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25 from different dogs were indistinguishable by both PFGE and RAPD. These isolates
26 were cultured from dogs with non-overlapping stays in the kennel, which may
27 indicate maintenance of some strains within kennels. A small number of isolates
28 showed much greater genetic heterogeneity and were genetically distinct from the
29 main group of *M. cynos* strains. There was also a high degree of similarity of the *M.*
30 *cynos* type strain (isolated from a dog with respiratory disease in Denmark in 1971) to
31 at least one of the United Kingdom isolates using PFGE analysis, which may suggest
32 possible conservation of pathogenic strains of *M. cynos*.

33

34

35 **Keywords**

36 *Mycoplasma cynos*; canine infectious respiratory disease (CIRD); kennel cough;
37 PFGE; RAPD

38

39

40 **1. Introduction**

41 Canine infectious respiratory disease (CIRD or kennel cough) is a multifactorial
42 disease complex and the agents traditionally associated with this disease are
43 *Bordetella bronchiseptica*, canine parainfluenza virus (CPIV), canine adenovirus
44 (CAV), and canine herpesvirus (Chvala et al.). Recently, a novel canine respiratory
45 coronavirus (CRCoV; (Erles et al., 2003) and *Streptococcus equi* subsp.
46 *zooepidemicus* (Chalker et al., 2003a) have also been found to be associated with the
47 disease.

48

49 Within this microbial complex, *Mycoplasma* spp. are found to be ubiquitous in the
50 upper respiratory tract of dogs and are thought to be normal flora (Rosendal, 1982;
51 Randolph et al., 1993). However, mycoplasmas have also been the sole bacterial
52 isolate in a number of clinical cases of canine respiratory disease, but unfortunately
53 these isolates were not speciated and viral causes of CIRDC were not investigated
54 (Kirchner et al., 1990; Jameson et al., 1995; Chandler and Lappin, 2002). The
55 involvement of *M. cynos* in CIRDC has been noted for some time (Rosendal, 1972,
56 1978, 1982). Evidence for this has been mounting recently, as Chalker et al. (2004)
57 found that *M. cynos* was the only mycoplasma significantly associated with canine
58 respiratory disease. In addition, dogs entering a re-homing kennel that developed an
59 antibody response to *M. cynos* were more likely to suffer respiratory disease (Rycroft
60 et al., 2007). *M. cynos* has been isolated from dogs with pneumonia (Rosendal, 1972,
61 1978; Chvala et al., 2007) and was particularly abundant in the most necrotic areas of
62 the lung (Chvala et al., 2007). Furthermore, *M. cynos* was the only detected agent in a
63 case of severe bronchopneumonia in a litter of young puppies which resulted in the
64 deaths of some puppies, but which was resolved in the surviving littermates after the
65 administration of appropriate antibiotics (Zeugswetter et al., 2007).

66

67 Recently molecular epidemiological studies of isolates of the *Mycoplasma* species *M.*
68 *bovis* (Kusiluka et al., 2000; McAuliffe et al., 2004), *M. ovipneumoniae* (Parham et
69 al., 2006) *M. gallisepticum* and *M. synoviae* (Feberwee et al., 2005) have been
70 conducted using the genetic typing techniques amplified fragment length
71 polymorphism (AFLP), random amplified polymorphic DNA (RAPD) and pulsed-
72 field gel electrophoresis (PFGE). This is the first genetic typing study performed on
73 *M. cynos*.

74

75

76 **2. Materials and Methods**

77 *2.1. Mycoplasma cynos isolates*

78 *Mycoplasma cynos* isolates cultured from respiratory samples from dogs with
79 moderate to severe respiratory disease were identified from an earlier large study.
80 Isolation and identification of these isolates has been previously described (Chalker et
81 al., 2004). Briefly, bronchoalveolar lavage (BAL) and trachea samples were obtained
82 from euthanized dogs from a re-homing centre with a history of endemic CIRDC
83 (population A). Alternately, BAL samples were taken from dogs with persistent
84 coughs at a training centre (population B). Dogs were graded for respiratory signs
85 prior to sampling or euthanasia. *Mycoplasma cynos* was cultured on Mycoplasma
86 media (Mycoplasma Experience) and identified by PCR specific for the 16S/23S
87 rRNA intergenic spacer region. Cultures of the single-cloned *M. cynos* isolates were
88 stored frozen at -70°C.

89

90 The type strain *M. cynos* H381 NCTC10142 was obtained from the National
91 Collection of Type Cultures (NCTC), Collindale, London.

92

93 *2.2. Bacterial and viral screening*

94 Bacteriological screening of the samples has been previously described (Chalker et
95 al., 2003a; Chalker et al., 2003b). Briefly, BAL and trachea samples were inoculated
96 onto MacConkey agar and two blood agar plates (incubated aerobically and
97 anaerobically) and incubated at 37°C. Gram positive, catalase negative, beta-
98 haemolytic colonies were identified as streptococci and sero-grouped into Lancefield

99 Groups, then identified to the species level with API 20STREP (Biomérieux). Oxidase
100 positive colonies with typical *B. bronchiseptica* growth characteristics were identified
101 as such with API 20NE.

102

103 Virus screening of the samples has been previously described (Erles et al., 2004).
104 Briefly, RNA and DNA were extracted from the respiratory tissue samples and PCR
105 and reverse transcription-PCR were used to detect canine parainfluenza virus (CPIV),
106 canine herpesvirus (Chvala et al.), canine adenovirus (CAV), canine distemper virus
107 (CDV), and canine respiratory coronavirus (CRCoV). In addition, RT-PCR for canine
108 influenza virus (CIV) was carried out using primers AMP227F and AMP622R
109 directed to the M gene (Ellis and Zambon, 2001). Equine influenza virus (H3N8)
110 served as a positive control.

111

112 2.3. Pulsed-field gel electrophoresis

113 Aliquots (20 ml) of stationary phase *M. cynos* culture (maximum absorbance A_{600} of
114 approximately 0.3) were used for PFGE analysis. Cells were harvested by
115 centrifugation (3,500 x g for 20 min at 4 °C), washed three times with PBS buffer
116 with 10% (w/v) glucose and resuspended in 300 ml cold PBS/glucose buffer. Agarose
117 plugs were made from a 1:1 mixture of 2% low-melting-point agarose (Biorad) and
118 the cell suspension. Plugs were incubated in lysis buffer (10 mM Tris- HCl, 1 mM
119 EDTA, 1% lauroyl sarcosine, 1 mg / ml proteinase K) for 48 h at 56 °C. Plugs were
120 washed four times with Tris-EDTA buffer for 30 min at 4 °C. Slices (2 mm) were cut
121 aseptically from plugs and equilibrated in restriction buffer (Promega) for 1 h.
122 Subsequently, restriction digestion was performed by using 30 U of *Sma*I (Promega)
123 for 16 h according to the manufacturer's instructions. The fragments were resolved on

124 1 % pulsed field certified agarose (Biorad) gels using a CHEF-DRIII system (Biorad)
125 at 6 V/cm, with a running time of 20 h at 14 °C; included angle of 120°; initial pulse
126 time of 4 sec; final pulse time of 40 sec. Gels were stained with ethidium bromide (0.5
127 mg/ml) for 15 min, destained in distilled water for 1h and photographed under UV
128 light. A lambda ladder PFGE marker (Sigma) was used for fragment size
129 determination. The Bionumerics package (Applied Maths) was used for gel analysis
130 and dendrograms were produced using the Jaccard Coefficient and unweighted pair
131 group method using arithmetic averages (UPGMA) cluster analysis.

132

133 2.4. RAPD

134 The single primer Hum4 5'- ACGGTACACT – 3' (Hotzel et al., 1998) was used for
135 the generation of RAPD profiles. Amplification was performed in a 50 µl total
136 reaction volume containing 100 ng of DNA sample, 10 mM Tris-HCl (pH 9.0), 1.5
137 mM MgCl₂, 50 mM KCl, 0.1% Triton X-100, 0.2 mM each deoxynucleoside
138 triphosphate, and 0.5 U of TaqGold (Perkin-Elmer). Cycling conditions included an
139 initial denaturation step at 94 °C for 5 min, followed by 40 cycles of 94 °C for 15 sec,
140 37 °C for 60 sec and 72 °C for 90 sec. The last cycle included a final elongation at 72
141 °C for 7 min. PCR products were resolved by electrophoresis on 10 cm 2% agarose
142 gels at 60 mA for 1.5 h, stained with ethidium bromide and visualized under UV
143 illumination. The Bionumerics package (Applied Maths) was used for gel analysis
144 and dendrograms were produced using the Jaccard Coefficient and unweighted pair
145 group method using arithmetic averages (UPGMA) cluster analysis

146

147

148 3. Results

149 3.1. Dogs

150 Six dogs with moderate to severe respiratory disease from which *M. cynos* was
151 isolated were identified from an earlier large study (Chalker et al., 2004). Four dogs
152 were housed at a re-homing centre with a history of endemic CIRDC (population A)
153 and two dogs at a training centre (population B). All six dogs had respiratory disease
154 with symptoms of either bronchopneumonia (respiratory score 5) or cough and nasal
155 discharge (score 3). Trachea and/or BAL samples were taken from the dogs within 4
156 weeks of the first symptoms of CIRDC. The dogs were 1-3 years old and of various
157 breeds. The group consisted of entire and neutered males and females (Table 1).

158

159 3.2. Bacteriology and virology screening

160 *Mycoplasma cynos* was cultured from the BAL of each dog and also the trachea
161 where that sample was available (Tables 1 and 2).

162

163 Testing of BAL samples from the two dogs from the training centre (B-1 and B-2)
164 was negative for the viruses CRCoV, CHV, CPIV, CAV, CDV and CIV. In addition,
165 these samples yielded no bacterial growth except that of *M. cynos*. In comparison, the
166 four dogs from the re-homing kennel had other bacteria cultured from the respiratory
167 samples (see Table 1).

168

169 3.3. PFGE analysis of *M. cynos* isolates

170 PFGE analysis of the *M. cynos* type strain and the 14 isolates from dogs with
171 respiratory disease, resulted in six different PFGE profiles (Figure 1). The PFGE
172 profiles consisted of 3-5 DNA bands, which ranged in size between approximately 6
173 and 425 kb. The PFGE profiles of the isolates can be divided by similarity into three

174 groups. Group 1 contains ten isolates and it is a genetically homogeneous group with
175 at least 78% similarity; isolates 185, 190, 191, 210, 253, 312, 387, 417, 428, and 429
176 all form this group. These are all of the isolates from the population A dogs except
177 isolate 214 from dog A-1.

178

179 Group 2 contains 491 and 492 and these isolates are indistinguishable from each other
180 but quite distinct to all the other isolates with only 28% similarity by cluster analysis.

181 These isolates are from two different dogs from the training centre population (dogs
182 B-1 and B-2).

183

184 The third group contains the type strain and isolates 510 and 214. The type strain and
185 510 are indistinguishable from each other, but 214 is distinctly different with only
186 about 46% similarity to the other two. Isolate 510 was from dog B-2 while isolate 214
187 was from dog A-1.

188

189 3.4. RAPD analysis of *M. cynos* isolates

190 When the same *M. cynos* isolates were subjected to analysis with RAPD with the
191 primer Hum4, 12 different profiles were obtained (Figure 2). The profiles consisted of
192 3-13 bands which ranged in size between approximately 240 and 2,200 bp. Two broad
193 groups of similar isolates were formed. The type strain and the 11 isolates 185, 190,
194 191, 210, 253, 312, 387, 417, 428, 429 and 510 had similar profiles and are
195 considered to be a homogeneous group with more than 68 % similarity (group 1). This
196 group comprises all of the isolates from the population A dogs, except for isolate 214,
197 but also includes 510 from dog B-2 and the type strain.

198

199 Isolates 214, 491 and 492 formed a heterogeneous group about 60 % similar to each
200 other, but only about 26% similar to the group 1 isolates. These isolates are from dogs
201 B-1, B-2 and isolate 214 from dog A-1.

202

203 The PFGE and RAPD grouping of isolates is summarised in Table 2.

204

205 **4. Discussion**

206 This is the first genetic typing study of *M. cynos*. The isolates from each kennel were
207 found to be genetically similar. Indeed, isolates from dogs that had been housed in the
208 same kennel 4 and 8 months apart were found to be indistinguishable using both
209 genetic analysis methods (isolates 417 and 191 from dogs A-2 and A-3, and isolates
210 429 and 387 from dogs A-1 and A-4, respectively). The dogs had stayed at the
211 kennels for between 8 and 16 days. This may suggest that there is maintenance of *M.*
212 *cynos* strains within a kennel situation. *M. cynos* can be isolated from the upper
213 respiratory tract of healthy dogs (Chalker et al., 2004) and it is probable that some
214 strains are passed between subsequent dogs, resulting in the survival of these strains.
215 In addition, environmental survival may aid the continued existence of some strains.
216 Although the environmental survival of *M. cynos* is not known, the environmental
217 survival of other mycoplasma species varies from a week to several months
218 (Nagatomo et al., 2001) and *M. cynos* can be isolated from the air (Chalker et al.,
219 2004). Recently it has been shown that biofilm formation is important for persistence
220 of mycoplasmas and may aid environmental survival (McAuliffe et al., 2006), it
221 seems feasible that *M. cynos* may be able to persist in the kennel environment as an
222 adherent biofilm layer.

223

224 The *M. cynos* type strain was isolated from the lung of a dog with CIRD in Denmark
225 in 1971 (Rosendal, 1973). This *M. cynos* type strain was indistinguishable by PFGE to
226 isolate 510 from dog B-2 and was more than 68 % similar by RAPD analysis to 11 of
227 the *M. cynos* isolates from both kennels. The high degree of similarity of the type
228 strain to these United Kingdom isolates from 1999 and 2000 suggests a low level of
229 diversity of this organism in CIRD. However, this study also shows that some isolates
230 have a relatively low level of similarity with each other (for example isolates 214, 491
231 and 492 appear to be dissimilar to the group 1 isolates). Indeed, this study suggests the
232 potential for mixed *M. cynos* infections, as the same bronchoalveolar lavage sample
233 from dog B-2 yielded *M. cynos* isolates 492 and 510, which are dissimilar strains.
234 Similarly, the BAL sample from dog A-1 resulted in the culture of the 214 isolate
235 which was dissimilar to the other isolates from this sample. A larger strain typing
236 study of more isolates is required to consolidate these observations.

237

238 *Mycoplasma cynos* was the only CIRD agent detected in two out of the six dogs (dogs
239 B-1 and B-2). Similarly, recently Zeugswetter et al. (2007) described lethal
240 bronchopneumonia in puppies where *M. cynos* was the only CIRD agent detected
241 from the puppies. Mycoplasmas have been the sole bacterial isolate in a number of
242 other cases of CIRD, but unfortunately these isolates were not speciated (Kirchner et
243 al., 1990; Jameson et al., 1995; Chandler and Lappin, 2002). However, in the current
244 study, both dogs were on a course of antibiotics preceding the sampling date (dog B-1
245 cephalosporin; B-2 erythromycin), which may have precluded the isolation of other
246 bacterial agents. Likewise, in the case of Zeugswetter et al. (2007), the puppies had
247 been treated with amoxicillin prior to isolation of *M. cynos* from the lung tissue.

248

249 *Mycoplasma cynos* has also been previously implicated in canine respiratory disease
250 along with other bacterial or viral pathogens (Rosendal, 1978; Chalker et al., 2004;
251 Chvala et al., 2007). This was also found in the current study as other respiratory
252 pathogens apart from *M. cynos* were detected in the four dogs from the re-homing
253 kennel, for example *B. bronchiseptica*, *S. equi* subsp. *zooepidemicus*, CHV and
254 CRCoV. Multi-pathogen respiratory disease is commonly reported and it has been
255 suggested that the pathogens may interact synergistically to produce disease
256 (Randolph et al., 1993).

257

258 *Escherichia coli*, which was detected in one dog in the present study, has been
259 previously isolated from BAL from a puppy with CIRD and was thought to be a
260 contaminant (Williams et al., 2006). This is likely to be the case in this study as
261 *Enterococcus* spp. was co-isolated from the same sample. In addition, *M. spumans*
262 was isolated from two dogs, one of which also had *M. canis* and *Ureaplasma* spp.,
263 however these species were not found to be significantly associated with respiratory
264 disease in dogs (Chalker et al., 2004).

265

266 In summary, the PFGE and RAPD genetic typing methods were in basic agreement
267 and showed that many of the isolates were highly similar. Strain maintenance is
268 suggested by strains which are indistinguishable by genetic typing, being isolated
269 from dogs housed months apart within the same kennel. There was also a high degree
270 of similarity of the *M. cynos* type strain (isolated from a dog with respiratory disease
271 in Denmark in 1971) to at least one of these United Kingdom isolates, which suggests
272 possible conservation of pathogenic strains of *M. cynos*.

273

274

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277 Bacteriology Lab for bacteriological screening of dog respiratory samples. We thank
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279

280

281 **References**

282 Chalker, V.J., Brooks, H.W., Brownlie, J., 2003a. The association of *Streptococcus*
283 *equi* subsp. *zooepidemicus* with canine infectious respiratory disease. *Vet*
284 *Microbiol.* 95, 149-156.

285 Chalker, V.J., Toomey, C., Opperman, S., Brooks, H.W., Ibuoye, M.A., Brownlie, J.,
286 Rycroft, A.N., 2003b. Respiratory disease in kennelled dogs: serological
287 responses to *Bordetella bronchiseptica* lipopolysaccharide do not correlate
288 with bacterial isolation or clinical respiratory symptoms. *Clin Diagn Lab*
289 *Immunol.* 10, 352-356.

290 Chalker, V.J., Owen, W.M., Paterson, C., Barker, E., Brooks, H., Rycroft, A.N.,
291 Brownlie, J., 2004. Mycoplasmas associated with canine infectious respiratory
292 disease. *Microbiology.* 150, 3491-3497.

293 Chandler, J.C., Lappin, M.R., 2002. Mycoplasmal respiratory infections in small
294 animals: 17 cases (1988-1999). *J Am Anim Hosp Assoc.* 38, 111-119.

295 Chvala, S., Benetka, V., Mostl, K., Zeugswetter, F., Spargser, J., Weissenböck, H.,
296 2007. Simultaneous canine distemper virus, canine adenovirus type 2, and

- 297 *Mycoplasma cynos* infection in a dog with pneumonia. Vet Pathol. 44, 508-
298 512.
- 299 Ellis, J.S., Zambon, M.C., 2001. Combined PCR-heteroduplex mobility assay for
300 detection and differentiation of influenza A viruses from different animal
301 species. J Clin Microbiol. 39, 4097-4102.
- 302 Erles, K., Toomey, C., Brooks, H.W., Brownlie, J., 2003. Detection of a group 2
303 coronavirus in dogs with canine infectious respiratory disease. Virology. 310,
304 216-223.
- 305 Erles, K., Dubovi, E.J., Brooks, H.W., Brownlie, J., 2004. Longitudinal study of
306 viruses associated with canine infectious respiratory disease. J Clin Microbiol.
307 42, 4524-4529.
- 308 Feberwee, A., Dijkstra, J.R., von Banniseht-Wysmuller, T.E., Gielkens, A.L.,
309 Wagenaar, J.A., 2005. Genotyping of *Mycoplasma gallisepticum* and *M.*
310 *synoviae* by Amplified Fragment Length Polymorphism (AFLP) analysis and
311 digitalized Random Amplified Polymorphic DNA (RAPD) analysis. Vet
312 Microbiol.
- 313 Hotzel, H., Schneider, B., Sachse, K., 1998. Investigation of *Mycoplasma bovis* field
314 isolates using PCR fingerprinting. In: Leori, G., Santini, F., Scanziani, E.,
315 Frey, J. (Eds.), *Mycoplasma of Ruminants: Pathogenicity, Diagnostics,*
316 *Epidemiology and Molecular genetics*, vol. 2, Brussels: European
317 Commission, pp. 17-19.
- 318 Jameson, P.H., King, L.A., Lappin, M.R., Jones, R.L., 1995. Comparison of clinical
319 signs, diagnostic findings, organisms isolated, and clinical outcome in dogs
320 with bacterial pneumonia: 93 cases (1986-1991). J Am Vet Med Assoc. 206,
321 206-209.

- 322 Kirchner, B.K., Port, C.D., Magoc, T.J., Sidor, M.A., Ruben, Z., 1990. Spontaneous
323 bronchopneumonia in laboratory dogs infected with untyped *Mycoplasma* spp.
324 Lab Anim Sci. 40, 625-628.
- 325 Kusiluka, L.J., Kokotovic, B., Ojeniyi, B., Friis, N.F., Ahrens, P., 2000. Genetic
326 variations among *Mycoplasma bovis* strains isolated from Danish cattle.
327 FEMS Microbiol Lett. 192, 113-118.
- 328 McAuliffe, L., Kokotovic, B., Ayling, R.D., Nicholas, R.A., 2004. Molecular
329 epidemiological analysis of *Mycoplasma bovis* isolates from the United
330 Kingdom shows two genetically distinct clusters. J Clin Microbiol. 42, 4556-
331 4565.
- 332 McAuliffe, L., Ellis, R.J., Miles, K., Ayling, R.D., Nicholas, R.A., 2006. Biofilm
333 formation by mycoplasma species and its role in environmental persistence
334 and survival. Microbiology. 152, 913-922.
- 335 Nagatomo, H., Takegahara, Y., Sonoda, T., Yamaguchi, A., Uemura, R., Hagiwara,
336 S., Sueyoshi, M., 2001. Comparative studies of the persistence of animal
337 mycoplasmas under different environmental conditions. Vet Microbiol. 82,
338 223-232.
- 339 Parham, K., Churchward, C.P., McAuliffe, L., Nicholas, R.A., Ayling, R.D., 2006. A
340 high level of strain variation within the *Mycoplasma ovipneumoniae*
341 population of the UK has implications for disease diagnosis and management.
342 Vet Microbiol. 118, 83-90.
- 343 Randolph, J.F., Moise, N.S., Scarlett, J.M., Shin, S.J., Blue, J.T., Bookbinder, P.R.,
344 1993. Prevalence of mycoplasmal and ureaplasma recovery from
345 tracheobronchial lavages and prevalence of mycoplasmal recovery from

- 346 pharyngeal swab specimens in dogs with or without pulmonary disease. Am J
347 Vet Res. 54, 387-391.
- 348 Rosendal, S., 1972. Mycoplasmas as a possible cause of enzootic pneumonia in dogs.
349 Acta Vet Scand. 13, 137-139.
- 350 Rosendal, S., 1973. *Mycoplasma cynos*, a new canine Mycoplasma species.
351 International Journal of Systematic Bacteriology. 23, 49-54.
- 352 Rosendal, S., 1978. Canine mycoplasmas: pathogenicity of mycoplasmas associated
353 with distemper pneumonia. J Infect Dis. 138, 203-210.
- 354 Rosendal, S., 1982. Canine mycoplasmas: their ecologic niche and role in disease. J
355 Am Vet Med Assoc. 180, 1212-1214.
- 356 Rycroft, A.N., Tsounakou, E., Chalker, V., 2007. Serological evidence of
357 *Mycoplasma cynos* infection in canine infectious respiratory disease. Vet
358 Microbiol. 120, 358-362.
- 359 Williams, M., Olver, C., Thrall, M.A., 2006. Transtracheal wash from a puppy with
360 respiratory disease. Vet Clin Pathol. 35, 471-473.
- 361 Zeugswetter, F., Weissenböck, H., Shibly, S., Hassan, J., Spargser, J., 2007. Lethal
362 bronchopneumonia caused by *Mycoplasma cynos* in a litter of golden retriever
363 puppies. Vet Rec. 161, 626-627.
- 364
- 365

366 **Figure Captions**

367

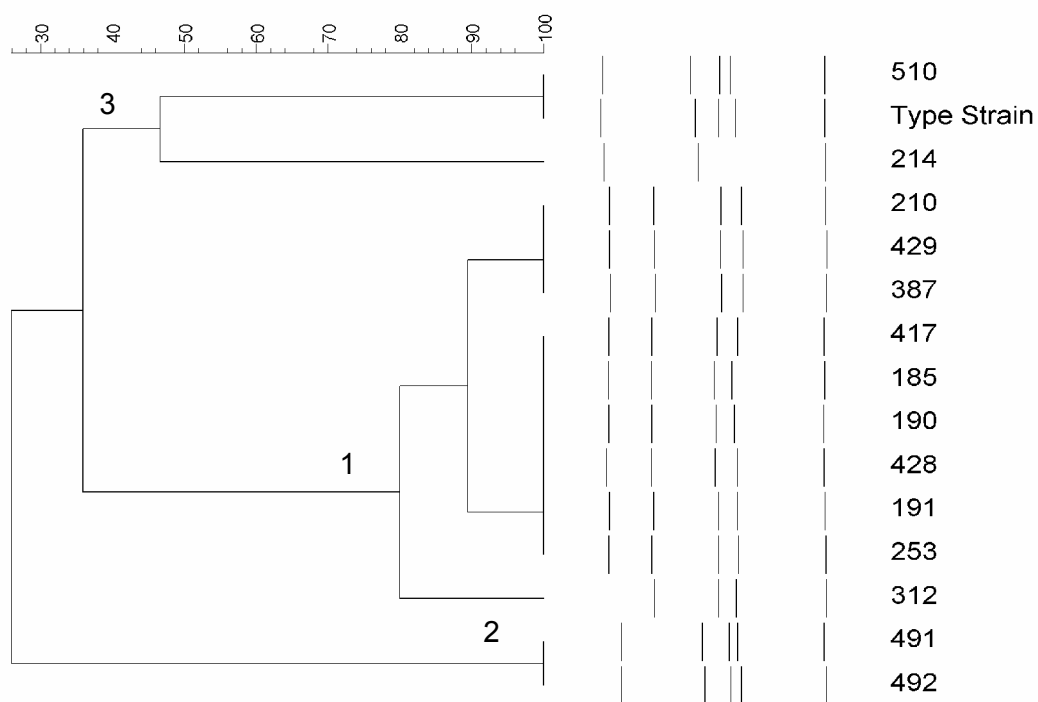
368 **Figure 1.** Similarity analysis of the *Sma*I PFGE profiles of the 14 *M. cynos* canine
369 respiratory isolates from six dogs. Numbers 1-3 denote the groupings of similar
370 profiles.

371

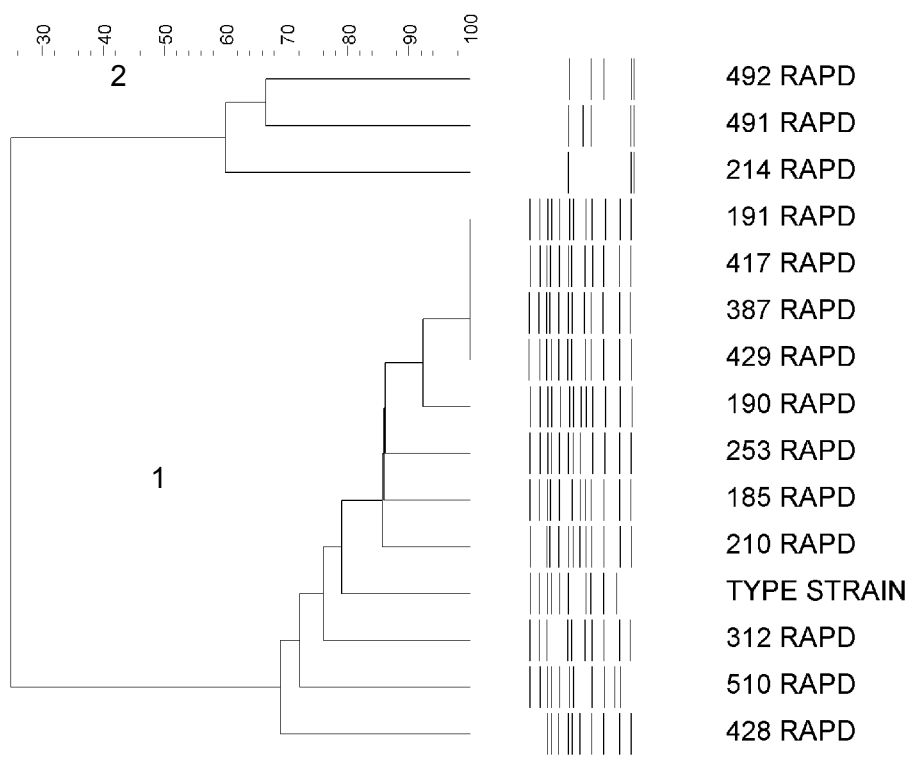
372

373 **Figure 2.** Similarity analysis of the profiles produced by RAPD with the primer
374 Hum4. Numbers 1 and 2 denote the groupings of similar profiles.

375



Accepted



ACQ

Table 1. Details of dogs with respiratory disease from which *M. cynos* isolates were cultured, and bacteriology and virology screening results of trachea (T) and bronchoalveolar lavage (BAL) samples.

Dog ¹	Age (years)	Sex ²	Breed	Respiratory score ³	Date sampled	Bacteriology	Virology
A-1	1	MN	German shepherd	3	03Jun99	<i>B. bronchiseptica</i> & <i>M. spumans</i> in BAL; <i>M. cynos</i> in BAL & T	negative
A-2	2	M	Staffordshire bull terrier	3	03Jun99	<i>B. bronchiseptica</i> & <i>Pasteurella</i> spp. in BAL; <i>M. cynos</i> in BAL & T	CRCoV in T
A-3	2	M	Dalmatian	5	11Oct99	<i>S. equi</i> subsp. <i>zooepidemicus</i> in BAL; <i>M. cynos</i> & <i>M. spumans</i> in BAL & T	negative
A-4	3	FN	Mongrel	5	24Feb00	<i>S. equi</i> subsp. <i>zooepidemicus</i> , <i>Enterococcus</i> spp., <i>Ureaplasma</i> spp. & <i>Escherichia coli</i> in BAL; <i>M. canis</i> & <i>M. cynos</i> in BAL & T	CHV in BAL
B-1	1	MN	Labrador	5	08Nov00	<i>M. cynos</i> in BAL ⁴	negative ⁴
B-2	1	F	Labrador	3	23Nov00	<i>M. cynos</i> in BAL ⁴	negative ⁴

¹A, re-homing centre; B, training centre.

²N, neutered.

³3, cough and nasal discharge; 5, bronchopneumonia.

⁴No trachea sample available.

Table 2. *Mycoplasma cynos* isolate source and genetic typing groups.

Dog	<i>M. cynos</i> isolate #	Source	PFGE group	RAPD group
A-1	185	BAL	1	1
	210	BAL	1	1
	214	BAL	3	2
	253	BAL	1	1
	428	T	1	1
	429	T	1	1
A-2	190	BAL	1	1
	417	T	1	1
A-3	191	BAL	1	1
A-4	312	BAL	1	1
	387	T	1	1
B-1	491	BAL	2	2
B-2	492	BAL	2	2
	510	BAL	3	1