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1 **Genetic diversity and associated pathology of *Pasteurella multocida* isolated**
2 **from porcine pneumonia**

3

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11

12 **Abstract**

13 *Pasteurella multocida* is a widespread respiratory pathogen in pigs associated with atrophic rhinitis
14 and contributing to aggravation of the pulmonary lesions. The aims of the present study were to
15 characterize isolates of *P. multocida* from porcine bronchopneumonia by pulsed-field gel
16 electrophoresis (PFGE), PCR based capsular typing and multilocus sequence typing (MLST) and to
17 compare clonal complexes outlined with the type of histological lung lesions to investigate if a
18 correlation between clonal lineages and lesions might exist. Isolates of *P. multocida* were obtained
19 from cases of cranioventrally located porcine bronchopneumonia. All lung lesions were described
20 and classified according to histological lesions. A total of 139 isolates, from lung (n=111),
21 pericardial sac (n=21) and kidney (n=7) of 111 pigs were described using PFGE with *ApaI* as the
22 restriction enzyme. Furthermore, 20 and 29 isolates were characterized by capsular serotyping and
23 multilocus sequence typing, respectively. PFGE demonstrated 15 different clusters showing 50% or
24 more similarity. All selected isolates were of capsular serotype A and only three main sequence

25 types (ST) were detected among the isolates. Associations were not found between histopathology
26 and clonal complexes of *P. multocida*. In conclusion, PFGE demonstrated a high diversity of
27 genotypes of *P. multocida* associated with porcine bronchopneumonia. However, isolates obtained
28 mainly belonged to few STs, indicating that isolates of *P. multocida* associated with porcine
29 bronchopneumonia originates from a limited number of clonal lineages and therefore might have
30 adapted to porcine hosts. No correlation was demonstrated between genotypes and types of lesions,
31 and extra-pulmonary spreading was only rarely demonstrated.

32

33 Keywords: Porcine bronchopneumonia, *Pasteurella multocida*, pulsed-field gel electrophoresis,
34 multilocus sequence typing

35

36 **1. Introduction**

37 Infectious bronchopneumonia is a widespread and well-known disease of pigs used for meat
38 production (Sørensen et al., 2006). In a Danish study, bronchopneumonia was demonstrated in 25%
39 of the pigs at slaughter (Christensen and Enoe, 1999). Similar reports have been published from
40 other countries (Gois et al., 1975; Osborne et al., 1981; von Altröck, 1998; Maes et al., 2001). Both
41 viruses and bacteria have been included in the aetiology of porcine pneumonia (Thacker, 2001;
42 Sørensen et al., 2006; Hansen et al., 2010). Among these, the Gram-negative bacterium *Pasteurella*
43 *multocida*, associated with a broad disease spectrum in animals (Hunt et al., 2000), constitutes a
44 frequent finding (Gois et al., 1975; Morrison et al., 1985; Falk et al., 1991; von Altröck, 1998; Ross,
45 2006), and it is suggested that *P. multocida* may play a central role in the porcine respiratory
46 disease complex (PRDC) (Thacker, 2001; Pijoan, 2006; Ross, 2006; Sørensen et al., 2006; Hansen
47 et al., 2010). Compared to bronchopneumonia and atrophic rhinitis, systemic infections with *P.*
48 *multocida* in pigs are infrequently reported, although acute septicaemia due to capsular serotypes B

49 and D has been reported from Asia and Australia (Mackie et al., 1992; Cameron et al., 1996; Pijoan,
50 2006). Finally, *P. multocida* has been associated with the porcine dermatitis and nephropathy
51 syndrome (PDNS), a disease of unknown aetiology recognized in many pig-producing countries
52 (Thomson et al., 2001; Lainson et al., 2002). A variety of lesions in porcine lungs have been
53 associated with *P. multocida*, including mild exudative as well as extensive necrotizing lesions and
54 abscess formation (Pijoan, 2006; Hansen et al., 2010). Factors influencing the outcome, however,
55 remain to be elucidated. In addition, only little is known about the host response to invasion in the
56 lungs, just as possible systemic spreading remains to be confirmed. The difference observed in
57 pathological manifestations might be explained as a result of differences in virulence of the bacteria
58 previously reported for avian isolates of *P. multocida* (Pedersen et al., 2003; Eigaard et al., 2006).
59 Although strains of *P. multocida* obtained from porcine bronchopneumonia have been typed and
60 characterized using several different approaches, such correlations between isolates and pathology
61 have not been reported for porcine isolates (Zucker et al., 1996; Fusing et al., 1999; Bowles et al.,
62 2000; Lainson et al., 2002; Moreno et al., 2003; Jamaludin et al., 2005). However, demonstration of
63 a small number of *P. multocida* variants associated with the majority of cases of porcine pneumonia
64 in the United Kingdom during a twelve year period might suggest that strains associated with lung
65 lesions represent primary pathogens rather than secondary invaders (Davies et al., 2003). Pulsed-
66 field gel electrophoresis (PFGE) has previously been used to investigate the genetic diversity of *P.*
67 *multocida* (Aalbaek et al., 1999; Lainson et al., 2002; Pedersen et al., 2003). However, results
68 obtained have not been compared with the type of lesions found in bronchopneumonia. For this
69 reason the aims of the present study were to characterize isolates of *P. multocida* from porcine
70 bronchopneumonia by capsular typing, PFGE and multilocus sequence typing (MLST) and to
71 compare clonal complexes outlined with the type of lesions found in the lungs and to examine if a
72 correlation between clonal lineages and lesions might exist. In addition, isolates from the lungs,

73 pericardial sac and kidney of the same animal were compared in order to investigate if isolates from
74 the pericardial sac and kidney were identical with those obtained from the lung.

75

76 **2. Materials and Methods**

77 *2.1 Cases of bronchopneumonia and bacterial isolates*

78 Cases of cranioventrally located porcine bronchopneumonia with positive cultivation of *P.*
79 *multocida*, randomly collected at two Danish slaughter houses during 2006, were included in the
80 present study (Hansen et al., 2010). The animals originated from 80 separate farms. In addition to
81 isolates from the lung, isolates of *P. multocida* from the pericardial sac and kidney of pigs
82 demonstrating bronchopneumonia with isolation of *P. multocida*, were included. All organs were
83 cultured using sterile cotton swabs after heat decontamination of the surface. The swabs were plated
84 on blood agar (Blood agar Base CM 55, Oxoid, Basingstoke, United Kingdom, added 5% bovine
85 blood) and incubated at 37°C in a sealed plastic bag for 24 h. Isolates showing typical colony
86 morphology of *P. multocida* were sub-cultured and identified using procedures as previously
87 reported by Hansen et al. (2010). Growth of *P. multocida* on the primary plates and gross lesions
88 were evaluated as previously reported (Hansen et al., 2010). Evaluation of histopathology was done
89 according to Hansen et al. (2010) and Pors et al. (2011) leading to the following groups of
90 histopathology: acute, subacute, chronic necrotizing, chronic suppurative, chronic mixed and
91 chronic non-suppurative bronchopneumonia.

92

93 *2.2 Pulsed-field gel electrophoresis*

94 Isolates of *P. multocida* were kept at – 80°C in brain heart infusion (BHI) broth (Difco, Heidelberg,
95 Germany) with 10% glycerol (LIFE Pharmacy, Copenhagen, Denmark) and were plated on blood
96 agar. Inoculated plates were incubated at 37°C in sealed plastic bags for 18-24 hours. From pure

97 cultures, a single colony typical of *P. multocida* was grown overnight in 10 ml BHI (Difco) at 37°C
98 with shaking. The optical density at 578 nm was measured using 200 µl of bacterial suspension
99 added to 800 µl SE-buffer (75mM NaCl (Merck, Darmstadt, Germany), 25mM EDTA (Merck) [pH
100 7.4]). Approximately 3 ml of culture was used for DNA preparation. The culture was centrifuged at
101 10,000 rpm for 25 min after which the supernatant was discarded and the pellet re-suspended in 4.5
102 ml SE-buffer followed by centrifugation. After discarding the supernatant and adding SE-buffer as
103 adjustment for density, the suspension of bacterial DNA was mixed with an equal amount of 1%
104 low-melting-point agarose (Seakem GTG agarose, FMC Bioproducts) and dispensed into a plug
105 mould. After solidification the blocks were digested overnight at 56°C in ES-buffer (1% N-
106 lauroylsarcosin (Sigma-Aldrich, Steinheim, Germany), 0.5M EDTA (Merck) [pH 9.5]) added
107 Proteinase K (0.5 mg/ml) (Roche, Basel, Switzerland). The blocks were then washed in cold (4°C)
108 TE-buffer. Subsequently, the purified DNA was washed in restriction buffer (NEBuffer 4, B7003S,
109 New England BioLabs, Massachusetts, USA) containing 10 mg/ml bovine serum albumin (B9001S,
110 New England BioLabs) and digested overnight with 2 µl *ApaI* (R0114, New England BioLabs) at
111 25°C as this has previously been used in PFGE analysis of *P. multocida* isolates (Lainson et al.,
112 2002). The fragments were separated in a 1% agarose gel (Pulsed Field Certified Agarose, Bio-
113 Rad, Richmond, California) in 0.5×TB-buffer (Tris/boric acid (Merck), 0.5 M EDTA (Merck) [pH
114 8.]) using a CHEF-DR III system (Bio-Rad) at 14°C for 20 hr. The electrophoresis conditions were:
115 an initial switch time of 1 sec, a final switch time of 15 sec, a potential difference of 5.6 V/cm and
116 an included angle of 120°C. Gel images were analyzed and banding profiles compared using
117 GelCompar II (Applied Maths, Belgium). Subsequently, the Unweighted Pair Group Method with
118 Arithmetic Average (UPGMA) was used for cluster analyses using the DICE similarity coefficient
119 and a band-matching index greater than 0.8% among the profiles. To determine clonality among the
120 isolates, guidelines presented by Lainson et al. (2002) and Tenover et al. (1995) were used.

121

122 *2.3. Capsular PCR*

123 Twenty isolates obtained from lung lesions, were randomly selected to represent major clusters
124 according to PFGE. A multiplex capsular PCR based upon capsule-specific primer pairs (CAPA,
125 CAPB, CAPD, CAPE, and CAPF) was used as described by Townsend et al. (2001).

126 *2.4. MLST*

127 Twenty-nine isolates were selected randomly to represent major clusters based upon PFGE. MLST
128 was performed according to Subaaharan et al. (2010). Seven housekeeping gene fragments *adk*
129 (coding for adenylate kinase), *est* (esterase), *pmi* (mannose-6-phosphate isomerase), *zwf* (glucose-6-
130 phosphate-1-dehydrogenase), *mdh* (malate dehydrogenase), *gdh* (glutamate dehydrogenase) and *pgi*
131 (glucose-6-phosphate-isomerase) were characterized. PCR conditions included an initial
132 denaturation of 5 min at 94°C; 30 cycles of (94°C, 30 s; 48°C, 30s; 72°C, 1 min) and a final
133 elongation at 72°C for 5 min. For the *gdh* gene the annealing temperature was 58°C instead of
134 48°C. Residual dNTPs and primers were removed by mixing 10 µl PCR-mixture with 1 µl
135 exonuclease I and 2 µl calf intestine alkaline phosphatase (Fermentas, St. Leon-Rot, Germany). The
136 samples were incubated for 15 min at 37°C and subsequently at 85°C to stop the enzymatic
137 reaction. Sequencing was performed at Macrogen Inc., Seoul, Korea, using the PCR primers.
138 Sequencing results were analyzed with Vector NTI 10.1.1 (Invitrogen, Carlsbad, USA) and
139 assembled sequences aligned and compared using ClustalW at www.ebi.ac.uk. Using the
140 *Pasteurella multocida* RIRDC MLST Database (http://pubmlst.org/pmultocida_rirdc/) the ST types
141 found was compared to previous findings.

142

143 *2.5. Statistical analysis*

144 Association between PFGE and lesion types demonstrated was done by Fisher's exact test.
145 Significance level was set to $P < 0.05$. All analysis was done using SAS version 9.1 (SAS Institute,
146 Cary, NC, USA). For statistical analysis, histopathological observations were pooled according to
147 the age of lesion (acute, subacute, chronic) to ensure adequate group sizes. Furthermore, analyses
148 were performed among the groups of lungs with chronic lesions (necrotizing, suppurative, mixed,
149 non-suppurative). Clusters with a number of isolates below ten were not included in the statistical
150 analyses.

151

152 **3. Results**

153 *3.1 Bacteriology*

154 A total of 111 isolates of *P. multocida* were obtained from the lungs of cases of cranioventral
155 located bronchopneumonia. Of these 96 were demonstrated in pure culture, while colonies of *P.*
156 *multocida* present in a mixed culture were obtained in 15 cases. Massive growth (more than 300
157 colonies) were found in 76 cases, dense growth (25-300 colonies) in 21 cases and weak growth
158 (<25 colonies) in 14 cases. Isolates of *P. multocida* were also obtained from the kidney in 7
159 animals. All isolates from the kidney were in pure culture, massive growth was found in 4 cases and
160 dense growth were found in 3 cases. Isolates obtained from the pericardial sac were kept and
161 included for further examination in 21 out of 40 cases with positive cultivation of *P. multocida* from
162 the pericardial sac. Of these 19 were demonstrated in pure culture, while colonies of *P. multocida*
163 present in a mixed culture were obtained in 2 cases. Massive growth (more than 300 colonies) were
164 found in 4 cases, dense growth (25-300 colonies) in 6 cases and weak growth (<25 colonies) in 11
165 cases. Four of the cases had simultaneous isolation of *P. multocida* from kidney and pericardial sac.
166 Isolates from lung, pericardial sac and kidney all shared the same colony morphology, typical of *P.*
167 *multocida*.

168

169 *3.2. Pathology*

170 Lung lesions from which *P. multocida* was cultivated and included for further characterization
171 (n=111) were grouped into six categories according to histopathology (Table 2). There were only
172 eight acute and 21 subacute cases of bronchopneumonia. The most frequent finding was chronic
173 bronchopneumonia (n=82) represented by different types of exudation in alveoli, including
174 neutrophils or a mixed cell population consisting of lymphocytes, macrophages and neutrophils.
175 Additionally, multifocal necrotic lesions were found in a single subacute case and in eight cases of
176 the chronic bronchopneumonia. A detailed description of the histopathological findings has been
177 reported by Hansen et al. (2010).

178

179 *3.3. PFGE analysis*

180 A total of 139 isolates of *P. multocida* obtained from lung (n=111), pericardial sac (n=21) and
181 kidney (n=7) were examined by PFGE. The PFGE patterns obtained with *ApaI* as the restriction
182 enzyme included 7-13 clear and well separated bands. A total of 15 clusters (A-O) demonstrated
183 more than 50% similarity (Table 1 and Fig. 1). Clusters A and B each contained 34 isolates. Within
184 these clusters accumulations of clonally related isolates representing several farms were observed.
185 Cluster C included 13 isolates, six isolates made up cluster D, while three (E, F and G), one (H) and
186 two (I and J) clusters contained four, three and two isolates, respectively. Five clusters (K, L, M, N
187 and O) only included a single isolate each. A total of 47 isolates formed 13 clusters (Fig. 1) each of
188 which demonstrated 90% or more similarity. The similarity of PFGE profiles of isolates obtained
189 from lung, pericardial sac and/or kidney is shown in Table 2. In nine out of 24 animals isolates
190 obtained demonstrated more than 90% similarity. Isolates from the remaining 15 animals were

191 different with the exception of isolates from three animals from which two out of three isolates
192 demonstrated an identical profile.

193

194 3.4. Association between PFGE clusters, pathology and cultivation of *P. multocida*

195 The nine isolates obtained from necrotic lung lesions belonged to 7 different clusters (Table 1) and
196 a significant association between PFGE clusters and necrosis was not observed (P=0.12).
197 Furthermore, an association between the clusters outlined and groups of histopathology (acute,
198 subacute, chronic) (P=0.49) or groups of chronic cases (necrotizing, suppurative, mixed, non-
199 suppurative) (P=0.32) was not demonstrated. Neither was a significant association between the
200 clusters outlined and isolation of *P. multocida* from the pericardial sac and/or kidney found
201 (P=0.40). Using the definition of clonal relationship between isolates defined by Tenover et al.
202 (1995) did not demonstrate clones that correlated with groups of histological lesions either
203 (P=0.15) (data not shown).

204

205 3.5. Capsular typing

206 All isolates selected randomly to represent major PFGE clusters for capsular PCR belonged to
207 capsular serotype A.

208

209 3.6. MLST

210 Three groups of sequence types (STs) were demonstrated (Table 3). The largest group made up the
211 clonal complex 13 and comprised ST13 (21 isolates) or ST149 (one isolate). Four isolates belonged
212 to clonal complex 74 including ST74 (3 isolates) or ST148 (one isolate), while the last group
213 comprised three isolates belonging to clonal complex 50 (ST50, ST150 and ST146). Within the
214 three clonal complexes, the STs share 6 out of seven alleles. ST50 and ST74 differ in five out of

215 seven alleles and ST13 demonstrates seven unique alleles compared to ST50 and ST74 (Table 3).
216 All three clonal complexes have been reported in the *Pasteurella multocida* RIRDC MLST
217 Database to be mainly associated with pigs in a worldwide distribution. Due to the uneven
218 distribution of STs among the 29 sampled animals, a statistical analysis of a possible correlation
219 with histopathology was not possible.

220

221 4. Discussion

222 A high prevalence of *P. multocida* is usually demonstrated from cases of porcine
223 bronchopneumonia (Gois et al., 1975; Morrison et al., 1985; Falk et al., 1991; Hansen et al., 2010).
224 However, strains of *P. multocida* associated with bronchopneumonia in pigs are usually considered
225 secondary pathogens being dependent on co-infections or immunosuppression of the host (Pijoan,
226 2006; Ross, 2006). The present study characterized isolates from naturally occurring porcine
227 bronchopneumonia to investigate the genetic diversity of the isolates and to investigate a possible
228 link between genotypes and the histopathology of the corresponding lung lesions. In addition,
229 isolates found in the kidney and pericardial sac was compared with lung isolates from the same
230 animal, to investigate the possibility of systemic spreading of the infection. The results showed that
231 *P. multocida* isolated from bronchopneumonia in pigs demonstrate a high genetic diversity. Based
232 upon PFGE a total of 15 clusters demonstrating 50% similarity or more were outlined. Using the
233 same cut of value Lainson et al. (2002) only demonstrated four clusters among 51 isolates. A high
234 genetic variation among isolates from porcine pneumonia has also been reported by others, although
235 other methods were used and based on a small number of isolates compared to this study
236 (Djordjevic et al., 1998). Application of restriction endonuclease analyses (REA) and ribotyping,
237 has demonstrated less genetic diversity among isolates from porcine pneumonia (Zhao et al., 1992;
238 Blackall et al., 2000; Bowles et al., 2000; Rubies et al., 2002). However, in the present study

239 affected pigs were randomly selected and represent isolates which are not epidemiologically
240 connected.

241

242 Demonstrations of a low number of STs and a few clonal complexes of *P. multocida*, however,
243 seems to indicate that only a limited number of clones seem to be associated with porcine
244 bronchopneumonia in Danish pig herds. The three major STs demonstrated in this study, ST13,
245 ST50 and ST74 and the subtypes (146-150) hereof, all belong to *P. multocida* subspecies *multocida*
246 (unpublished data). These STs have previously also mainly been reported associated with pigs
247 (http://pubmlst.org/pmultocida_rirdc/) indicating an adaptation to this host. MLST results also
248 demonstrate that several evolutionary lines of *P. multocida* independently may have adapted to the
249 porcine host since the sequence types differ in most of the alleles. These observations confirm
250 previous findings by Davies et al. (2003), who showed that the majority of isolates of *P. multocida*
251 from porcine pneumonia only made up four different outer-membrane protein (OMP) types.

252

253 Previous studies have used different molecular methods to describe the genetic diversity of *P.*
254 *multocida* obtained from porcine pneumonia (Pijoan et al., 1983; Zhao et al., 1992; Zucker et al.,
255 1996; Blackall et al., 2000; Rubies et al., 2002). However, none of these studies examined the
256 possible association between the genotypes and histopathological findings in the lung. In the present
257 study a connection between PFGE clusters of *P. multocida* and histopathological findings was not
258 demonstrated, indicating the important role of other infectious agents and/or management factors in
259 the outcome of porcine bronchopneumonia (Sørensen et al., 2006; Hansen et al., 2010). With an
260 uneven distribution of the STs among the 29 sampled animals it was not possible to carry out any
261 meaningful statistical analyses of the association between ST and histopathological findings.
262 However, it was observed that ST13 did not occur in animals with chronic necrotic lesions and

263 ST50 and ST13 did not occur in animals with chronic suppurative lesions (data not shown). This
264 observation should be further investigated in future studies.

265

266 All isolates of *P. multocida* selected for capsular serotyping, were found to be of capsular type A in
267 agreement with previous studies (Pijoan et al., 1983; Zhao et al., 1992; Djordjevic et al., 1998;
268 Davies et al., 2003). In contrast, strains found in porcine atrophic rhinitis often belong to capsular
269 serotype D (Sakano et al., 1992; Gardner et al., 1994; Davies et al., 2003), indicating that isolates of
270 *P. multocida* associated with atrophic rhinitis and bronchopneumonia, respectively, seem to
271 represent different clonal lineages. Investigations of the tonsillary flora of apparently normal pigs
272 demonstrated a high prevalence of capsular serotype A (Townsend et al., 2000; Jamaludin et al.,
273 2005) and typing these strains might give insight as to whether the clones found in this study
274 originate from the tonsils. Comparison of isolates from lung and extra-pulmonary sites, including
275 the kidney and pericardial sac, showed the same PFGE profile in only nine out of 24 animals.
276 Previous studies of paired isolates from lung and kidney demonstrated similar results for eight out
277 of 14 animals based on REA (Buttenschon and Rosendal, 1990). Lainson et al. (2002) demonstrated
278 identical PFGE profiles of isolates of *P. multocida*, obtained from different isolation sites, including
279 kidney and lung, in pigs suffering from PDNS. These results indicate that a spreading of infection
280 might be possible. However, it could be speculated whether the discrepancies observed might be
281 due to contamination, infection with multiple clones or clearance of the bacteria in chronic cases. A
282 majority of the histopathological findings in the lungs was characterized as chronic and therefore it
283 could be speculated whether a systemic spread was cleared by the host at the time of examination,
284 thereby leading to the low isolation rate of bacteria from the kidney. As *P. multocida* is reported as
285 a zoonotic organism (Bisgaard et al., 1994; Iaria and Cascio, 2007), systemic spreading of the

286 organism in pigs used for human consumption might represent a potential source of human
287 infection. Consequently the risks and rate of the spreading should be further examined.

288

289 In conclusion, the results of the present study clearly demonstrated the presence of highly diverse
290 genotypes of *P. multocida* in porcine bronchopneumonia. However, isolates obtained mainly
291 belonged to a few STs, indicating that isolates of *P. multocida* associated with porcine
292 bronchopneumonia originates from a limited number of clonal lineages which and therefore might
293 have adapted to porcine hosts. No relation was demonstrated between genotypes outlined and type
294 of lesion, and extra-pulmonary spreading was only rarely demonstrated.

295

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299

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Table 1. Isolates of *P. multocida* characterized by PFGE. Distribution according to herd, isolation from kidney and/or pericardial sac and type of bronchopneumonia for clusters demonstrating more than 50% similarity by PFGE analysis.

Cluster	Cases with isolation of <i>P. multocida</i> from lung	No. of herds	Isolation from kidney/pericardial sac	Type of bronchopneumonia					
				<i>Acute</i>	<i>Subacute</i>	<i>Chronic necrotizing</i>	<i>Chronic suppurative</i>	<i>Chronic mixed</i>	<i>Chronic nonsuppurative</i>
A	34	28	14	3	4	2	14	5	6
B	34	27 (3 ND)	14	1	5	1	12	9	7
C	13	11 (1 ND)	2	2	2	1	1	4	2
D	6	6	3	0	2	1	1	0	2
E	4	4	2	1	1	0	1	0	1
F	4	2 (1 ND)	2	0	1	1	1	1	0
G	4	4	1	0	1	0	1	1	1
H	3	4	2	1	0	2	0	0	0
I	2	ND	1	0	2 ^a	0	0	0	0
J	2	2	0	0	1	0	0	1	0
K	1	1	0	0	0	0	1	0	0
L	1	1	0	0	1	0	0	0	0
M	1	1	0	0	0	0	1	0	0
N	1	1	0	0	0	0	0	1	0
O	1	1	1	0	1	0	0	0	0
Total	111		42	8	21 ^a	8	33	22	19

ND: herd origin not reported. a: One case of necrotizing pneumonia

Table 2. Clonality of PFGE profiles between isolates from different organs in the same pig.

Similarity of PFGE profiles of isolates of *P. multocida* obtained from lung, pericardial sac and/or kidney from the same pig. Clonality of profiles were defined as 90% or higher similarity

Organ with positive culture of <i>P. multocida</i>	PFGE profile		
	PFGE cluster	No. of animals with clonal isolates obtained from different organs	No. of animals with unrelated isolates obtained from different organs
Lung Pericardial sac 17 animals	A	2	4
	B	4	3
	D	-	1
	E	-	2
	G	1	-
Lung Kidney 3 animals	B	1	1
	I		1
Lung Kidney Pericardial sac 4 animals	B	1	1 ^a
	D	-	2 ^{b,c}

a: Isolates from kidney and lung were identical.

b: Isolates from kidney and heart were identical.

c: Isolates of lung and heart were identical.

Table 3. Clonal complex, sequence type (ST) and allelic profile demonstrated among 29 isolates of *P. multocida* randomly selected to represent the genetic diversity outlined by PFGE.

Clonal complex	Sequence type	Gene and allelic profile							Clusters represented (isolates with ST /total isolates typed from cluster)
		<i>adk</i>	<i>est</i>	<i>pmi</i>	<i>zwf</i>	<i>mdh</i>	<i>gdh</i>	<i>pgi</i>	
13	13	7	11	9	10	4	7	8	A(4/6) B(6/7) C(2/3) D(1/2) E (1/1) F(1/1) G(1/1) J(1/1) K(1/1) M(1/1) O(1/1)
	149	7	11	9	10	4	30	8	L(1/1)
50	50	14	10	20	19	8	3	20	D (1/2)
	146	14	10	20	19	8	29	20	C(1/3)
	150	14	10	20	19	8	3	53	N(1/1)
74	74	22	13	8	29	8	3	31	A(2/6) B(1/7) H(1/1)
	148	22	13	46	29	8	3	31	I(1/1)

Fig. 1

Dendrogram derived from the Unweighted Pair Group Method with Arithmetic Average (UPGMA) used for cluster analyses using the DICE similarity coefficient and a band-matching index greater than 0.8% among the PFGE profiles of all isolates of *P. multocida* obtained from porcine bronchopneumonia. Clusters (A-O) was determined by 50% similarity between profiles.

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Figure 1

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