# Adherence of *Bordetella bronchiseptica* and *Pasteurella multocida* to Porcine Nasal and Tracheal Epithelial Cells

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## ABSTRACT

**The ability of 19 different *Bordetella bronchiseptica* isolates and 25 *Pasteurella multocida* isolates to adhere *in vitro* to porcine nasal and tracheal epithelial cells was examined. It was found that *B. bronchiseptica* adhered well to upper respiratory tract cells. In contrast the number of *P. multocida* organisms which adhered was four to six times less than the number of *B. bronchiseptica* adherent organisms. This difference was statistically significant ($p < 0.0001$). Both microorganisms adhered in greater numbers to nasal cells than to tracheal cells ($p < 0.005$). The data indicated that *B. bronchiseptica* possesses a greater ability than *P. multocida* to attach to porcine upper respiratory tract cells.**

## RÉSUMÉ

**Cette expérience consistait à vérifier la capacité de 19 souches de *Bordetella bronchiseptica* et de 25 de *Pasteurella multocida* d'adhérer *in vitro* aux cellules épithéliales nasales et trachéales du porc. Les auteurs constatèrent que *B. bronchiseptica* adhérait bien aux cellules épithéliales des voies respiratoires supérieures, alors que quatre à six fois moins de *P. multocida* manifestaient cette propriété adhésive. Cette différence se révéla significative, du point de vue statistique ($p < 0,0001$). Les deux bactéries expérimentales adhérèrent en plus grand nombre aux cellules épithéliales nasales qu'à celles de la trachée ($p < 0,005$). Il semble par conséquent que *B. bronchiseptica* possède une meilleure capacité d'adhérer aux cellules épithéliales des voies respiratoires du porc, que *P. multocida*.**


Porcine atrophic rhinitis (AR) is a multifactorial disease complex. It is generally agreed that *Bordetella bronchiseptica* and *Pasteurella multocida* are capable of causing atrophy of nasal turbinates in pigs, but the severity and persistence of the changes they induce are different (1). Infection with *B. bronchiseptica* permits colonization by toxigenic *P. multocida* and leads to more severe lesions than does infection with either microorganism alone. There is now good evidence that colonization and toxicity are the most important virulence determinants of these organisms (1).

Adherence is a complex interaction between the bacterium and the target cell which enables colonization to occur and allows the bacterium to exert its pathogenic and immunogenic effects. There is evidence that *B. bronchiseptica* becomes closely associated with the ciliated epithelium of the respiratory tract of pigs (2), dogs (3) and rabbits (4). Little research has been done on the attachment of *P. multocida* to porcine respiratory epithelium. Frymus *et al* (5) observed poor adherence of type D *P. multocida* isolates to porcine nasal epithelial cells. We recently showed that type A *P. multocida* isolates adhered in greater numbers to porcine tracheal epithelial cells than type D isolates (6). It is generally believed that *P. multocida* is a poor colonizer, compared to *B. bronchiseptica* (1). To our knowledge, no studies have compared the adherence of a large number of isolates of both microorganisms under the same experimental conditions. Thus, the purpose of the present study was to compare the adherence of *P. multocida* capsular types A and D, and *B. bronchiseptica* to porcine nasal and tracheal epithelial cells.

Nineteen fresh isolates of *B. bronchiseptica*, retrieved from the nasal cavities of pigs affected (8 isolates) or unaffected (11 isolates) with AR, were kindly supplied by R. Higgins (Faculté de Médecine vétérinaire, Université de Montréal, St-Hyacinthe, Québec). All these isolates were intermediate-phase (smooth colonies which are nonhemolytic), as described by Bemis *et al* (7). Twenty-five isolates of *P. multocida* were also retrieved from the nasal cavities of pigs with (18 isolates) or without (7 isolates) AR. Their phenotypes have been recently described (6). Bacteria were grown in tryptic soy broth (Difco Laboratories, Detroit, Michigan) at 37°C for 18 h, collected by centrifugation (2000 x *g* for 10 min), and suspended in phosphate-buffered saline (PBS, pH 7.2) at the desired concentration of $10^8$ colony-forming units (CFU)/mL.

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**TABLE I. Adherence of *B. bronchiseptica* and *P. multocida* to Porcine Nasal and Tracheal Epithelial Cells**

| Bacterial Isolates | n[a] | Mean Number of Adherent Bacteria per Epithelial Cell ± SD | |
|---|---|---|---|
| | | Nasal | Tracheal |
| *B. bronchiseptica* | 19 | 23.4 ± 11.6 | 15.3 ± 8.1[b] |
| *P. multocida* type A | 10 | 4.4 ± 0.8 | 3.8 ± 0.9[b] |
| *P. multocida* type D | 15 | 3.5 ± 1.0 | 2.8 ± 0.7[b] |

[a]n: number of isolates
[b]Significant difference ($p < 0.005$) between adherence to nasal and tracheal epithelial cells

These suspensions were diluted and 0.1 mL aliquots were plated on blood agar to obtain exact inoculum counts.

Tracheal cells were recovered from newborn, colostrum-deprived piglets, as previously described (6). Nasal epithelial cells were collected by scraping the mucosa of the nasal septum. Both suspensions were adjusted to contain $10^5$ cells/mL as determined by microscopic count using a hemacytometer. The adherence assay has been described in detail (6). Briefly, a mixed suspension of bacteria and epithelial cells was incubated at 37°C for 30 min with agitation (100 rpm). The epithelial cells were centrifuge-washed twice in buffer to remove nonadherent bacteria, spread on glass slides, and stained with the Diff-Quik stain set (American Scientific Products, McGraw Park, Illinois). The mean number of adherent bacteria per ciliated epithelial cell was calculated by examining 20 epithelial cells per slide. Results were compared for statistical significance using the Student's t-test.

The results obtained for the 19 *B. bronchiseptica* isolates and the 25 *P. multocida* isolates regarding their ability to adhere *in vitro* to porcine upper respiratory tract cells are given in Table I. It was found that *B. bronchiseptica* adhered well to porcine tracheal and nasal epithelial cells after the 30 min incubation period. The mean number of adherent *B. bronchiseptica* organisms per epithelial cells was four to six times greater than the number of adherent *P. multocida* organisms ($p < 0.0001$). Capsular type A isolates of *P. multocida* adhered in significantly ($p < 0.005$) greater numbers to porcine epithelial cells than capsular type D isolates. Both microorganisms adhered in larger numbers to nasal cells than to tracheal cells. No significant differences were observed between the adherence of *P. multocida* and *B. bronchiseptica* isolates from pigs with or without AR. Bacteria were attached to the cilia of ciliated cells (Fig. 1), whereas few bacteria were seen attached to nonciliated cells or to the body of ciliated cells.

The present *in vitro* study has demonstrated that *B. bronchiseptica* adheres in higher numbers to porcine respiratory tract cells than does *P. multocida* when tested by the same assay. These findings are consistent with the observations that *B. bronchiseptica* adheres well to porcine nasal epithelial cells (2,8), and porcine tracheal ring explants (9), and that *P. multocida* adheres rather poorly to porcine upper respiratory tract cells (5,6), and rabbit pharyngeal cells (10).

Both microorganisms attach preferentially to the cilia of ciliated epithelial cells. Although adherence of both microorganisms to nasal cells was slightly higher than to tracheal cells, the latter were more easily recovered. Therefore, unless required by a particular experimental design, we suggest that tracheal cells be used when studying the *in vitro* adherence of such microorganisms.

Our comparative study indicates that *B. bronchiseptica* isolates possess a greater ability than *P. multocida* isolates to adhere to porcine upper respiratory tract cells. Our findings may explain, in part, why *B. bronchiseptica* first colonizes porcine upper respiratory tract, and supports the view that *P. multocida* is probably far more important as an aggravating agent in AR than as an initiator of the disease. Further studies are needed to understand how *B. bronchiseptica* assists colonization by *P. multocida*.

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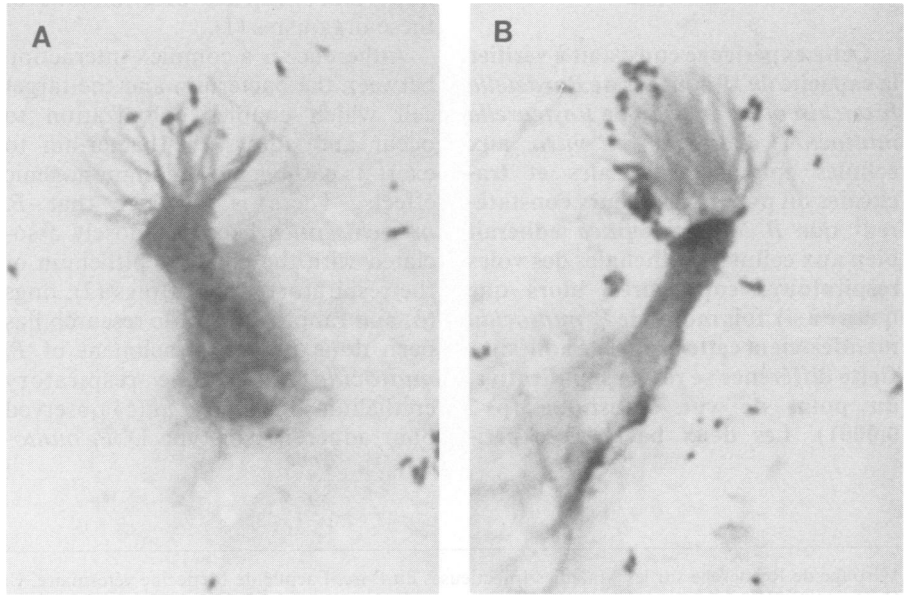


**Fig. 1. Light micrographs of porcine tracheal ciliated cells with adherent bacterial cells. (A) *Pasteurella multocida* 28-6 (capsular type A). (B) *Bordetella bronchiseptica* 402. (magnification: X1000).**

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# BOOK REVIEWS/ANALYSE DE VOLUME

## ELECTRON MICROSCOPY IN DIAGNOSTIC VIROLOGY. A PRACTICAL GUIDE AND ATLAS. *Frances W. Doane and Nan Anderson. Published by Cambridge University Press, New York, N.Y. 1987. 178 pages. Price $52.50.*

Frances Doane and Nan Anderson are faculty members in the Department of Microbiology in the Faculty of Medicine at the University of Toronto who enjoy an established reputation for their pioneering studies on the application of electron microscopy to diagnostic virology. This excellent book brings together in admirably concise but extensively illustrated form many of the techniques developed by the authors during the last fifteen years, as well as methods which have been developed elsewhere. The book will be of interest to both veterinary and medical virologists.

The first five chapters comprise a practical guide to the use of electron microscopical procedures in diagnostic virology. The topics covered include advice on setting up an electron microscopy unit, methods for the preparation and examination of clinical specimens and virus isolates by negative staining, immunoelectronmicroscopy and thin sectioning, as well as the screening of cell cultures for adventitious agents. The methods are all clearly described with helpful diagrams, although the description of techniques for the preparation of thin sections of infected cells is somewhat fragmentary. The latter procedure, however, is much less useful in a diagnostic context than negative staining and immunoelectronmicroscopy, in which the authors have made their main original contributions. Of special interest is the recently developed immunoelectronmicroscopical procedure which utilizes protein A-gold to enhance the visibility of the antigen-antibody reaction. This section concludes with a brief appendix which details methods for the preparation of specimen grid support films and negative stains.

The remainder of the book consists essentially of an atlas of the major families of animal viruses. After an introductory chapter covering the basic features of viral morphology and morphogenesis, the DNA and RNA viruses are dealt with in turn, family by family. Each family of viruses is illustrated by micrographs of negatively stained virions and thin sections of infected cells, for which a brief commentary describing the major features of morphology and morphogenesis is provided. The micrographs, many of which were prepared in the authors' laboratory, are of generally high quality, and they have been carefully selected. Appropriate references to the literature are provided for each virus family. The final chapter contains examples of some of the confusing structures which may be encountered in biological specimens during the search for animal viruses. These include bacteriophages, cellular membranes, filaments and tubules, and various artifacts which may sometimes be found in both negatively stained specimens and thin sections of cells and tissues, and which may challenge the interpretive ability of the microscopist.

While several texts which have appeared in recent years cover some aspects of the application of electron microscopy to diagnostic virology, none better combines clearly described methods with excellent illustrations. This book should be readily available at the bench in all diagnostic virology laboratories, where it will be an invaluable and constant source of reference both on the techniques required for electron microscopical examination of viruses and on the morphological and morphogenetic features of viruses which are useful for their identification.

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