Restriction Endonuclease Analysis of Porcine *Pasteurella multocida* Isolates from Quebec

Josée Harel, Sandra Côté and Mario Jacques

ABSTRACT

We have used restriction endonuclease analysis (REA) of genomic DNA to classify porcine *Pasteurella multocida* isolates with similar capsular and somatic serotypes, and to monitor the distribution of isolates from 12 different herds in Quebec. Within herds, *P. multocida* isolates of similar capsular and somatic serotypes showed similar REA fingerprints. Between herds, some isolates had similar REA fingerprints. However, differences in REA enabled subtyping of many *P. multocida* isolates with the same antigen types. Our data indicate that REA would enable accurate epidemiological typing of *P. multocida* in conjunction with classical capsular and somatic typing.

RÉSUMÉ

Cette étude visait à classifier les isolats porcins de *Pasteurella multocida,* provenant de 12 troupeaux du Québec, à l'intérieur de leur sérotype capsulaire et somatique et à déterminer leur distribution. Les auteurs ont analysé à cette fin l'empreinte génomique obtenue par digestion des génomes des différents isolats de *P. multocida* avec des endonucléases de restriction. Il a été observé que, dans un même troupeau, les isolats ayant le même serotype capsulaire et somatique avaient un patron d'empreinte génomique similaire. Dans plusieurs cas, des isolats possédant un patron d'empreinte génomique similaire se retrouvaient dans différents troupeaux. Les différents patrons d'empreinte génomique obtenus par enzymes de restriction ont permis de classifier les isolats de *P. multocida* qui avaient les mêmes antigènes capsulaires et somatiques. Les résultats obtenus ont suggéré que l'empreinte génomique des endonucléases de restriction s'avère un très bon outil pour un typage épidémiologique de *P. multocida* lorsque utilisé en conjonction avec les sérotypages capsulaires et somatique.

INTRODUCTION

Atrophic rhinitis (AR) is an important disease of pigs and is characterized by rhinitis, atrophy or hypoplasia of the nasal turbinate bones, snout deformation, and poor growth rates (1). *Pasteurella multocida* and *Bordetella bronchiseptica* are recognized as the etiological agents of porcine AR (2,3).

Serotyping of *P. multocida* strains is based on differences in capsular polysaccharides (4) and in O-antigen groups of lipopolysaccharides (LPS) (5,6). Several capsular and somatic serogroups are involved in pasteurellosis (3). Leblanc *et al* (7) have shown a great heterogeneity among strains of porcine *P. multocida* isolated in Quebec, based on both capsular and somatic antigens and the presence or absence of dermonecrotoxin. The major groups were represented by nontoxigenic strains of serotype A 3,16 followed by toxigenic and nontoxigenic strains of serotype D 3,16 (8). Toxigenic *P. multocida* were mainly found in herds with clinical AR or with history of AR (7,8).

Recent investigations have shown that the progressive changes in the snout and turbinate bones can be reproduced by using certain strains of *P. multocida*, capsular serotype A and D which produce a novel protein toxin (3). The toxin of *P. multocida* which is mostly associated with capsular type A and D isolates produces turbinate atrophy and snout deformation after inoculation of pigs (3). Current evidence indicates that toxin-producing *P. multocida* organisms are involved in the etiology of naturally-occurring progressive atrophic rhinitis of swine (1). The toxin gene has been cloned in *Escherichia coli* (9-11).

Restriction endonuclease analysis (REA) has proved valuable for strain discrimination among bacteria (12-17). Restriction endonuclease analysis fingerprinting permits the identification of unique strains within groups of related organisms (16). Restriction endonuclease analysis fingerprinting did permit the differentiation of isolates of *P. multocida* from turkeys in serotype A:3 (17). To study the occurence and distribution of various porcine isolates of *P. multocida* from 12 herds in Quebec, we used REA to classify the isolates within a capsular and somatic serogroup and to monitor the distribution of strains within a particular herd and among herds. Twenty-three *P. multocida* isolates, including 18 from pigs associated with an outbreak of atrophic rhinitis, were typed on the basis of capsular and somatic antigens, biochemically char-

Département de pathologie et de microbiologie, Faculté de Médecine vétérinaire, Université de Montréal, C.P. 5000, Saint-Hyacinthe, Québec J2S 7C6.

This study was supported by a grant from Fonds pour la formation de Chercheurs et l'Aide à la Recherche (90EQ3725) and from Fonds de la Recherche en Santé du Québec (870046).

Submitted August 22, 1989.

acterized and examined by total cellular DNA restriction endonuclease digest analysis.

MATERIALS AND METHODS

BACTERIAL STRAINS

A total of 23 isolates of *P. multocida* were examined in this study. The isolates were retrieved from the nasal cavities of pigs from herds with (18 isolates) or without (5 isolates) clinical signs of atrophic rhinitis in the herd. Some isolates (1354-5, 1354-4, 1354-10, 1354-12) were recovered from animals showing severe atrophic rhinitis in 1988. All other strains were isolated in 1984 (18). The isolates studied as well as their farm identification are listed in Table I. The farms were located in the St-Hyacinthe area, Quebec. Bacteria were grown in brain heart infusion broth (Difco, Detroit, Michigan) at 37°C for 18 h in an Orbit environment shaker (New Brunswick).

Identification of capsular types A and D was done by hyaluronidase and acriflavine tests (6). Somatic antigens were identified serologically by gel precipitin tests (5). Production of a dermonecrotoxic toxin were determined by the guinea pig skin test as described by Lugtenberg *et al* (19). Capsular and somatic characterization and toxigenic properties of the isolates are presented in Table I.

BIOCHEMICAL CHARACTERIZATION

The methods and media used to determine the biochemical characterization of the bacterial strains have been described by Muuters *et al* (20). *Pasteurella multocida* subsp. *multocida* HIM 975-1 (NCTC 10322) was used as a reference strain.

TOTAL CELLULAR PREPARATION

The DNAs were extracted using a procedure based on the method of Marmur (21). Briefly, bacteria were grown in 5 mL of brain heart infusion broth on a shaker. Cells were harvested, washed and resuspended in 1.2 mL buffer (0.5 M NaCl, 0.01 M ethylenediaminetetraacetate (EDTA) containing lysosyme (1 mg/mL) and incubated at room temperature for 30 min. Sodium dodecyl sulfate (SDS) was added (0.8 mL of 10% solution) and the mixture incubated for 5 min prior to the addition of proteinase K (final concentration: 1 mg/mL), the lysates were shaken for 2 h at 37°C. Following several phenol and chloroform extractions, purified DNA was dialyzed against 10 mM Tris HCl, 1 mM EDTA, pH 8.0, at 23°C for 24 h and at 4°C for 36 h.

TOTAL CELLULAR DIGESTS

Approximately 1.0 to 2.0 μg of DNA sample was used for each restriction endonuclease digest. The bacterial DNA was digested with *Eco*RI or *Hin*dIII restriction endonucleases separately according to the manufacturer's instructions. Restriction enzymes were purchased from Pharmacia LKB Biotechnology Inc., Baie d'Urfe, Quebec. Restriction fragments were separated by electrophoresis for 12 h on 0.8% agarose gels in Tris-acetate buffer (0.04 M Tris-acetate, 0.002 M EDTA), at 25 volts. DNA bands were visualized by staining the gel with ethidium bromide (1 μg/mL) (22). Polaroid photographs were taken during exposure to 256 nm ultraviolet light illumination.

Bacteriophage lambda DNA (strain cl857 Sam7) cut with *Eco*RI and *Hin*dIII was used as a molecular weight marker. Digestion of the lambda DNA produced fragments of 21.226, 5.148, 4.973, 4.266, 3.530, 2.027, 1.904, 1.584, 1.375 kilobases, 947, 831, 564, and 125 basepairs.

The bacterial DNAs were digested with *Eco*RI and *Hin*dIII restriction endonucleases separately. The REA classification is based on both the *Eco*RI data and the *Hin*dIII data. Restriction endonuclease analysis fingerprints of the isolates were compared within serotypes and comparisons were made within gels. Among each capsular and somatic serogroup, REA fingerprints were compared and isolates with similar profile were grouped. Within and among herds, REA fingerprints were compared and isolates with similar profiles were grouped.

TABLE I. Source, serological and toxigenic characterization of the *P. multocida* isolates

Herd	Isolate	AR[a]	Antigen type		Dermonecrotic toxin[c]
			Capsular	Somatic[b]	
16	1604	+	D	4,7	+
17	1703	+	D	4,7	+
23	23-3	+	D	4,7	–
23	23-4	+	A	4,7	–
23	2305-A[d]	+	A	4,7	+
23	2305-B[d]	+	A	4,7	+
23	23-8	+	D	1,7,15,16	–
24	24-6	+	D	3,16	–
26	26-2	–	A	3,16	–
26	26-5	–	D	3,16	–
27	27-1	+	D	4,7	+
28	28-1	+	A	3,16	–
28	28-9	+	D	15	–
30	3001	+	D	3,16	+
30	30-1	+	D	3,16	+
30	3002-1	+	D	3,15,16	–
32	32-1	–	A	3,16	–
36	3601	–	D	3,16	–
37	3750	–	D	3,16	–
1354	1354-5	+	A	ND[e]	–
1354	1354-4	+	D	ND[e]	+
1354	1354-10	+	D	ND[e]	+
1354	1354-12	+	D	ND[e]	+

[a]+, from a pig from herd with clinical signs of atrophic rhinitis;
–, from a pig from herd without clinical signs of AR
[b]Typing system of Heddleston *et al* (5)
[c]Determined by the guinea pig skin test of Lugtenberg *et al* (19)
[d]Strains isolated from the same animal
[e]Not determined

RESULTS

BIOCHEMICAL TESTS

All *P. multocida* isolates tested were classified as *P. multocida* subsp. *multocida*. The biochemical reactions were common to all isolates (Orni-

thine+, Indole+, Urease-, Maltose-, D-Xylose+, L-Arabinose+, Mannitol-, Sorbitol+, Dulcitol+) except for acid production from the trehalose. Only two isolates, 28-1 and 32-1 fermented trehalose.

GENOMIC REA

REA patterns: Seven of the 23 compared isolates had unique fingerprints. Only isolates with identical REA patterns are presented in Table II. Sixteen of the 23 compared isolates were grouped into six REA patterns.

Comparison of fingerprints within serotypes: In serotype A 4,7, three strains from the same herd, 23-4, 2305-A and 2305-B (Fig. 1, lane A, lane B, and lane C respectively) had the same REA fingerprint (Table II) although phenotypically they differed in their ability to produce toxin (Table I)). It is not known whether strain 23-4 has lost its capacity to produce dermonecrotoxic toxin because of *in vitro* manipulations, or if the strain is genotypically nontoxigenic because of a mutation or presence of a possibly lysogenic phage.

In serotype A 3,16, two isolates from different herds, 28-1 and 32-1 (Fig. 1, lane E and lane F, respectively) had the same REA fingerprint (Table II) which differed from that of 26-2 (Fig. 1, lane D). It is of interest to note that the biochemical reactions were common to all isolates except two, 28-1 and 32-1 which were the only isolates to ferment trehalose.

In serotype D 3,16 (Fig. 2), the isolates had very similar REA fingerprints (Fig. 2, lanes A to G); however, distinct bands were apparent in isolates 3750, 30-1, 3001 and 3002-1 (Fig. 2, lane D, lane E, lane F and lane G respectively), which are due to plasmid bands (Coté *et al,* Am J Vet Res (in press)). The molecular weight of the plasmid bands generated by *Eco*RI digestion from isolates 30-1, 3001 and 3002-1 were 5.6, 2.0, 1.65 and 1.35 Kb. The molecular weight of the plasmid band generated by *Eco*RI digestion from isolate 3750 was 5.9 Kb. The isolates 30-1, 3001 and 3002-1 had the same REA fingerprint (Table II) although phenotypically they differed in their ability to produce a dermonecrotic toxin (Table I). Other isolates from the same serotype D 3,16, isolates 26-5, 24-6, and 3601 showed a similar REA fingerprint (Fig. 2, lane A, lane B and lane C, respectively) (Table II).

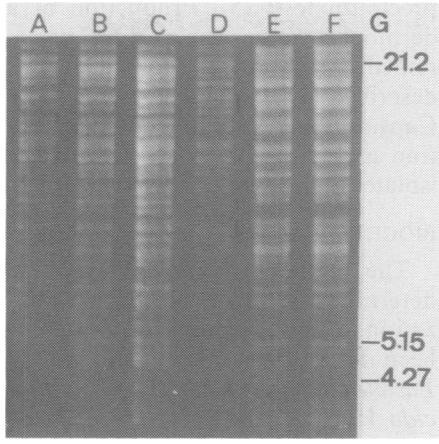

Fig. 1. Restriction endonuclease analysis fingerprints of genomic *Eco*RI digested DNAs from *P. multocida* isolates of capsular serotype A. A-C, isolates of serotype A 4,7; D-F, isolates of serotype A 3,16. Isolate 23-4 (lane A), isolate 2305-A (lane B), isolate 2305-B (lane C), isolate 26-2 (lane D), isolate 28-1 (lane E), isolate 32-1 (lane F), lambda *Eco*RI-*Hin*dIII molecular weight marker (lane G). Black lanes in the figure point some major differences in the patterns.

In serotype D 4,7 two isolates from different herds, 23-3 and 27-1 (Fig. 2, lane K and lane L, respectively) showed a similar genomic REA fingerprint (Fig. 2) (Table II). The isolates 1604 and 1703 (Fig. 2, lane I and lane J, respectively) from different herds had their own genomic pattern. The presence of plasmid bands generated by *Eco*RI digestion is probably responsible for the major differences between their REA pattern.

In serotype D 15, isolates 28-9 and 23-8 (Fig. 2, lane N and lane O, respectively) showed difference in their REA fingerprint only due to the presence of a 1.6 Kb plasmid band generated by *Eco*RI digestion in isolate 28-9.

Comparisons of fingerprints within herds: Within three different herds with AR, isolates with similar serotypes shared similar REA fingerprints (Table II). In herd 23 (Fig. 1, lane A, lane B, lane C), three isolates (2305-A, 2305-B, 23-4) out of five isolates shared the same genomic fingerprint (Table II). In herd 30, the isolates of serotype D 3,16 (3001, 30-1, 3002) had the same REA fingerprint (Fig. 2, lane E, lane F, and lane G, respectively). In herd 1354, three isolates (1354-4, 1354-10, 1354-12) (Fig. 3, lane B, lane C and lane D) shared the same REA fingerprint (Table II) and they were of serogroup D while 1354-5 did not show a similar REA fingerprint (Fig. 3, lane A) and was of serogroup A.

Comparison of fingerprints among herds: Isolates 28-1 and 32-1 (Fig. 1, lane E and lane F respectively) showed identical REA fingerprints as did isolates 24-6, 26-5 and 3601 (Fig. 2, lane A, lane B and lane C respectively) (Table II), although these isolates were found in herds with clinical sign of AR (herds 24 and 28) and without clinical signs of AR (herds 26, 32 and 36) (Table I).

DISCUSSION

Restriction endonuclease analysis of genomic DNA is now an established technique for the study of molecular epidemiology of bacterial infections. The use of direct nucleic acid analysis has been particularly valuable for strain differentiation and allows the monitoring of distribution of strains (12-17).

TABLE II. Restriction endonuclease analysis classification of *Pasteurella multocida* isolates[a]

REA classification	Capsular and somatic types	Isolates
1	A 3,16	28-1, 32-1
2	A 4,7	23-4, 2305-A, 2305-B
3	D 3,16	30-1, 3001, 3002-1
4	D 3,16	24-6, 26-5, 3601
5	D 4,7	23-3, 27-1
6	D ND[b]	1354-4, 1354-10, 1354-12

[a]Isolates with identical REA patterns are presented. Isolates with similar profile were grouped
[b]Not determined

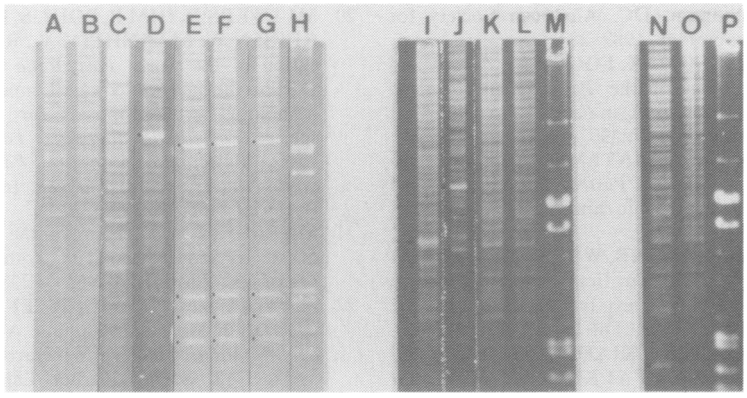

Fig. 2. Restriction endonuclease analysis fingerprings of genomic *Eco*RI digested DNAs from *P. multocida* isolates of capsular serotype D. A-G, isolatesof serotype D 3,16; I-L, isolates of serotype D 4,7; N-O, isolates of serotype D15. Lanes H, M, and P represent the lambda *Eco*RI-*Hin*dIII molecular weight marker. Squares in the figure point some major differences in the patterns. Isolate 26-5 (lane A),isolate 24-6 (lane B), isolate 3601 (lane C), isolate 3750 (lane D), isolate 30-1 (lane E), isolate 3001 (lane F), isolate 3002-1 (lane G), isolate 1604 (lane I), isolate 1703 (lane J), isolate 23-3 (lane K), isolate 27-1 (lane L), isolate 28-9 (lane N), isolate 23-8 (lane O).

In Quebec, great heterogeneity has been found among porcine isolates of *P. multocida* (7,8). In Leblanc's study (8), 19 different patterns of *P. multocida* based on capsular serotypes, somatic serogroups, and dermonecrotoxicity, were observed. Procedures which combine serotyping, plasmid profiles and chromosomal restriction patterns can be used to identify epidemic-causing strains. Bacterial restriction endonucleases were used to produce DNA cleavage patterns that could be useful as tools to study the relatedness among porcine *P. multocida* serogroup A and D isolates. This study shows that there is some heterogeneity as demonstrated by REA, among strains with the same capsular and somatic serotype and biochemical pattern but isolated from different herds. Seven isolates had unique fingerprints. Sixteen isolates could be grouped in six REA patterns (Table II). Similar REA fingerprints were observed for some strains presenting identical capsular and somatic antigens.

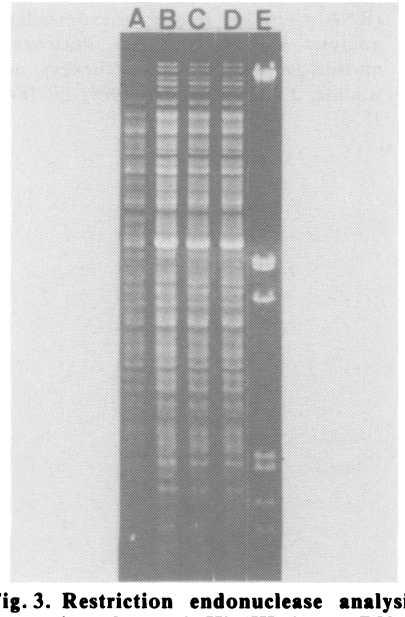

Fig. 3. Restriction endonuclease analysis fingerprints of genomic *Hin*dIII digested DNAs from *P. multocida* isolates from animals from herd 1354. Isolate 1354-5 (lane A), isolate 1354-4 (lane B), isolate 1354-10 (lane C), isolate 1354-12 (lane D), lambda *Eco*RI-*Hin*dIII (lane E). Squares in the figure point some major differences in the patterns.

Little is known about the epidemiology of nasal pasteurellosis. It is assumed that transmission readily occurs in the field among populations of pigs in which the susceptibility to infection is increased by concurrent bordetellosis or interaction with other determinants (23). Whether the introduction of toxigenic strains of *P. multocida per se* is the principal event preceding an outbreak awaits clarification. The precise role of the dam in the transmission of *P. multocida* to her suckling pigs remains unknown (24), but the recognized infectious determinants pass readily between populations of young weaned pigs (25). We observed that isolates having similar somatic and capsular antigens from different animals of the same herd (herds 23 and 1354) also have the same REA fingerprint, suggesting that the isolates are clonal and could be involved in the transmission of the disease.

Another point of interest is that isolates of *P. multocida* with the same REA fingerprint are found in different herds. It is interesting to note that isolates with similar REA fingerprints were found both in herds with clinical sign of AR and in herds without any clinical sign of AR. But isolates with different REA profiles were found in the same herd.

Although *P. multocida* is recognized as one of the etiological agents of porcine AR, the genetic elements encoding the virulence factors involved in the disease remain unknown (9,26). Pathogenicity of *P. multocida* is correlated with exotoxin activity. The production of dermonecrotoxin by *P. multocida* serotypes A and D may not be plasmid mediated. Recently the toxin gene was cloned in *E. coli* and it has been suggested to be present in the chromosome (10,11). Toxigenic *P. multocida* are mainly found in herds with clinical AR or with a history of AR (7,8,25). However we have found isolates with the same serotype and with similar REA fingerprints that differ in their ability to produce the dermonecrotic toxin. At present we do not know whether these nontoxigenic strains have lost their ability to produce the toxin because of *in vitro* passage, because of subtle differences at the chromosomal DNA level or because of the presence of a phage. Small differences in profiles are not easily detected by REA (27).

We have found interesting trends in REA patterns of *P. multocida,* although these results must be confirmed with a larger number of herds and animals. Restriction endonuclease analysis enables accurate epidemiological typing of *P. multocida* isolates, as a complement to classical capsular and somatic typing. Analysis of bacterial genomic digests by *Eco*RI and *Hin*dIII restriction endonucleases is a practical method for differentiating *P. multocida* isolates that have been previously serotyped. Moreover ribotyping, a method of highlighting DNA restriction site heterogeneity by using a rRNA probe, has recently shown to work well for differentiating avian strains of *P. multocida* (28).

ACKNOWLEDGMENTS

We thank J. Moore for reviewing the manuscript and Dr M. Denicourt for providing us with some isolates.

REFERENCES

1. **RUTTER JM.** Atrophic rhinitis in swine. Adv Vet Sci Comp Med 1985; 29: 239-279.
2. **BLACKSTROM L.** *Bordetella bronchiseptica* and *Pasteurella multocida* in swine respiratory diseases. Proc Am Assoc Swine Pract 1984: 116-130.
3. **RUNNELS LJ.** Infectious atrophic rhinitis of swine. Vet Clin North Am [Large Anim Pract] 1982; 4: 301-319.
4. **CARTER GR.** Serotyping of *Pasteurella multocida*. I. A hemagglutination test for the identification of serological type. Am J Vet Res 1955; 16: 481-484.
5. **HEDDLESTON KL, GODSON T, LEIBOVITZ T, ANGSTROM CI.** Serological and biochemical characteristics of *Pasteurella multocida* from free-flying birds and poultry. Avian Dis 1972; 16: 729-734.
6. **CARTER GR.** Serotyping of *Pasteurella multocida*. Methods Microbiol 1984; 16: 247-258.
7. **LEBLANC L, LARIVIERE S, MARTINEAU GP, MITTAL KR.** Characterization of *Pasteurella multocida* isolated from the nasal cavities of piglets from farms with or without atrophic rhinitis. Proc 9th Cong Int Pig Vet Soc 1986b: 225.
8. **LEBLANC L.** Études des souches de *Pasteurella multocida* provenant des cavités nasales de porcelets. Master's thesis, Faculté de Médecine vétérinaire, Université de Montréal, 1986.
9. **RUTTER JM.** Bacterial toxins as virulence determinants of veterinary pathogens: an overview. In: Roth JA, ed. Virulence Mechanisms of Bacterial Pathogens. Washington, DC: American Society for Microbiology, 1988: 224.
10. **PETERSON SK, FOGED N.** Cloning and expression of the *Pasteurella multocida* toxin gene, *tox*A, in *Escherichia coli*. Infect Immun 1989; 57: 3907-3913.
11. **LAX AJ, CHANTER N.** Cloning of the toxin gene from *Pasteurella multocida* and its role in atrophic rhinitis. J Gen Microbiol 1990; 136: 81-87.
12. **MARSHALL RB, WILTON BE, ROBINSON AJ.** Identification of leptospira serovars by restriction endonuclease analysis. J Infect Dis 1981; 149: 251-256.
13. **KAPER JB, BRADFORD HB, ROBERTS NC, FALKOW S.** Molecular epidemiology of *Vibrio cholerae* in the US Gulf Coast. J Clin Microbiol 1982; 16: 129-134.
14. **BRADBURY WC MURRAY RGE, MANCINI C, MORRIS VL.** Bacterial chromosomal restriction endonuclease analysis of the homology of *Bacteroides* species. J Clin Microbiol 1985; 21: 24-28.
15. **TOMPKINS LS, TROUP N, WOODS T, BIB W, McKINNEY RM.** Molecular epidemiology of *Legionella* species by restriction endonuclease and alloenzyme analysis. J Clin Microbiol 1987; 25: 1875-1880.
16. **SMART NL, MINIATS OP, MacINNES JI.** Analysis of *Haemophilus parasuis* isolates from southern Ontario swine by restriction endonuclease fingerprinting. Can J Vet Res 1988; 52: 319-324.
17. **KIM CJ, NAGARAJA KV.** DNA fingerprinting for differentiation of field isolates from reference vaccine strains of *Pasteurella multocida* in turkeys. Am J Vet Res 1990; 51: 207-210.
18. **FORTIN M, JACQUES M.** Hemagglutination by *Pasteurella multocida* of porcine origin. J Clin Microbiol 1987; 25: 938-939.
19. **LUGTENBERG B, BOXTEL R, JONG M.** Atrophic rhinitis in swine: correlation of *Pasteurella multocida* pathogenecity with membrane protein and lipopolysaccharide patterns. Infect Immun 1984; 46: 48-54.
20. **MUUTERS R, IHM P, POHL S, FREDERIKSEN W, MANNHEIM W.** Reclassification of the genus *Pasteurella* Trevisan 1887 on the basis of deoxyyribonucleic acid homology, with proposals for the new species *Pasteurella dagmatis, Pasteurella canis, Pasteurella stomatis, Pasteurella anatis,* and *Pasteurella langaa*. Int J Syst Bacteriol 1985; 35: 309-322.
21. **MARMUR J.** A procedure for the isolation of deoxyribonucleic acid from microorganisms. J Mol Biol 1961; 3: 208-218.
22. **MANIATIS T, FRITSCH EF, SAMBROOK J.** Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory, 1982.
23. **FARRINGTON DO.** Pneumonic pasteurellosis. In: Leman AD, Straw B, Glock RD, Mengeling WL, Penny RHC, Scholl E, eds. Diseases of Swine. Ames: Iowa State University Press, 1986: 436-468.
24. **BERCOVICH Z.** Contamination and age as factors in the pathogenesis of atrophic rhinitis. Tijdschr Diergeneeskd 1978; 103: 833.
25. **NIELSEN SC, RIISING HJ, BILLE N.** Experimental reproduction of atrophic rhinitis in pigs reared to slaughter weight. Proc 4th Int Congr Pig Vet Soc 1976: 202.
26. **CHANTER N, RUTTER JM.** Pasteurellosis in pigs and the determinants of virulence of toxigenic *Pasteurella multocida*. In: *Pasteurella* and Pasteurellosis. Academic Press, 1989: 170-173.
27. **VAN KETEL RJ.** Similar DNA restriction endonuclease profiles in strains of *Legionella* from different serogroups. J Clin Microbiol 1988; 26: 1838-1841.
28. **SNIPES KP, HIRSCH DC, KASTEN RW, HANSEN LM, HIRD DW, CARPENTER TE, McCAPES RH.** Use of an rRNA probe and restriction endonuclease analysis to fingerprinting *Pasteurella multocida* isolated from turkeys and wildlife. J Clin Microbiol 1989; 27: 1847-1853.