JOURNAL OF CLINICAL MICROBIOLOGY, June 1992, p. 1398–1401
0095-1137/92/061398-04$02.00/0
Copyright © 1992, American Society for Microbiology
Vol. 30, No. 6

# Characterization of *Pasteurella multocida* from Nasal Cavities of Piglets from Farms with or without Atrophic Rhinitis

S. LARIVIERE, L. LEBLANC, K. R. MITTAL,* AND G.-P. MARTINEAU

*Department of Pathology and Microbiology, Faculty of Veterinary Medicine, University of Montreal, St. Hyacinthe, Quebec, Canada J2S 7C6*

Received 25 November 1991/Accepted 10 March 1992

**A total of 137 strains of *Pasteurella multocida* isolated from the nasal tracts of pigs with and without clinical atrophic rhinitis (AR) were studied for their biochemical, antigenic, and toxigenic characteristics. There were no major biochemical differences among the *P. multocida* isolates. Capsular antigen types A and D were both present in the nasal cavities of the pigs with or without clinical AR. However, the prevalence of type D was higher on farms with pigs with AR. Types A and D with different somatic antigens could both be present in the same pig. There was no correlation between somatic types and/or capsular types with the clinical AR status of the pigs on the farm. Toxigenic isolates were found only in pigs which had a problem of clinical AR, and a great majority of these isolates belonged to type D. Since there was a high level of heterogeneity of the strains in the *P. multocida* population on a farm, several strains should be characterized before the diagnosis of AR could be excluded on the basis of the absence of isolation of rhinopathogenic *P. multocida* strains.**

*Pasteurella multocida* is often isolated from the nasal cavities of piglets from farms that have pigs with or without clinical signs of atrophic rhinitis (AR). AR is present in a large number of pig farms in Quebec. Since the work of De Jong et al. (7), several workers have demonstrated that only certain strains of *P. multocida* are associated with AR and that rhinopathogenicity is linked with the presence of a dermonecrotoxin (8, 16, 18–20). The studies described here were carried out in order to ascertain whether *P. multocida* strains with only certain patterns based on antigenic and toxigenic characteristics are associated with clinical AR.

## MATERIALS AND METHODS

**Collection of nasal secretions.** Nasal swabs (Calgi swab type 1 Inolex) were used to collect the nasal mucus from 4- and 8-week-old piglets. The nasal swabs were introduced into the piglet's nasal cavities as far as possible and were then placed in 2 ml of transport medium containing 50% fetal calf serum (Difco) in phosphate-buffered saline solution (pH 7.3; Oxoid). The samples were kept on ice during transport and, on the same day, were examined by culture for the presence of *P. multocida*.

**Isolation of *P. multocida*.** Two methods were used for the isolation of *P. multocida*; one used a selective culture medium and the other used inoculation of *P. multocida* into mice. Blood agar medium (tryptose soy agar [Difco] containing 5% bovine blood) was initially used for the isolation of *P. multocida*. Later, it was replaced by the media described by Smith and Baskerville (24) and Knight et al. (14).

Mice were used for the isolation of *P. multocida* by the method reported by Pedersen and Barfod (18). A 0.5-ml portion of transport medium containing nasal secretion was injected directly into one young adult Swiss albino mouse by the intraperitoneal route. The mice that died within 7 days of inoculation were autopsied, and their livers were examined for the presence of *P. multocida* by culture of liver samples on blood agar. Both selective culture media as well as mice were used to isolate *P. multocida*.

* Corresponding author.

**Selection of the isolates.** *P. multocida* colonies were selected on different media according to their morphological characteristics. A maximum of two isolates were selected from each piglet, and a maximum of 20 isolates from each farm were kept. A total of 266 isolates were conserved, of which 137 isolates were studied for their biochemical, antigenic, and toxigenic characteristics. Isolates were thus obtained from 89 pigs on nine farms with pigs with clinical signs of AR and from 48 animals on eight farms with pigs without any clinical signs of AR.

**Rapid identification of isolates.** Initially, the isolates were rapidly identified as *P. multocida* by using the minimum number of necessary characteristics. Isolates which were gram-negative, nonmotile, small rods that were unable to grow on MacConkey agar and that were positive for oxidase and indol were identified as *P. multocida*.

**Conservation of the isolates.** *P. multocida* isolates were conserved by the desiccation method of Gherna (9) by using sterile disks of 6.5 mm in diameter (Can Laboratories).

**Biochemical characterization.** A total of 137 isolates were subjected to a battery of different biochemical tests in order to characterize them in detail. The following tests were used: production of urease; nitrate reduction; decarboxylation of ornithine; and utilization of various sugars such as arabinose, glucose, glycerol, lactose, maltose, mannose, sorbitol, trehalose, and xylose. The production of urease was determined on Christensen's medium (urea agar base; Difco). Decarboxylation of ornithine was evaluated by using Moeller decarboxylase broth medium (Difco), and utilization of sugars was tested in phenol red broth medium (Difco). After inoculation, the media were incubated aerobically at 37°C. The readings were taken after 24 h for all tests. A second reading was taken after 48 h only for tests with sugars which were negative after the first reading. Results were interpreted according to the recommendations of MacFaddin (15).

**Antigenic characterization. (i) Determination of capsular antigens.** Capsular antigen type A was identified by the hyaluronidase test (4), and capsular antigen type D was identified by the acriflavine test (5). Isolates that were nontypeable by these two nonserological methods and some

isolates that were typeable by these methods were examined for their capsular antigen types by indirect hemagglutination tests by using antisera against reference strains of capsular antigen types A, B, D, and E produced in rabbits. Reference strains (TS8, type A; C81 [401/63], type B; P.27, type D; Buniya II, type E) were obtained from G. R. Carter (Regional College of Veterinary Medicine, Virginia-Maryland). A whole-cell saline extract that was obtained after the whole-cell suspension was heated at 56°C for 30 min was used for sensitization of human type O group erythrocytes for the indirect hemagglutination test. The test was performed as described by Carter (3).

**(ii) Determination of somatic antigens.** The somatic antigen groups of the isolates were determined by the immunodiffusion test (11). Reference strains of *P. multocida* representing somatic groups of 1 to 16 (X-73 [group 1], M-1404 [group 2], P-1059 [group 3], P-1662 [group 4], P-1702 [group 5], P-2192 [group 6], P-1997 [group 7], P-1581 [group 8], P-2095 [group 9], P-2100 [group 10], P-903 [group 11], P-1573H [group 12], P-1591H [group 13], P-2225 [group 14], P-2237 [group 15], and P-2723 [group 16]) were obtained from K. R. Rhodes (North Central Region, National Animal Disease Center, Agricultural Research Service, Ames, Iowa). Hyperimmune sera against all the type strains were produced in 10-week-old chickens (11).

*P. multocida* strains were tested for somatic group antigens by an immunodiffusion test by using 0.9% special Noble agar (Difco) dissolved in 8.5% sodium chloride solution (11).

**Toxigenic characterization.** The production of dermonecrotoxin by *P. multocida* isolates was determined by using a guinea pig skin test as described by De Jong et al. (7). Briefly, each isolate was cultivated in brain heart infusion broth and centrifuged. The supernatant was sterilized using a 0.22-μm-pore-size Millipore filter. A 0.2-ml portion of the filtrate was injected intradermally into the shaved skin of an adult guinea pig. Six to eight samples were tested for dermonecrotoxin production on a single guinea pig. Readings were taken 3 days after injection. Positive and negative controls were always used.

## RESULTS

**Biochemical characterization.** All 137 isolates tested were positive for glucose, mannitol, and sorbitol utilization and nitrate reduction. They were all negative for lactose and maltose utilization and urease production. One isolate was positive for arabinose utilization, 28 were positive for glycerol utilization, 135 were positive for xylose utilization, and 8 were positive for trehalose utilization. Only two isolates were negative for ornithine decarboxylation. Finally, 11 isolates were considered biovariants which did not form a predominant group on any of the farms (data not shown).

**Antigenic characterization. (i) Capsular antigens.** All except eight isolates examined in this study were classified into capsular antigen group A or D by nonserological methods. However, the strains which remained untypeable by nonserological methods were easily serotyped by the indirect hemagglutination test. Both nonserological and indirect hemagglutination tests correctly identified the capsular antigens of 21 strains selected at random. Capsular antigen types A and D were isolated from pigs with or without clinical AR. None of the isolates examined belonged to capsular antigen type B or E. However, capsular antigen type D strains were more frequently isolated from pigs coming from farms with pigs with clinical AR than from pigs coming from farms with pigs without clinical AR (Tables 1 and 2).

TABLE 1. Distribution of *P. multocida* of different capsular and somatic antigen types on farms with pigs with or without clinical signs of AR

| Farm status[a] | No. of isolates tested[b] | % of strains positive for: Capsular antigen type: A | D | Somatic antigen type: 3, 16 | 15 | 4, 7 | 1 | Other[c] | NT[d] | ND[e] |
|---|---|---|---|---|---|---|---|---|---|---|
| $AR^+$ | 89 (9) | 38 | 62 | 44 | 11 | 15 | 8 | 8 | 12 | 2 |
| $AR^-$ | 48 (8) | 67 | 33 | 73 | 0 | 4 | 8 | 13 | 2 | 0 |

[a] $AR^+$, farm with pigs showing clinical signs of AR; $AR^-$, farm with pigs showing no clinical signs of AR.
[b] Values in parentheses are numbers of farms.
[c] Other indicates other somatic antigens (1, 15, 7; 1, 3, 7, 16; 4, 7, 15; 1, 4, 7, 16; etc.).
[d] NT, nontypeable.
[e] ND, not determined.

**(ii) Somatic antigens.** *P. multocida* isolates were classified into six serogroups on the basis of somatic antigens. The majority of the isolates tested belonged to serotypes 3 and 16 and were found in pigs on 76% of the farms examined. Most of the nontypeable strains were also isolated from farms with pigs with clinical AR. Generally, the strains isolated from any one farm belonged to more than one serogroup (Table 1).

**Toxigenic characterization.** Toxigenic strains of *P. multocida* were isolated from pigs on five of nine farms with pigs with clinical AR and from none of the farms with pigs which were free of AR. Thirty-eight percent of all strains isolated from pigs on farms that had pigs with clinical AR were toxigenic. The prevalence of the toxigenic strains on five farms with pigs that were clinically affected by AR varied from one farm to another, ranging from 18 to 100%. Only one isolate belonging to type A was recognized as toxigenic. None of the isolates from pigs on four farms that had pigs with clinical AR was toxigenic, even though type D isolates were found on these farms (Table 2).

TABLE 2. Distribution of *P. multocida* isolates with different characteristic patterns on farms with pigs with or without clinical signs of AR

| Isolate patterns: Capsular antigen | Somatic antigen[a] | Toxigenicity | No. of isolates | No. of farms[b]: $AR^+$ | $AR^-$ |
|---|---|---|---|---|---|
| A | 3, 16 | − | 52 | 6 | 4 |
| A | 4, 7 | − | 7 | 2 | 0 |
| A | NT | − | 1 | 1 | 0 |
| A | 1, 15, 7 | − | 1 | 1 | 0 |
| A | 1, 3, 7, 16 | − | 1 | 0 | 1 |
| A | 4, 7 | + | 2 | 1 | 0 |
| D | 3, 16 | − | 21 | 3 | 5 |
| D | 4, 7 | − | 5 | 3 | 1 |
| D | 15 | − | 10 | 3 | 0 |
| D | 4, 7, 15 | − | 1 | 0 | 1 |
| D | 1, 4, 7, 16 | − | 1 | 0 | 1 |
| D | NT | − | 3 | 2 | 1 |
| D | 3, 16 | + | 20 | 1 | 0 |
| D | 4, 7 | + | 2 | 2 | 0 |
| D | NT | + | 8 | 1 | 0 |
| D | ND | + | 2 | 1 | 0 |

[a] NT, nontypeable; ND, not determined.
[b] See footnote *a* of Table 1 for definitions of $AR^+$ and $AR^-$.

**Establishment of *P. multocida* patterns.** On the basis of studies of the characteristics of the 137 *P. multocida* strains isolated from pigs on 17 farms, it was possible to differentiate them into at least 15 different patterns, 12 for farms with pigs with clinical AR and 7 for farms with pigs without clinical AR. The predominant patterns were type A toxin negative (type 3,16), representing 38% of the isolates, and type D toxin negative (type 3,16), representing 15% of the isolates. These two patterns were found on farms with pigs with and without clinical AR. However, 15% of the total isolates were type D toxin positive (type 3,16) and were isolated only from farms with pigs with clinical AR (Table 2).

## DISCUSSION

Results of biochemical characterization of the *P. multocida* isolates were in accordance with those reported by various workers (10, 12, 25). Most of the isolates possessed similar biochemical profiles except for their reactions in glycerol. Because the glycerol utilization test in particular is difficult to read and interpret, it is not certain whether results of this test represented true variation among the strains. It is emphasized that most of the biovariants were isolated from pigs on four farms.

Characterization of the strains on the basis of their capsular antigens did not permit us to demonstrate any association with clinical AR or the predominance of one type over the other. Both type A and D strains were present in the nasal cavities of the pigs from farms with pigs with or without clinical signs of AR. These results are similar to those reported by Soderlind and Bergstrom (26) and Sawata et al. (21). Characterization of the strains on the basis of their somatic antigens revealed a high level of heterogeneity among the isolates. At least a dozen somatic serogroups were present in isolates from pigs on farms in Quebec. Serogroup 3 was most commonly found in association with serogroup 16 but not with serogroup 4 or 5, as reported by Backstrom et al. (1). Toxigenic isolates were found only on farms with pigs with clinical AR, and most of these isolates belonged to capsular antigen type D (Table 2). However, the frequency of isolation of toxigenic isolates was much less in the current studies than has been reported elsewhere. The high frequency of isolation of toxigenic isolates reported by Pedersen (17), Schoss and Thiel (22), Schoss et al. (23), and Hoffman et al. (13) could be due to the increased severity of AR in pigs on the farms that they studied. Bechmann and Schoss (2) studied 548 strains of *P. multocida* isolated from tonsillar and nasal swabs collected from 468 piglets on 30 farms with pigs that were completely free of clinical AR. They reported that most of the isolates belonged to capsular antigen type D, and none of the isolates produced exotoxin in cell cultures. Cowart et al. (6) cultured nasal swab and lung samples from 163 pigs on nine farms representing various levels of AR for *Bordetella bronchiseptica* and *P. multocida*. Toxigenic type D strains of *P. multocida* were isolated from nasal swabs of only seven pigs (4%) and from cultures of a lung sample from only one pig. Somatic serovar 3 was found in 93% of the nasal isolates and in all lung isolates. Serovars 4, 5, 7, and 12 were found in low numbers, either alone or in combination with serovar 3. They further reported that somatic serovars showed no relationship with either capsular antigen serotype or pathological findings.

On the basis of the overall results of the characterization studies of *P. multocida*, it was interesting to find such a great heterogeneity among *P. multocida* isolates. As shown in Table 2, at least 15 different patterns were found, and 89 isolates coming from farms with pigs with clinical AR could be grouped into 12 different patterns and 48 isolates from farms with pigs without AR could be grouped into 7 patterns. It is possible that each pattern may represent one strain. Most of the farms had between three and six strains with different patterns. Pigs on 11 of 15 farms from which a minimum of two isolates were found harbored strains with more than one pattern. Thus, the results of this study provide strong evidence that several isolates must be characterized in order to exclude the possibility of AR on a farm on the basis of the absence of isolation of rhinopathogenic strains of *P. multocida*.

## REFERENCES


1. **Backstrom, L., D. Hoefling, A. Morkoc, R. Sinson, and A. R. Smith.** 1982. Atriphic rhinitis in swine. II. Bacteriology, *Pasteurella multocida* serotypes and pathogenicity in mouse virulence tests, preventive medications and vaccinations, p. 122. Proceedings of the 7th IPVS Congress, Mexico.
2. **Bechmann, G., and P. Schoss.** 1988. Investigations on the occurrence of toxigenic *Pasteurella multocida* strains in the tonsils and noses of piglets in breeding herds not suspected of atrophic rhinitis. Dtsch. Tierarzt. Wochenschr. **95:**283–285.
3. **Carter, G. R.** 1955. Studies on *Pasteurella multocida*. I. A hemagglutination test for the identification of serological types. Am. J. Vet. Res. **16:**481–484.
4. **Carter, G. R., and S. W. Rundell.** 1975. Identification of type A strains of *Pasteurella multocida* using staphylococcal hyaluronidase. Vet. Rec. **96:**343.
5. **Carter, G. R., and P. Subronto.** 1973. Identification of type D strains of *Pasteurella multocida* with acriflavine. Am. J. Vet. Res. **34:**293–294.
6. **Cowart, R. P., L. Backstrom, and T. A. Brim.** 1989. *Pasteurella multocida* and *Bordetella bronchiseptica* in atrophic rhinitis and pneumonia in swine. Can. J. Vet. Res. **53:**295–300.
7. **De Jong, M. F., H. L. Oei, and G. J. Tetenburg.** 1980. Atrophic rhinitis pathogenicity tests for *Pasteurella multocida* isolates, p. 211. Proceedings of the 6th IPVS Congress, Copenhagen, Denmark.
8. **Foged, N. T., J. P. Nielsen, and A. L. Schirmer.** 1988. Use of monoclonal antibodies in the diagnosis of atrophic rhinitis, p. 33. Proceedings of the 10th IPVS Congress, Rio de Janeiro, Brazil.
9. **Gherna, R. L.** 1981. Preservation, p. 209. *In* Manual of methods for general bacteriology. American Society for Microbiology, Washington, D.C.
10. **Ghoniem, N., B. Amtshey, and W. Bisping.** 1973. Comparative studies of the biochemical reaction spectrum strains of *Pasteurella multocida* in the dog and pig. Zentralbl. Vet. Med. Teil B **20:**310–317.
11. **Heddleston, K. L., J. E. Gallaher, and P. A. Rebers.** 1972. Fowl cholera: gel diffusion precipitation test for serotyping *Pasteurella multocida* from avian species. Avian Dis. **16:**925–936.
12. **Heddleston, K. L., R. D. Shyman, and F. L. Earl.** 1954. Atrophic rhinitis. IV. Nasal examination for *Pasteurella multocida* in two swine herds affected with atrophic rhinitis. J. Am. Vet. Med. Assoc. **125:**225–226.
13. **Hoffman, L. J., T. Klirefelter, and J. Upah.** 1989. Recovery of toxigenic *Pasteurella multocida* from Iowa swine herds affected with atrophic rhinitis, p. 98. Proceedings of the World Association of Veterinary Laboratory Diagnosticians, Guelph, Ontario.
14. **Knight, D. P., J. E. Paine, and D. C. E. Speller.** 1983. A selective medium for *Pasteurella multocida* and its use with animal and human specimens. J. Clin. Pathol. **36:**591–594.
15. **MacFaddin, J. F.** 1980. Biochemical tests for identification for medical bacteria, p. 527. The Williams & Wilkins Co., Baltimore.
16. **Nakai, T., A. Sawata, M. Tsuji, Y. Samejima, and K. Kume.** 1984. Purification of dermonecrotic toxin from a sonic extract of *Pasteurella multocida* SP-72 serotype D. Infect. Immun. **46:**429–434.

17. **Pedersen, K. B.** 1982. The occurrence of toxin-producing strains of *Pasteurella multocida* in SPF herds, p. 82. Proceedings of the 7th IPVS Congress, Mexico.
18. **Pedersen, K. B., and K. Barfod.** 1981. The etiological significance of *Bordetella bronchiseptica* and *Pasteurella multocida* in atrophic rhinitis of swine. Nord. Vet. Med. **33:**513–522.
19. **Pijoan, C., A. Lastra, C. Ramirez, and A. D. Leman.** 1984. Isolation of toxigenic strains of *Pasteurella multocida* from lungs of pneumonic swine. J. Am. Vet. Med. Assoc. **185:**522–523.
20. **Rutter, J. M., N. J. Rolley, and A. MacKenzie.** 1984. The toxigenicity of *Pasteurella multocida* in atrophic rhinitis, p. 156. Proceedings of the 8th IPVS Congress, Ghent, Belgium.
21. **Sawata, A., T. Nakai, M. Tsuji, and K. Kume.** 1984. Dermonecrotic activity of *Pasteurella multocida* strains isolated from pigs in Japanese field. Jpn. J. Vet. Sci. **46:**141–148.
22. **Schoss, P., and C. P. Thiel.** 1984. Occurrence of toxin producing strains of *Pasteurella multocida* and *Bordetella bronchiseptica* in pig herds with atrophic rhinitis and in unaffected herds, p. 162. Proceedings of the 8th IPVS Congress, Ghent, Belgium.
23. **Schoss, P., C. P. Thiel, and H. Schimmelpfennig.** 1985. Atrophic rhinitis of swine: occurrence of toxin producing strains of *Pasteurella multocida* and *Bordetella bronchiseptica*. Dtsch. Tievarzt. Wochenschr. **92:**316–319.
24. **Smith, I. M., and A. J. Baskerville.** 1983. A selective medium for the isolation of *Pasteurella multocida* in nasal specimens from pigs. Br. Vet. J. **139:**476–487.
25. **Smith, J. E.** 1958. Studies on *Pasteurella septica*. II. Some cultural and biochemical properties of strains from different host species. J. Comp. Pathol. **68:**315–323.
26. **Soderlind, D., and G. Bergstrom.** 1984. Bacteriological investigations as a background for vaccination in herds, p. 175. Proceedings of the 8th IPVS Congress, Ghent, Belgium.