938-939.
18. **RIMLER RB, RHOADES KR.** *Pasteurella multocida*. In: Adlam C, Rutter JM, eds. *Pasteurella* and pasteurellosis. London: Academic Press, 1989: 37-74.
19. **FRYMUS T, WITTENBRICK M-M, PETZOLD K.** Failure to demonstrate adherence of *Pasteurella multocida* involved in atrophic rhinitis to swine nasal epithelial cells. J. Vet Med [B] 1985; 33: 140-144.
20. **JACQUES M, PARENT N, FOIRY B.** Adherence of *Bordetella bronchiseptica* and *Pasteurella multocida* to porcine nasal and tracheal epithelial cells. Can J Vet Res 1988; 52: 283-285.
21. **NAKAI T, KUME K, YOSHIKAWA H, OYAMADA T, YOSHIKAWA T.** Adherence of *Pasteurella multocida* or *Bordetella bronchiseptica* to the swine nasal epithelial cell *in vitro*. Infect Immun 1988; 56: 234-240.
22. **PEDERSEN KB, ELLING F.** The pathogenesis of atrophic rhinitis in pigs induced by *Pasteurella multocida*. J Comp Pathol 1984; 94: 203-214.
23. **CHANTER N, MAGYAR T, RUTTER JM.** Interactions between *B. bronchiseptica* and toxigenic *P. multocida* in atrophic rhinitis of pigs. Res Vet Sci 1989; 47: 48-53.
24. **McGEE ZA, WOODS ML Jr.** Use of organ cultures in microbiological research. Annu Rev Microbiol 1987; 41: 291-300.
25. **RUTTER JM, FRANCIS LMA, SANSOM BF.** Virulence of *Bordetella bronchiseptica* from pigs with or without atrophic rhinitis. J Med Microbiol 1982; 15: 105-116.
26. **COLLIER AM.** Techniques for establishing tracheal organ cultures. Tissue Cult Assoc Manual 1976; 2: 333-334.
27. **DUGAL F, GIRARD C, JACQUES M.** Adherence of *Bordetella bronchiseptica* 276 to porcine trachea maintained in organ culture. Appl Environ Microbiol 1990; 56: 1523-1529.
28. **ARP LH, BROOKS EE.** An *in vivo* model for the study of *Bordetella avium* adherence to tracheal mucosa in turkeys. Am J Vet Res 1986; 47: 2614-2617.
29. **BELANGER M, DUBREUIL D, HAREL J, GIRARD C, JACQUES M.** Role of lipopolysaccharides in adherence of *Actinobacillus pleuropneumoniae* to porcine tracheal rings. Infect Immun 1990; 58: 3523-3530.
30. **HEDDLESTON KL, GALLAGHER JE, REBERS PA.** Fowl cholera: gel diffusion precipitin test for serotyping *P. multocida* from avian species. Avian Dis 1972; 16: 925-936.
31. **LUGTENBERG B, van BOXTEL R, de JONG M.** Atrophic rhinitis in swine: correlation of *Pasteurella multocida* pathogenicity with membrane protein and lipopolysaccharides. Infect Immun 1984; 46: 48-54.
32. **VENA MM, BLANCHARD B, THOMAS D, KOBISCH M.** Adherence of *Pasteurella multocida* isolated from pigs and relationship with capsular type and dermonecrotic toxin production. Ann Rech Vét 1991; 22: 211-218.
33. **HORIGUCHI Y, NAKAI T, KUME K.** Purification and characterization of *B. bronchiseptica* dermonecrotoxin. Microb Pathogen 1989; 6: 361-368.
34. **LETELLIER A, DUBREUIL D, ROY G, FAIRBROTHER JM, JACQUES M.** Determination of affinity of *Pasteurella multocida* isolates for porcine respiratory tract mucus, and partial characterization of the receptors. Am J Vet Res 1991; 52: 34-39.